Biochemical Engineering

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Module No. # 01
Lecture No. # 23
Microbial Growth: Phases and Models

Biochemical engineering that we are doing and today we do is start with the new chapter which is called microbial growth. But, before we start with the chapter let us try and understand that what we have been doing and how this translates or how this flows into what we are trying to do in the next few classes. So, we have been looking at the concept of enzymes you know and enzyme in mobilised and immobilised forms and how that affects the process that, catalyses the process. What that how does that relate to is that in any chemical, biochemical systems enzyme catalyses the process and so on? But, many of the process that are involved or some of the process at least that are involved the growth processes for example, the cell growth process. And what we are going to look at today and in the next couple of lectures is essentially the cell growth process.

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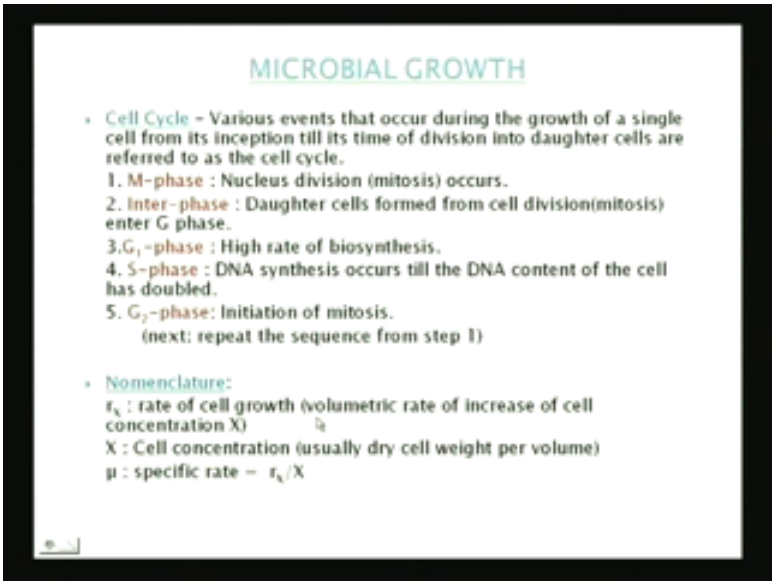

So, let us start with the screen and what we have over here the microbial growth. Now what is microbial growth or cell growth in general? Something called the cell cycle and cell cycle is essentially the various events that occur during the growth of a single cell from its inception till its time of division into daughter cells. And that whole thing is referred to as cell cycle. So, from the point where single cell inception occurs to the time when it divides into daughter cell that is known as one cell cycle and there are several parts of the cell cycle. And let us try and understand what these different parts of the cell cycle are. So, the first part is known as M-phase. Why it is called M-phase? Because this is where nucleus nucleus cell division or mitosis occurs and that is way it is called M-phase. So, this is when when the mitosis occurs. Next is inter-phase. So, after mitosis occurs, daughter cells are formed from from cell division that is mitosis. And so, two daughter cells formed and the cells enter into g phase or the growth phase. So you start with the m-phase of mitosis phase. Then, you go what to what is known as the inter-phase which is the phase between the m-phase and the mitosis phase and the growth phase or the g phase.

Then, you move on to what is known as so after the inter-phase. You move on to what is known as the G phase. Now, G phase there are two G phases; one is called the G one phase and then there is another one will come into a minute which is known as the G 2 phase. Now, what happens in g one phase there is a higher rate of biosynthesis so essentially the once the daughter cells are formed so there is growth of these daughter

cells. So, it is like you know a baby being born and you you give nourishment nutrition to the baby so that the baby grows into fully mature adult. So, that is what it happens so the adult cells mother cells generate the daughter cells which are which now need to be replenished through growth and therefore, there is a higher rate of biosynthesis and this phase is known as G one phase.

The next phase is known as s-phase so because what is what happens in this phase is, DNA synthesis occurs. So, the first G phase, G one phase is essentially there is all part of the growth phase again. But, the first G one phase that we talked about is essentially biosynthesis of you know different elements. And and and and these different elements come into the cell and the next S-phase is very important cell with DNA synthesis occurs till the DNA content of the cell has doubled. So, that is the DNA synthesis occurs until the DNA content of the cell doubles.

And then is the G phase, G 2 phase the final phase which is the initiation of the mitosis. So, what happens in the G 2 phase is so, you have you are at 5 number 5 so from here it goes back again to number 1. So, this is cell cycle and that is why it is called the cycle. So, it started with M-phase when nucleus division or mitosis occurs followed by the inter-phase with the daughter cells formed from cell division and it enters the growth phase. The growth phase is the higher rate of biosynthesis G one then, the S phase which is the DNA synthesis. And then the G 2 gain which is initiation of the mitosis and it goes back to the M-phase. So this is the entire cell cycle.

Ah now the nomenclature that we are going to use for much of today and in the next few lectures is r x would be the rate of cell growth. So why is this nomenclature? Because what we are essentially trying to do is, we are trying to quantify the rate of cell growth. Now, what happens? We are doing an experiment in the lab. For example, you know you do a process of what is known as incubation of cell right. So you take a petri dish or chemostat or something like that and you start off with so what is known as starter. So, you start off with some cells and then you incubate them, you provide nourishment nutrients to the cell, you you retain the certain level of carbon dioxide and oxygen supply and then you maintain the p h level at which the cells can grow and the temperature so the required required elements are at certain temperature. So say up to 25 30 at most 35 degree 37 degree at most so that kind of temperature. You cannot increase the temperature to say 40 42. The cells are going die. So, each cell depending on what cell

you are trying to grow would have temperature ranges like we had in the case of enzyme. If you remember, the enzyme was functional within a temperature range in a p h range. Similarly, so here for example, a cell would be for every cell there is a temperature range within which the cell is functional so the temperature range could be say 25 to 37 or forty and so, 38 so on not not more than that. So, and then in that p h range of say 6 point 7 to 7.2 or 6 point 8 to 7.2 or 7 to 7.4 the p h range where it will be effective. Then you would have to maintain a carbon dioxide concentration say 5 percent or something like that. A certain oxygen concentration and then you have to give nutrient. So, that all the elements that are needed for the cell to grow would be there. So what are all the elements that are needed for the cell to grow? The same elements that are needed for for a human body to grow because you know the cells are what constitutes human body. So what could be the element that required for the the cell to grow? Oxygen I already mentioned, carbon one is one of the essential ingredients, nitrogen, hydrogen, hydrogen right. So, essentially these are the nitrogen, hydrogen, carbon, oxygen. So, these are the essential elements that you require. So, you have to provide those elements in terms of nutrients. Oxygen supply is of course, there and carbon dioxide supply is of course, there in addition you need to provide in terms of nutrients like for example, what would be the most common nutrient that you provide to the cell? What would be the most common? For example, if you dehydrated what what is that, what is the thing that the doctor or everybody tells you to take?

