Aspects of Biochemical Engineering
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Lecture – 31
Kinetics of Substrate Utilization, Product Formation and Biomass Production of Microbial Cells – I

Welcome back to my lecture through the course on aspects of biochemical engineering; till now I was discussing that chemical reaction kinetics then, chemical reactor analysis the enzymatic reaction kinetics both by using free enzyme and the immobilized the enzyme system. Now today I am going to discuss the new topic that is kinetics of substrate utilization product formation and biomass production of microbial cells.

So, what I told you can remember that you know in which way this you know biochemical system is differ from chemical system. I told you the beauty of the biochemical system is that from a particular raw materials, we can produce n number of products. As for example, if you look at glucose we can convert it to ethanol, we can convert it to citric acid, we can convert to lactic acid, we can convert to acetic acid the different type of products we can form one compound.

But, if you look at the chemical process that as your product changes your raw material changes, but here in the how question come how it is possible in the biochemical system, how it is possible I mean due to the fact that, you know that microorganism that plays very important role though different microorganism has the different capability; because, 1 that is where I can give the example that yeast as for example, saccharomyces cerevisiae they can convert glucose to ethanol am I right.

Now, if you look at that this as per gillus Niger; it can convert glucose to citric acid. Now lactobacillus case your lactobacillus Del Brooke it can convert this glucose to this lactic acid. So, you know that indicates that you know that your raw material is same, but you are getting the different type of products, so this is very interesting; I hope that you will understand this thing if you understand them you can have better idea on the biochemical reaction kinetics. So, first thing that I want to tell here that is that how the enzymatic system differ from the microbial system.

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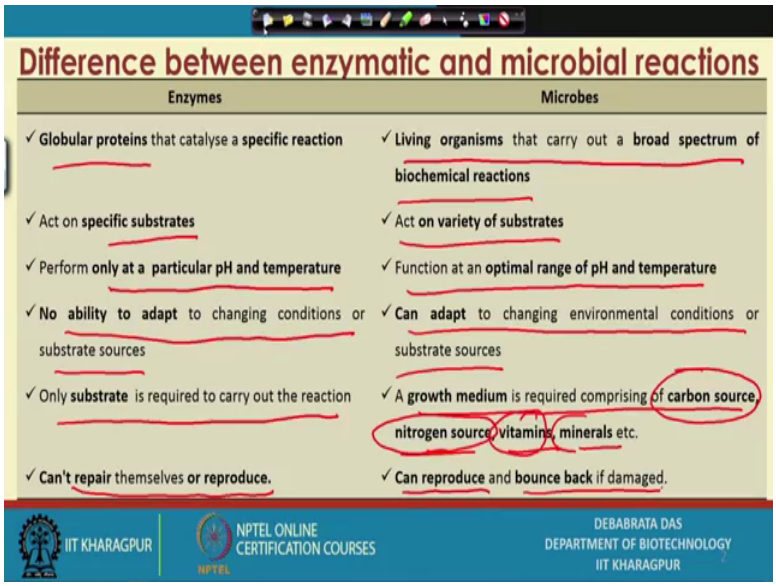

Now, what is what do we have in the enzymatic system? Enzymatic system suppose I just now I explained that glucose is converted to fructose with the help of glucose isomers enzyme am I right now. So, when if you use other than glucose, your glucose isomers enzyme will not add act, so you required the specific substrate, but the enzymes are very specific with respect to substrate, you cannot varies the one particular specific substrate only it will attack and give the product.

But in case of microbial system is totally different. Micro, when microbes they grow they grow in a media and in the media like we are all human beings for our survival, what we required? We required carbon source, we require nitrogen source, we require minerals we require vitamins, but similarly microbes also they required all this they required carbon source, they require nitrogen source, they required minerals there is the vitamin, so totally that you know. So, what were the reaction mixture for the enzymatic system and reaction mixture for the microbial cell growth that is totally different.

So, here I want to emphasize that if you look at the difference the I told you the enzymes, basically they are globular protein, they are randomly folded and in process they developed the some kind of active site and this active site is responsible for the enzymatic reaction and they are very specific with respect to substrate, they only at a particular temperature and pH, no ability to adapt to the changes condition or substrate sources it does not have the ability only substrate is required to carry out the it, does not

required anything else you required only the substrate and proper pH and temperature and cannot repair themselves or reproduce.

So, this is the enzyme that is the characteristics of the enzyme that we have. Now if you look at the microbial system, the living organism that carry out broad spectrum of biochemical reaction. I already mentioned that you know that different type of the same substrate can produce different type of products and then acts on a variety of substrate they can act on not only glucose, they can act different substrate they can use as a raw materials and function optimum range of pH and temperature can adapt to change the environmental condition and substrate.

Let me tell you that this is the typical characteristics of the living system, one what is the typical characteristics of the living system. The typical characteristic of living system is the acclimatization property, what is the acclimatization property? That means that therefore, suppose I can give the example that we are living in the tropical country, now when you go to the western country there very cold.

So, by immediately after going there we will be having kind of setback because, we required some time to acclimatize with the environment then we can work properly. Organism also like this, organism when you inoculate it has the new environment for the organism. So, they required some time for this acclimatize, but you know slowly they will acclimatize. I can give a typical example the astronaut when they go to the planet, that moon and other places then in between the temperatures suited very high, maybe 50 60 degree centigrade.