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Yeah. what is that what is that contain

Saline

What is that contain not saline and water

Glucose

Glucose essentially, yes so water containing sugar and salt. So, what would happen is that what is the element in glucose? Essentially carbon, hydrogen, oxygen. So, that is the reason that you are because your essential nutrients are here. So, you can provide some kind of nutrients like that and you know you need nitrogen and also, some ammonia or some other some other constituent which has nitrogenise. You provide these so then

what you do? What is the process of a cell growth? Has any of you have done have done cell growth in the lab? So, the process of cell growth is essentially you know, at home your excuse me yeah this is the process of cell growth that is used at home. Your mother does it probably and what is it? Can you tell me what what is that process of cell growth is

(( )) curd

For curd yeah

Curd

Yeah yeah for curd or yogurt. So, pleasing yogurt so what what you do? It takes little, it is the same process in the experiments also in the lab. You take a little bit of starter right. So, what does starter contain? The starter would contain a little bit of yeast, little bit of bacteria that is necessary. And then you provide, what is the process? You know if you think from engineering point of view the process of say curd formation yogurt formation what is the process you will take? A little bit of starter which contains the microorganisms that you need to grow and then you provide suitable environment, suitable environment suitable temperature, suitable acid conditions, suitable p h, enough oxygen and nutrients, some water and so on. So, that the thing grows and the microorganism grows and within a period of time, you will see that the entire milk has been curdled right. So, essentially the the it is a growth of microorganism and it what is the time scale for that process say around 24 hours curdling process would be around 24 hours. So similarly, is the exact same procedure that we do in the in in our homes everyday is the procedure that is transported to the labs when you incubate cells. It is may be a slightly longer procedure. So, why do this? Because some of these microorganisms for example yeast or some other microorganisms that you use may be expensive you know. You do not if you want to buy them all the time it is pretty expensive. So, what done is you grow these microorganisms in the lab.

So for example, you know, one of the microorganism yeast of course, things like macrophages. I talked about macrophages earlier in different point of time. So, these are immune cells and you want study for example, the properties of immunes. This so what I am trying to say is that two aspects one is microorganism that are grown that are beneficial for for human you know scientific whatever activity or whatever and you want

to grow those microorganisms for your use. That is the utilisation purpose and that that is something that you can do in lab, that is something that you can do in these factories and so on. And you can do in at home also. But, then there are other kinds of microorganisms which you do not necessarily need to utilise. But, you need them for scientific enquiry. for So, for one of them is this, for example, immune cells so if you want to buy these immune cells; monocytes, macrophages from the lab, they are pretty expensive. So, you buy some of them and then you regrow this over a period of time so that you have a steady supply of these during your experiments. So if you are forming your experiments everyday so we will now looking it for from very local lab scale point of view. So, if you are performing your experiments everyday, you need a steady supply of say 10 million or 20 million or 30 million macrophages. So what you do? You put a little starter or say million or couple of millions in a petri dish and then you allow the suitable environment which is that, so you put it inside in incubator. So, you have these incubators you know, many of us have in the lab. So you put put it into the incubator and you allows some of these incubators will allow certain carbon dioxide flow. So, you allow a certain carbon dioxide flow of 5 percent or something and you know oxygen flow is of course, there and then you put the nutrients in there. So, this is the process. I am trying to describe this cell growth process before we go into modelling and more complicated thing because it is important to understand that what really happens. So, you put in the nutrients whatever is required. Nutrients for the for the cell which should include carbon, hydrogen, oxygen, nitrogen and so on and allow it to be there for say around 24 hours, the same time scale that you would use for the curdling process.

Now, after you come back for for after 24 hours and you started with say 5 million cells you would expect more or less doubling say, you know if the doubling time is you will do that you know what is the doubling time you need to figure out what is the doubling time for the cells? If your doubling time is around say 20 4 hours so after 20 4 hours you would expect to have 10 million cells. But, what you do do you do you just take the petri dish and expect to have 10 million cells and start working with 10 million cells? No you do not do that. You take you take out the sample out of that little bit out of that liquid that you have and then you you spin it in a centrifuge. You spin it in a centrifuge. what Why spin it in a centrifuge? Just to get rid of the everything else surrounding it and isolate the cells. Then you take those cells below under microscope. Why do you take those cells under microscope? Can anybody? Into it so you just took a sample, you did

not take the whole thing in a petri dish or in a in a in a beaker or something you grow these cells. So, you started with 5 million cells you expect 10 million cells to be there. You provided the right nutrients, the right temperature, the right p h, right carbon dioxide, and oxygen, oxygen flow and everything was fine and you expected up to 24 hours to have 10 million cells. But, what will you do? You just take the things and assume that there are 10 million cells. No you are not going to do that. So, what are you trying to test? What I am trying to test here? Whether there are 10 million cells or not right that is what I am trying to tell and what other possibilities? Because this is very related to what we are going to do just now and what are the possibilities? For, let us talk about that.

So, one of the possibilities is that the cell did not grow at all right. The second possibility is that cell grew but, the cell did not grow at the rate that I thought it would. But, then what is the, what is what could be the reason for that possibility? The reason could be that I did not provide the right environment, the right nutrient, the right temperature, the right p h. Now, I might have provided. Then you might argue that well, I provide all the right environment. Then why is the cell not growing or why should not the cell grow. So, if you provided all the right ingredients then what is the third possibility that you say? So, first possibility is that cells did not cells did not grow. That would happen only if when grossly wrong. So if your say p h range was 7 to 7.4 for the, for the cell growth and you put your p h itself 7.8 then no cell growth. That that is something that will happen if you go grossly wrong. The second is the cell growth is reduced which means that the growth kinetics is retarded you know, is decelerated. There may be reason for that again. P h temperature can be effect but, small variation so if you are p h range was say 6 7 point 7 to 7 point 4 and you put your p h at 6 point 9 or 6.9 5 then, if the cell growth would happen but, it would be slightly retarded. But, what else could happen? What could be the third possibility?

So the cell grew you know, the third possibility could be cell grew but, something else happens cell grew at the rate that you that was supposed to grow because see the calculations typically are not wrong. That if doubling time has been ascertained for a particular kind of cell it has been ascertained through series of experiments and specific set of conditions have been specified right. Particular set of conditions have been specified. Now, if you maintain those conditions there is no reason for the cells not to

double. What is the other possibility? Just think about that if the cells grow at the rate that it was suppose to grow what else is possibility?

The third possibility is that cells are dying. It is very simple you know possibility. Cells are growing at the rate that are suppose to grow but, after growing they are dying and what could be one of the reasons? One could one of the reasons could be population explosion. That is, what is that what I mean by population explosion is that the amount of nutrients that you provided was not enough for 10 million cells? That is a very important point that you you thought the amount of a nutrient that you provided was alright for 10 million cells. But, amount of nutrient that was provided was not enough for 10 million cells and you know the same the the this biological principles that apply for for different animals or whatever applies for cells also because this is survival of the fittest. And so these cells will start to fight with each other if there is a there is there is less amount of nutrient that is present. So this start cells will compete with each other for nutrients. And the the cells that depleted of nutrients will die right. Is it clear? So, this is a very important thing that you have to understand that just as there is growth of cells or birth of cells that there is death of cells. And this is a this is a thing that is there inherently there. This is the thing that you cannot get rid of that you cannot assume that you put an put in nutrients and everything and then cells will grow grow as it is. So, the one of the possibility of cell death could be that there, there is not enough nutrient for that population; the second possibility is that the, you know there is defects in some of these cells that are growing as a result of which they are dying. So, what I am supposed to do now? That is why I take out a sample from there and centrifuge it and first of all, I drain the liquid around it, centrifuge it and then I put it in the microscope. What I do under microscope? I try to measure the amount of dead cells amount of dead cells that is that is there. So, how do I measure it? Let me will show you.

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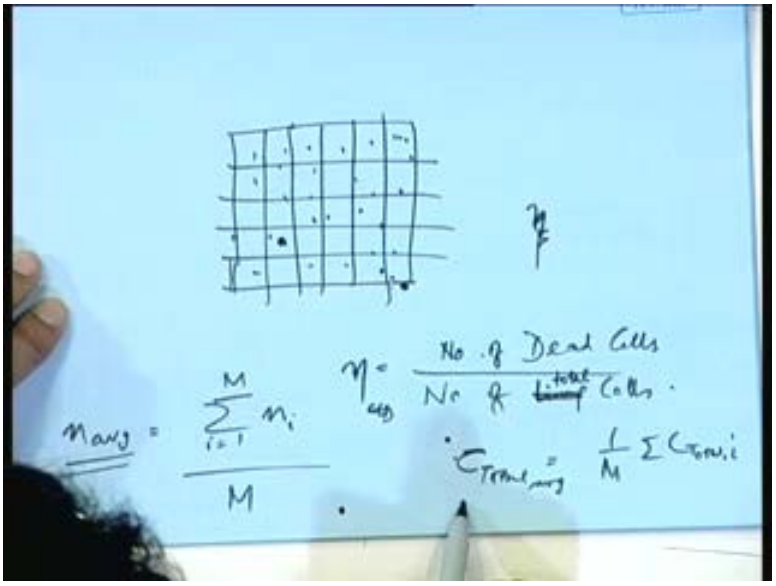

So what you do is, you put your so you take your cells. These are you take a dish like this. These there are these dishes that are available in the lab, flat dishes with markers on them. So, what you do is in it put that sample. After you centrifuged you put that sample here and and by the way what you have to do is after centrifuged, you dye it, you dye the cells. And so what happens is that, when you put the cells in like this, these cells would be so these dots that I put over here can you see the dots? So the dots that I put over here are the cells. Now the cells when you dye a cell with a certain dye; cells that are living would be of a certain colour and the cells that are not living would be of another colour. Because the cells that are living with the dye would get diffused into the cytoplasm of the cells and the cell would have certain colour. The living cells are red then the other cells could be blue or some other thing. Now, you put this under a microscope over here and then you look at the cell. So, you can figure out so you you have a count so these these number, these blocks are there so that it is easier to count. So you have a cells, count of the first you have to count the total number of cells and then you have to count the number of living cells or the number of dead cells whichever. People typically count the dead cells. So the total number of cells and the and the number of dead cells. So then you will have a average fraction say n equals number of an average is number of dead cells over number of living cells. So, you will have a average for the sample. Now assuming that this is the random sample, you need to do this a few times. You just cannot do it once and trust that you know it do it three or four times and based on this these n basically lets called this n then so n average would be. So n I I going from say one to m

if you have done this m times over m. So that would be your total, average average fraction of dead cells in the system. So, from that you can just multiply by if you are suppose to have like 10 million cells or something then you just multiply that what is what would be my total total number of living cells. Now, how do I how do I know whether actually I have 10 million cells? Right that is the question. I expect 10 million cells does not necessarily mean that you'll i will have 10 million cells. So how do I know that? What I do is I take say for example, if my total liquid is like hundred millilitre or 50 millilitres; say I take a 5 millilitre sample and now what I have done over here is I have counted. If you look at this, I have counted the number of cells not just number of dead cells, I have also counted the number of total number of cells in the system right. So, you can just multiply if my 5 million, 5 millilitre gives me one millilitre, whatever. You know or one millimetre you take one millilitre sample out of that so one millilitre gives me this amount of cells then 50 or something would give you hundred millilitre was suppose to give that and because this I am doing this again and again. So, then this there is an average thing that is, there as averaging that is coming out. So, calculate the n average which is fraction of dead cells. Actually this should be number of living over number of total cells. And similarly, you calculate the total c. c total let us call it. c total average of that, so if you taking if you did it for 5 times and you calculate the c total for each. So, one over m sigma of c total. I So that would give you the average number, average number of cells within that and you can calculate, multiply that by the number to find what is the total number of cells in your system. So then what you find? For example, at the end of the day is that, while you are suppose to have like 10 million cells you end up having say 8 million cells or something. And what what you do next day is that you take out out of those 8 million living cells whatever you have take out whatever you need for the experiment or say you you might need lot more you might need 50 million for your experiments. So you use the 50 million cells or say forty million cells and you recycle 10 million cells. So you put those 10 million cells again for next day put in nutrients and put it in the incubator for 24 more hours and recycle it. Why do you recycle it? Because if you are running your experiment everyday you need a steady supply of cells everyday fine. Now what do you with the 8 million cells or whatever forty million cells that you have? Can can use it? No. You have to wash them. So you need to wash the cells because the cells are were was with the nutrients. Now, if you are going to do your experiment, you need to wash it you need to wash it and then you know centrifuge it possible that if drain away the liquid and then you can use the cells for your

purpose. So this is the essential process of cell growth in the lab in the in the lab while you do your experiments.

What we are going to study here in today's lecture and part of today's lecture and next lectures is what is the mechanism, what is the quantitative way you can measure these cell growth. So these are experimental ways of I talked to you about how we actually go and measure the cell growth under in experiment. But, what would be quantitative mechanism to measuring cell growth, what other models of cell growth and what if a much more complex system is involved. For example, you do not have one kind of cell and one kind of nutrients that are growing but, you have multiple cells of kind that are growing and same set of nutrients can faster the growth of different cells right. So, if you are trying to incubate two or three different cells at the same point of time. Then what happens? So, are there interactions between these cells? Possible, there could be interaction between each cell. Some of these complicated things. Why? Because see when you are doing these experiments in the lab; it is more of pristine controlled atmosphere right. But, when these things happen in real life you really do not know what kind of interactions there could be. There could be varied complicated interaction between these cells. For example, you know many of these bacterial diseases that we get especially in in countries like India, tropical countries or subtropical countries. We get get these lot of diseases in summer, late summer and then in the monsoon. Can you can you can you (( )) why we get all of these some of diseases or things like that more here then say then say in the colder countries?

Environment

What kind of environment yeah that is right but

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Yeah It is a hot, humid yeah humid climate. So, essentially what is happening is this, for example, these bacteria you know many of the bacterias that are responsible for gut infection and other kinds of infections even there you know upper respiratory tract infection, lot of these different kinds of infections that are there. So what happens? In colder climate because of temperature is very very low, these cells cannot grow. So temperature is the major hindrance in in their growth process whereas, for us if you think the temperature is actually very, very ideal for the cell growth process. This is a ideal

temperature that around 25 to 37 degree centigrade or 20 to 35, 37 degree centigrade and the temperatures that are offered in that are available in these tropical and subtropical countries are actually ideal for that. So that is one and because of humidity in many of the, you know, oxygen and oxygen is available everywhere in general. But, because of humidity, the moisture that is there in the air is also, something that can, that the bacteria can use.

Now, why am I talking about this because in the human body in physiological systems so when you are doing a controlled experiment in the lab with one single cell it is a straightforward process and that is something that we are going to study today. But, in the later lectures may be tomorrow or in the next lecture one after that we will study what happens when there is a complex dynamics between these cell growth processes that occur and why are we talking about complex dynamics between the cell growth processes. Because, in physiological systems, in human body that is what happens. You do not have a controlled atmosphere where you are very carefully growing one cell and and looking it under the microscope right. So, that is not possible. So, all these cell growths are connected to each other. So, the growth of one cell might suppress the growth of another cell and the amount of nutrient is that is available you known for example, e coli you know, colitis e, coli b, coli some of these colitis bacteria that that are very well known to grow inside the human body. When they grow, they will take away much of the nutrients that is available right and they will therefore, suppress the growth of other bacteria beneficial bacteria that is necessary for for example, producing enzymes for digestion. So you know, the the digestive enzymes are often produced by microorganisms in the system you you one of the things that you might know is that you know might might or might do not know but, lets I will talk about it. For example, when the doctor if you have a if you have a say upper respiratory tract infection some of us getting in these days because of season change and all that stuff. So, the doctor gives you some antibiotic right. For example, as erythromycin is there, typical antibiotic for upper respiratory tract infection. So, doctor gives you that. So, when you take those antibiotics have you ever noticed if you take those for 3 or 5 or 7 days that you have bad stomach right. You have bad stomach and why is that?

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Useful bacteria in the gut. Exactly, that is the reason. See because in the guts human guts is useful beneficial bacteria that are that are used for digestion and all gut related you know intestine related activity are being constantly formed. Now the antibiotic is the one that kills bacteria and most of the antibiotics not all but, most of the antibiotics do not have the capability to decide or differentiate between a good bacteria and bad bacteria. So, why that kills the bad bacteria? That is, there in your lung or elsewhere and these go through the blood right. So they are everywhere. So even if it wants to kills now the nowadays we have localised antibiotics but, despite that so because it flows through the blood so why it is suppose to kill only the bad bacteria in your lung or upper respiratory tract? It also kills the good bacteria that are there elsewhere. It can kill the macrophages that are there reduce your immune system. That is a very bad part of it. It can kill bacteria beneficial bacteria that help in digestion in the guts. So, that that is why you have these problems. So what is the antidote? One of the things that usually doctor say tells you or gives an addition medicine when you have a bad stomach and you take because of antibiotics so when you take antibiotics for long, you you are bound to have bad stomach you know. This is something that will happen to you so what is the antidote? Have you ever thought about it? Somebody ever told you that? You eat yogurt curd why because curd regenerates that those microorganisms and sometimes what happens is that, so these science, this pharmaceutical companies are getting smarter so the doctor use to give you or may be may be use to give you one with antibiotic another medicine and but, these days you see so, some of the antibiotics will say saccharomyces cerevisiae. So, which means the yeast is there in the antibiotic. So, so while the antibiotic is killing the yeast it, it also has a ready supply of yeast in it to regenerate these antibiotics. So, this is something that is coming up new you know, probably in the last couple of years (( )). And you would have seen that pharmaceutical companies produce these drugs which come, antibiotic which come with yeast. If it does not come with yeast, doctor either gives you a separate antibiotic, separate tablet for generation of yeast or tells you that you need lot of yogurt. So, that to regenerate the yeast so that is the kind of system that I am talking about, where there is complex interactions between between bacteria. So one cell is growing and the other at the expense of another cell may be. So or may be one cell is killed along with that another cell is killed. So, some of these complex interactions we will study and we will try and see that how this could be extended to other complicated systems okay.

So but, let us start with the simplest thing today, first which is the cell growth as I described in a experimental environment with a very simple controlled experiment. So the the nomenclature that we are going to use they are written out here. r x is the rate of cell growth which is the volumetric rate of increase of cell concentration. So just you had the rate rate of reaction in an enzyme this is the rate of cell growth. x is the cell concentration we will use the notation x for it usually dry cell weight per volume. This is how it is defined. Dry cell weight that is you drain away the liquid just as I said you drain away the liquid centrifuge it and take the dry cell and dry cell weight for liquid, for volume and mu is the specific rate. That is growth of cell cell per unit concentration of cell. Cell growth per unit concentration of cell. So these are the nomenclatures that we are going to use and you may want to note them down. So, r x is the rate of cell growth, volumetric x is the cell concentration, dry cell weight per unit volume and mu is the specific cell rate of growth that is r x per unit concentration. ((no audio 31:02 to 31:33)) So, one of the reasons we are studying this cell growth process in this lecture is of course, that this is a reactive process.

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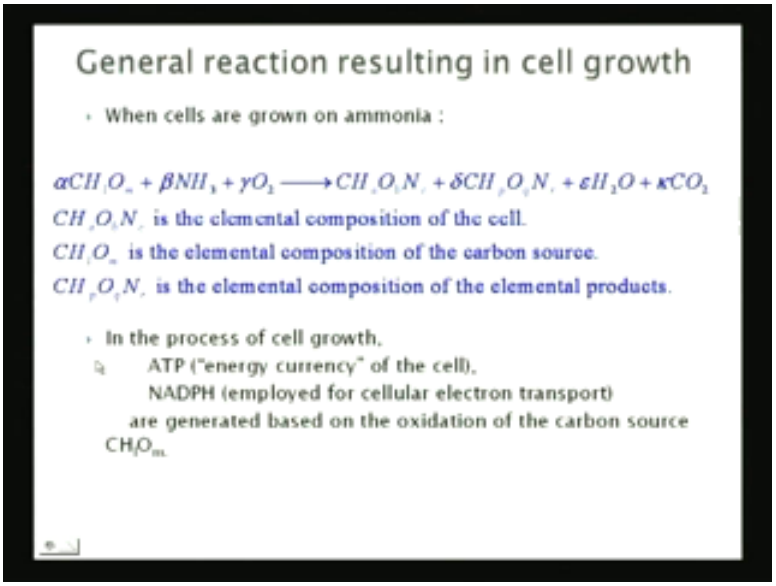

So what happens? What is the reaction that occurs during the cell growth that we need to understand? So, as I told you that what are the, what are the nutrients what are the components that are necessary? Nitrogen, hydrogen, carbon, oxygen right and one of the main sources that we give it through is glucose. Glucose of course, does not contain the nitrogen source we need to add a nitrogen source. So, the nitrogen source that we are

adding is ammonia over here. It is as it is written and this is a kind of reaction that happens. Let us look at this. So, first one that you have alpha c h l o m this is, lets called this glucose source, the carbon source could be glucose or some form of carbon. Then use the ammonia, which is the nitrogen source. Oxygen is plentiful you know any any time you do a cell incubation, oxygen is there. In addition what has not been described here you can give a little bit of carbon dioxide because the incubator that you use typically has a has the possibility that you can use two percent, three percent, five percent carbon dioxide and people give give that often because that acts as an additional source of carbon. But, you may not also but, because carbon is there in this carbon source that you have give glucose or whatever. So, then what happens is this carbon source changes itself and you get from that carbon source some other form so because the part of it is being utilised. So, this is the c a is the elemental composition of the cell that that is going in and sorry yeah this this is this is the source (( )) changes itself but, this is the elemental composition of the of the cell c h a o b n c. So, this is not the cell itself but, the constituents of all these chemicals inside the cell. So, we assume that carbon hydrogen, oxygen, nitrogen are present in the ratio one is to a is to b is to c. That is the assumption here and then this is what is left and then water is left and carbon dioxide is left. These are very straightforward things but, this is what we are more interested in. And this is as I said, this is what is the left so you start with this and this so you add carbon source so essentially to remember that use the glucose or some kind of carbon so nitrogen in terms of ammonia say here and the oxygen and this is the one that is important to us, this is what you get inside the cell and this is what is outside all of it fine.

So, what happens in the cell cell growth process? the In the cell growth process ATP is generated and this something we discussed before. ATP is adenosine triphosphate and I think we have done this in professor (( )) would have talked about this a lot. So, it is essentially a energy currency of the cell. So when we talking of matter the cell growth process the nutrients what we are talking, what we are essentially interested in is energy right. That all of us essentially interested in in it starting from the human body to the to the human civilization everybody is interested essentially in the energy.

So but, what is the process? The process is you taken matter you burn the matter to generate the energy right. So ATP is known as energy currency of the cell. So, it is like

money. It is like bank notes. How much money you know you have? You measure in terms of bank notes. The cell measure how much measure, how much energy is that it has in terms of the amount of energy? ATP it has and it all does not spends all the energy together. So, it converts from ATP adenosine triphosphate, adenosine diphosphate, adenosine diphosphate to a t a m p adenosine monophosphate and these are minor things so it kind of spend money, spend the energy money little slowly. So ATP is the energy currency of the cell and ADP is is you know it is part of the cellular electron transport mechanism. NADP, NADPH plus so essentially this is a very important thing that we have done this in some other course before. So, I do not want to repeat that. Some many of the cellular transport processes if you remember from what we did in other course before are governed by NDPH and because of the cellular electronic transport that is there. And and this is based on the oxidation of the based on the oxidation of the carbon source. So, apart from the nutrients; so that are three outputs that are there in the cell. So this is one c a c h a o b n c this is the material output. So the cell (( )) this material but, what is the cell is essentially interested in energy and that energy is stored in terms of ATP. So, there will be a material or bulk increase in the size of the cell. So I was explaining you know the cell growth process like the growth of a baby after it is born. So initially you know, if I am to go back here so initially this is a cell cycle. So, initially the cell are are born through mitosis and daughter cells formed. So you start with the M-phase of mitosis phase then the inter-phase when the with daughter cells are born and then this is the phase that we are looking at high rate of biosynthesis G one phase. So, the M-phase is the nucleus cell division, mitosis formation of the daughter cells. Now this is like a birth of the baby and then when the baby is born there is a higher rate of biosynthesis goes on for years together may be fifteen or sixteen or eighteen years, till the baby turns into an adult. So there is a, what is happening? So two things are happening in this high rate of biosynthesis; one is a material increase of the volume of the cell just like a baby grows into mature adult and the size of the baby grows and also the energy that is used and stored by the cell increases that is the ATP of the system increases. If it stores more energy you can use more energy right. So then it is DNA synthesis phase that we are not looking at and the DNA synthesis occurs till the DNA content of the cell has doubled and then again it goes back to phase one over here. Initiation of mitosis, so it is a cyclical process. So, what we are looking at here is the phase three, the G one phase, rate of cell biosynthesis okay.

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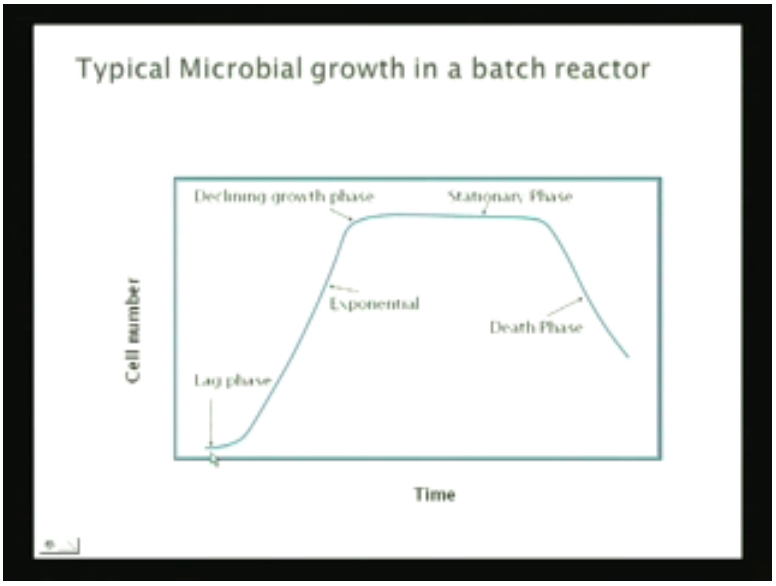

So, let us this is an interesting thing. So this is an experiment that had been done in a batch reactor. the this experiment that I described to you is now at the beginning of this lecture that is done in the in a large scale. So what really if I am monitoring if I am taking out sample from time to time what I talked to you about is I start here and I talked about the end end product right. I started here 0 hours and this is say 24 hours then this is where I measured the cells fine or somewhere around here 24 hours I measured the cells. What if I am taking out samples time to time from my petri dish or wherever I am incubating and measuring the concentration of the cell. How do I measure the concentration of the cell? Just as I described you take it out centrifuge it put it under microscope and measure the concentration of the living cell. Then what do I find? And it is very interesting thing. So, this is what it shows your cell number versus time and this time is same in the length scale of hours. So, 24 hours or 20 hours or whatever. So, initially what you find is look here, initially what you find this is known as lag phase. So initially what you find is, you put the cells in and nothing happens for a period of say one hour or two hours nothing happens. Because why is it because because once it before you you go to the phase where it starts to divide. What is the cell growth? Let us understand the cell growth process. So, what we are looking at is that the rate of biosynthesis what I just showed you. The reaction that is, that I showed you right. But, you want these cells to double right and that means that it has to go to the initiation of mitosis phase. So initially is it clear to everybody initially you have the cell growth phase which is the high rate of biosynthesis phase and once a high rate of biosynthesis is over;

that is the cell has attained the mature adult stage; then it starts to divide forming daughter cells. Now, what you are measuring over here is the total number of cells in the system right. So, if you want the cells to double or say increase in in number then, first it has to go through higher rate of biosynthesis phase and generate all the energy that is required and then then it starts to divide. So initial lag phase this when the only biosynthesis happening and there is no cell division. And then after the cell division go gets over then you start the growth phase. That is growth means cell growth phase cell number growth phase growth of cell numbers or in other words the mitosis of the cell division occurs. Is that clear? okay.

So, this turns out to be an exponential phase. So, this this growth is an exponential phase. So growth is occurring then you have a stationary phase. That is the cell number reaches so cell number is constant initially then it increases very fast exponentially then it reaches a stationary phase. So, if you would like to have (( )) and then what it what it what happens? It starts to decrease. If you have like to have maximum maximum output from your culture; so the culture process that I described what you do you would stop somewhere here right. You stop somewhere here and the take cells out fine. So, that is why it is important for us to figure out what is the dynamics of the process because if I know the dynamics of a certain process, so if I know certain cell a or b has a certain dynamics then I would stop my culture at say I would no after sixteen hours this this stationary phase starts so I stop at sixteen hours. I stop my incubation of the culture in sixteen hours, take it out. Now you have to tell me now, I told you that this is the growth that is happening. You have to tell me that how this stationery phase is attained? What happens in the stationery phase? Why do we stop cell growth stops? And if so, why?

(( ))

What is that?

(( ))

No (( )) not deficient in this phase

Cell (( ))

What is that?

Cell (( )) stop

No, cell division does not stop. If nutrient is not deficient why would cell division stop? Cell division stops, will stop only if nutrient is deficient. Why this why this cell number is not growing any further?

(( ))

Right, correct the growth rate and the death rate are equal in the stationary phase. It is not that nutrients are deficient. If nutrients are deficient then cell cell division wouldn't occur. The growth rate and the death rate are the same in this phase. So if you look here what is happening is that this is the that is why this stationary phase then comes the death phase where what you said is correct that the nutrient is deficient in the death phase and then the cell start to fight for each other. As a result not that cell growth or cell division completely stops but, the death is higher death rate is higher than the growth rate as a result the death phase is there. So, these are the these are the total process. Now, if I want to model this dynamics of the cell growth process; I have to find a way of incorporating all these different phenomena; the Lag phase, the growth phase, the stationary phase and the death phase. So, these four phases. So what we will try to do is, try and see what kind of model we can develop or what kind of model have been developed over the last 50 years to try and model this. So we will go slowly. Let us understand one by one.

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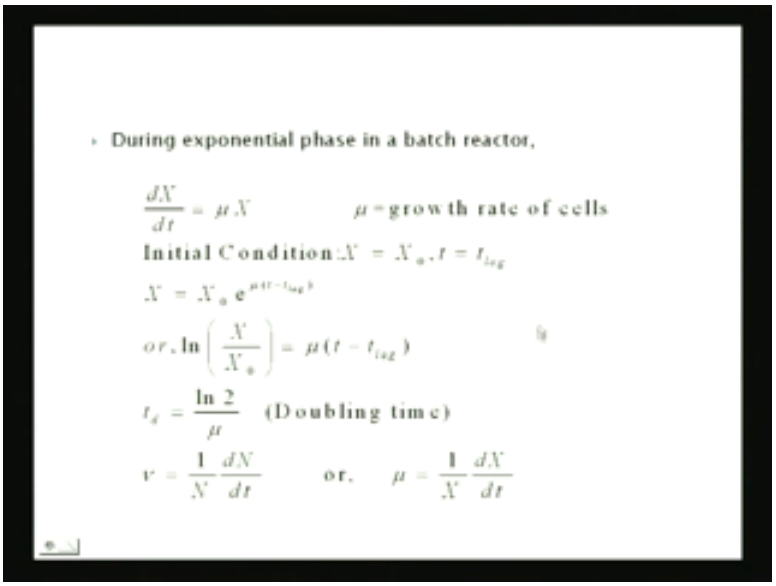

So, the first thing that we look at is the exponential exponential phase, the exponential growth phase which is the easiest to model. Now this exponential growth phase is given as d x d t equals mu x. You may want to make a note of this. So, d x d t equals mu x what is mu mu is a specific growth rate of cells. Now, we are just modelling the only the exponential growth rate part, not the other other three phases only the exponential growth growth phase. So, the d x d t equals mu x mu is the specific growth rate. It is like a reaction rate constant and x is the cell concentration. So, what this means is that more is the number of cells you start with the more is the possibility that you will generate cells. Why is that? Because every cell would give rise to two cells through mitosis at each point of time. So if you start with the 5 million cells you expect to have 10 million if you start with 10 you expect to 20 fine. So, that is why it is linearly dependent and you can integrate it like we have done over here before here. So, you after integration you get x equals x 0 e to the power mu t minus t lag t lag. Why? Because you assume that up to the time equals t lag. There is no cell growth, so x equals x 0 what is the initial concentration of the cell that you have started? So that would be the starter you know, when you you doing the curd making the curd or the yogurt so the amount of cell microorganism or yeast that you put into starting that is your x naught fine. So, that is the t equals t lag that up to t equals t lag there is no increase in the cell number. So, x equals x 0 e e to the power mu t minus t lag. And once you integrate it, you get this.

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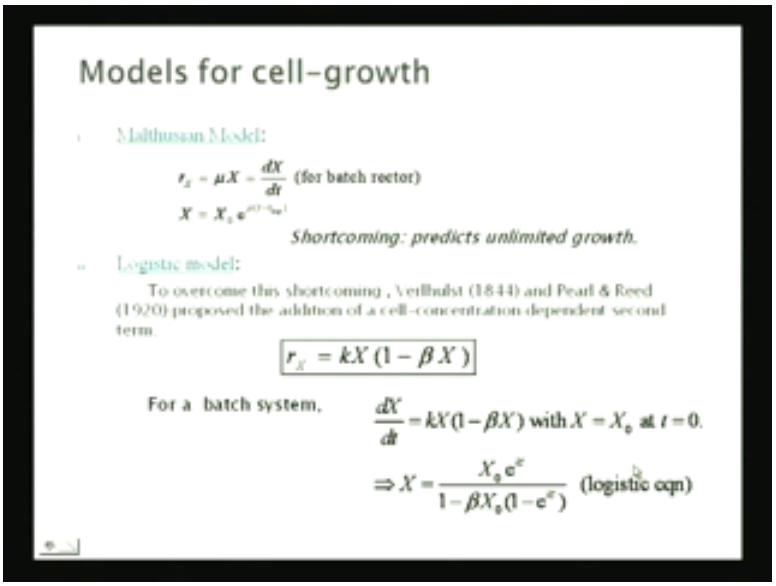

Now, what we are interested in is doubling time. What we are interested is doubling time it is like a half time in in radioactive decays your half time and here because it is a growth process and not a decay process you have something called the doubling time. And that is what we are interested in so t d is the doubling time and is given as l n 2 over mu. Mu being the specific growth rate of cells. So, mu if you go back to reaction just like your reaction engineering where you had k is defined as one over x del x del t here you have mu as defined as one over x del t x being the cell concentration. So this is we have the exponential phase.

Now what we have looked at is that look at how to evaluate this mu is this mu a constant? No, it is not a constant. Is it something like reaction first order reaction rate constant? No, I am afraid it is not. Then, what does it depend on? Because it depends on number of factors. So, what does it depend upon and how we obtain a model for this? So, there is several models for evaluating this mu, the specific growth rate and I will go through these models one by one and I may may not finish in this lecture but, let us see. So, the first model that we look at is known as Malthusian model, m a l t h u s I a n malthusian model. So, this model assumes that r x is time mu times x del x del t and x 0 is given as e to the power mu t minus t lag and this model assumes that mu is constant. What is the shortcoming of this model if mu is a constant? Then what happens is exponential growth phase continuous forever. There is no way to predict this stationary or the decay phase over here. The only way is to be able use this model and cut it off at a certain time scale.

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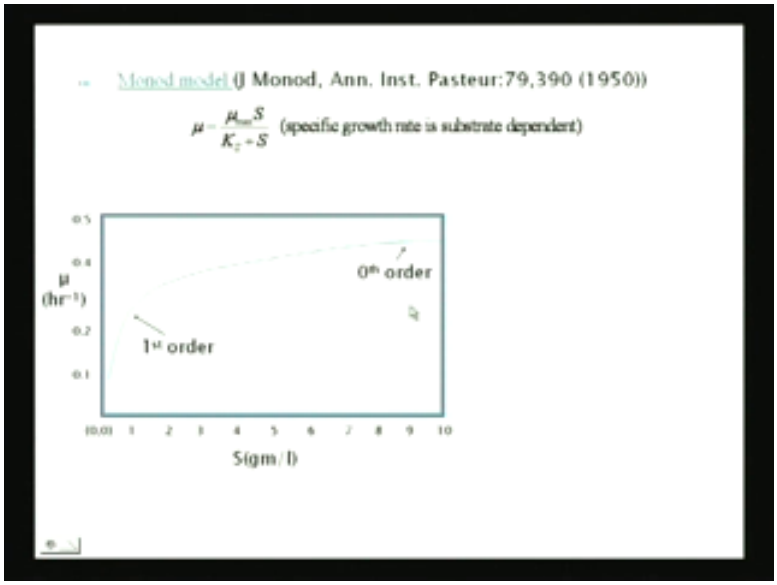

The next model is known as the logistic model and to overcome this shortcoming of the Malthusian model that was predicted. So you see these are very old models actually. These people Verhulst and Pearl and reed in nineteen eighteen forty four and nineteen twenty proposed the addition of the cell concentration as a dependent second term which means that the the reaction is no longer first order in x. That is what they said so that was an intuitive idea. What what was the idea? That let us go back here to the picture and that will so they said well it is not decreasing after a certain certain value of the x cell number; so why not make it a make it a a part part of it to be dependent on cell number but, inversely. So as cell number increases, then your growth rate decreases. So, this was intuitive thing so they they were doing experiment and they are figuring out but, after a certain cell number it starts to decrease. Then they thought why not make it like such that initially it is dependent on cell number but, after a certain cell number it starts to decrease with that with that cell number. It is not a bad idea actually if you think of it. It may may not work but, it it is not a bad idea.

So it is like this, so you know initially for small values of x. Then this number is does not kick in the the quadratic part does not kick in. It it goes almost linearly and then when x reaches a certain value this number becomes larger and therefore, the effect of quadratic because of negative quadratic effect, it starts to decrease. So, this if you integrate and you can still integrate it by the method of, method of what?

(( ))

Not separation of variable, partial fraction. yeah so at x equals x 0 t equals t equals 0 at x equals x 0 and actually you can remove this t equals 0 and you can put t equals t lag if you want to. That way you can include the lag phase also. And when you integrate it this is this is what you get x 0 e to the power k t one minus beta x 0 one minus e to the power k t. One of the things you can do is I do not think I have that is, you can go and plot this in the computer using some numbers of k and try to see that how, whether that predicts the experimental. So what I showed you the plot I showed you is experimental plot and whatever model we come up with has to has to kind of predict that experimental plot properly. Is that right? Shall we go on?

The next model is the most important model that we are going to do and this is something that you need to remember is known as the Monod model. This was found by J Monod and it was in nineteen fifty in this (( )) of institute of Pasteur in nineteen fifty he founded it this model. And the reason it is the most important model is because it was most successful one. And almost all growth processes that are there in the human body today are modelled by the Monod model or what is known as the modified Monod model and I will teach them right one after another today.

So the Monod model says that and it is an important thing. See what Monod model says is that no the x itself this this thing is right, r x is mu times x and you do not need to include in other part. But, let this mu be dependent on the substrate. That is what if there is a depletion depletion of the substrate what happens is the rate constant for growth decreases. And if you look at this interestingly what have they done with the rate constant? They have put a Michaelis-Menten kinetic phase in the rate constant.

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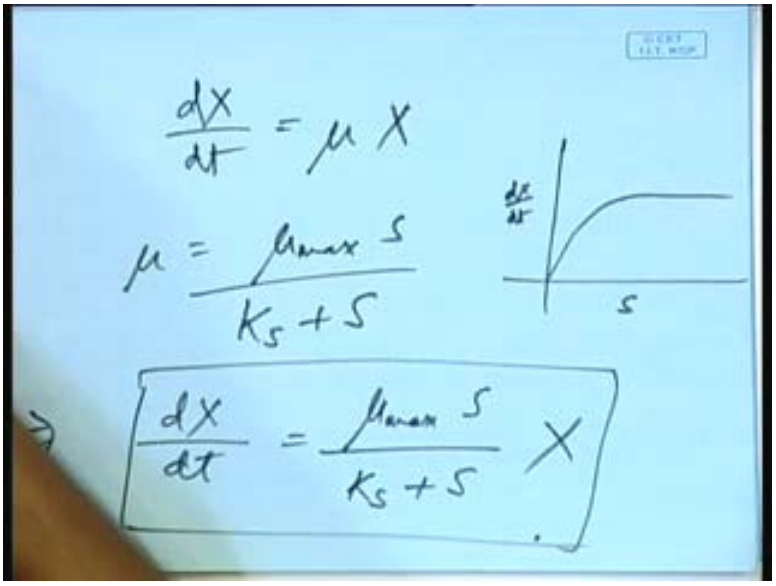

Now, my d x d t according to the Monod model as a result so, this is my model. So, the model is still first order in x but, Michaelis-Menten in substrate concentration or in terms of nutrient concentration. Is it clear? So what means what this means is that, if you look at d x d t versus s then it would have thing like this. So it it goes grows as it increases but, beyond a certain point, beyond beyond a certain point in s it it saturates out. So this is the Michaelis-menten (( )) x it increases first order as we have done before and then it saturates out to a zeroth order which ensures that the rate constant itself decreases. But, still it does not include the death phase remember of the stationary phase but, it ensures some kind of saturation beyond. It does not grow unlimited fine. This is the Monod model. Is that clear with everybody? And this is something that you need to remember by the way what I what I gave you today. this This if I what I wrote over here on the sheet yeah this formula d x d t for, this is for Monod model. This is what you need to remember and you also need to remember the modified Monod model because that is also something that is used. So two forms of Monod model, the rest are you do not necessarily need to remember for your for everything. But, well you know for the test of course, you need to remember.

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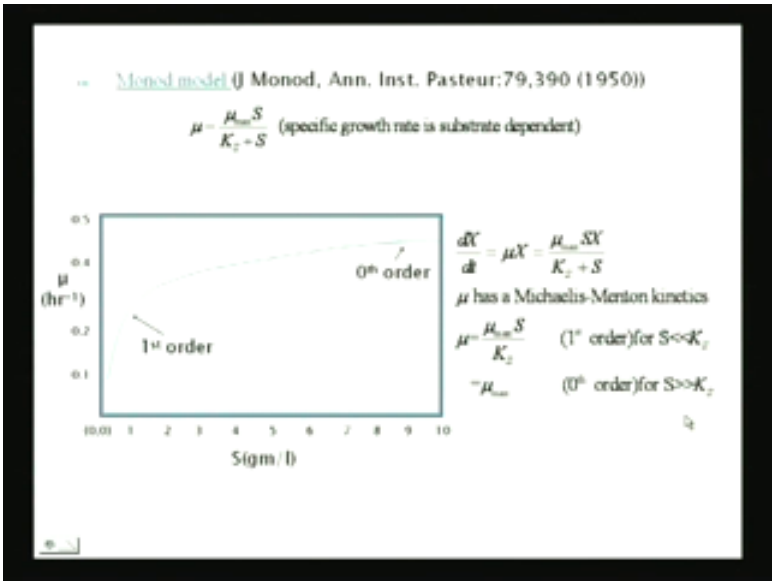

So d x d t for the Monod model is mu x times mu max s x over k s plus s and mu is Michaelis-Menten kinetics, it goes to first order that is mu max s over k s for s very, very small and goes to a constant mu max for s very large. Please make sure you make a note of this. Done? So this is the Monod model. ah

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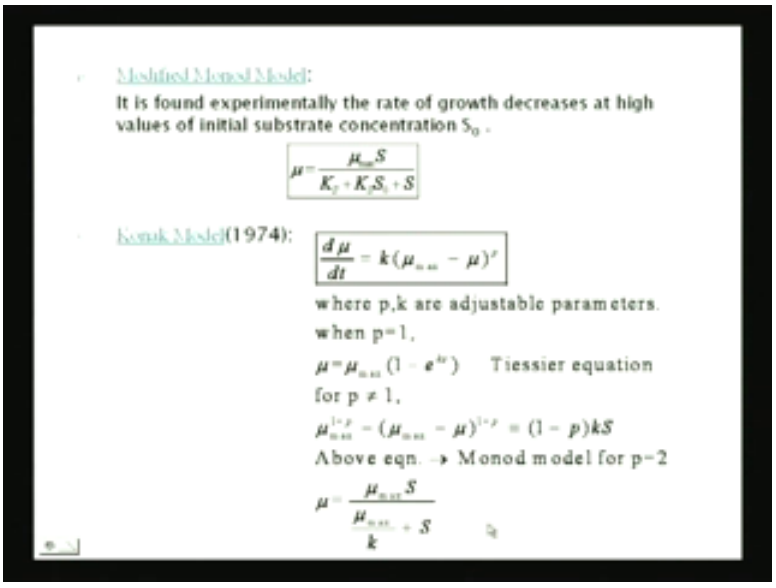

And what I want to do next is probably the last thing today is modified Monod model. So the modified Monod model is slight modification in the Monod model. It is found experimentally that the rate of cell growth decreases at high value of initial substrate

concentration s naught. So, it was strange thing that was seen that if if you increase the substrate concentration is good but, if we increase it to a very high value then, that instead of helping the cell growth process retarding the cell growth process and how do we include that? So that was included in mu and what was done is the simple thing. So monod Monod model had been mu max s over k s plus s and the modified Monod model simply put this k s s naught. So if if the substrate concentration is very high s 0 say, then decreases the mu. Mu itself decreases because you put it in the denominator and as a result it would have decreasing effect. The rest is fine that is the mu max that is d x d t is still equals mu times x where mu instead of mu max s over k s plus s is given as mu max s over k s plus s plus k s k s 0. So, this is the modified Monod model and it sometimes works because you know, so too much nutrient is not good either too much of substrate concentration is not good here okay.

So the let us finish this, Konak model and so this was found little later than the Monod model and this was how this is how it is. And I stress that you make a note of this as well. So, in the Konak model again you know, again the major in all these models except for the for the second one that we did here the just a second except for the logistic model all the models follow this, r x equals mu x. This should be very clear in your head that all the models follow r x equals mu x and all we are doing except for this logistic model all you are doing is trying to figure out how mu is. So, the Monod model gives me mu max s over k k s plus s. The modified Monod model gives mu max s over k s plus s k s s 0 plus s and the Konak model gives mu as the differential equation itself. So, what this says is that mu is a function of time. The specific growth rate itself is a function of time and that equation is given as, so the model stays the same but, mu is the function of time also. So mu is not a constant any more it is not necessarily dependent on the concentration also substrate concentration also. It is a function of time time which you need to integrate. So when you are integrating d x d t you need to integrate it the in coupled with the mu equation right. So d x d t equals something and d mu d t equals something so you need to integrate these two equations together.

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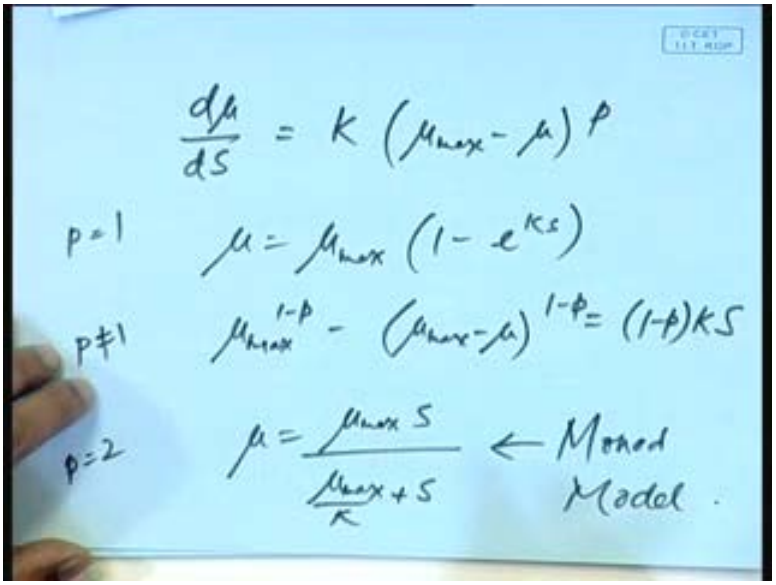

So, this equation is given d mu d t equals k times mu max minus mu to the p where p and k are adjustable variables. Now for p equals one mu max becomes linear so mu equals mu max e to the power minus k s. And and this becomes mu for p equals one and for p 0 equals one. This is what what you get actually I think I am sorry you know this is the typo over here it should be d del mu del s not del mu del t over here this should be del mu del s the Konak model. this Just make the change. So, this is del mu del s equals k times mu max. So, minus p mu to the power p so for p equals one you get mu equals mu max one minus e to the k s so p equals two. This is what you get, p 0 not equals one this is what you get and for p 0 equals one, the formula that you get if you put p equals two then you get the Monod model. Get back to the Monod model. Is that clear?

So, this this is slightly complicated so let me run through this and there is typo out here also. So, this is del mu del s equals k times mu max minus mu to the p and so let me write since there is typo, I will this write so del mu del s equals k times mu max minus mu to the p for p equals one, you get mu equals mu max one minus e to the k s p equals p 0 equals one you get mu max one minus p minus mu max minus mu into one minus p equals one minus p k s. And for p equals two you get, mu equals mu max s mu max over k plus s. This is the Monod model. So what we need to do is we need to kind of remember the Monod model the modified Monod model and the Konak model which is the generalised form of these. And how you get the Monod model from here? So, we will continue in the next lecture. Let us stop here and then thank you.