Now, if we increase the temperature of the room this suppose 45 50 degree centigrade we will run away, because we cannot our body cannot tolerate but our body can tolerate, when we increase the temperature slowly and slowly and our body will be adjusted at 1 time, when we can adjusted. So, in acclimatization property is the typical property of the living system and another is the, they are very sensitive to the environment, the growth media is required comprising of carbon source, nitrogen source, vitamin and mineral this I already mentioned can produce the and bounce back if it damages.

So, that you know the reproduction that is a typical characteristics, now here in the growth media the here I want to emphasize that this carbon source has 3 different

purpose, what is that 3 different purpose? It is use as a as body building material, it is the use as a energy source, it is use for the production of typical product..

Now nitrogen source mostly contribute for the cell mass formation, now vitamin and minerals they mostly have mostly take part in the metabolic reaction because, if you look at our metabolic pathways we require different enzymes and all enzymes they required cofactor and these cofactors mostly they are minerals or the vitamins. So, this is the how they take part in this reaction.

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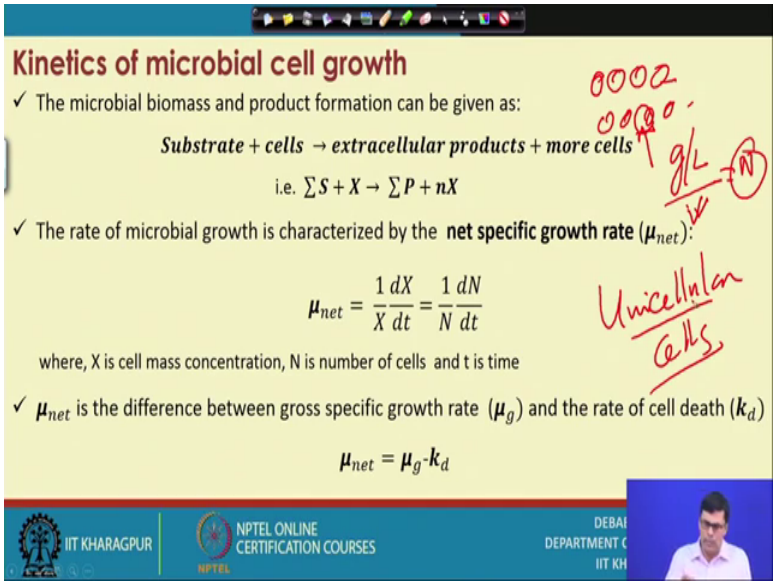

Now, let us discuss about the kinetics because, the when you talk about the kinetics of the microbial cell growth, now question comes that how we shall have to monitor. We monitored the concentration of the cells am I right? Now when we have the microbial cell the microbial cells might be of 2 types, one is the unicellular cell and there is the multi cellular cell or in a filamentous type of cell.

Now, in case of unicellular cell, the numbers is proportional to the mass. So, as for example, if we talk about the bacteria suppose the bacteria there mostly there unicellular cells. So, we know that what is the mass of one particular cell. So, if you even you know that concentration of the cell maybe grams per liter or milligram per liter and if you know the mass of individual cells, if you divide by mass of individual cells will get the number of the cells.

So, you know number and mass there will inter convertible in case of unicellular cells am I right? But in case of multi cellular cell it is not possible; you have to consider only the mass of that cells. Now the substrate plus cells how the reaction take place? Substrate plus cells, the extracellular product and one cells the reproduction is the cell is the factor. So, how you can write this a substrate is consumed with the cell mass with this added and this gives the product and this is the more cell mass is produced.

Now, net specific growth rate of the cell, how you can write this is 1 by x dX by dt. So this is x, x is when we express is mass per unit volume am I right.

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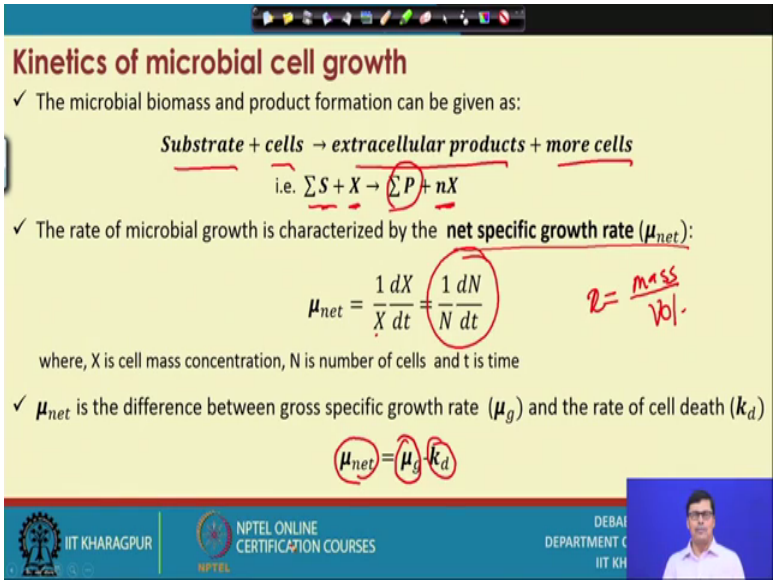

So, what I told you in case of unicellular cell, mass and number they are in inter convertible. If it is in case of unicellular cell we can express this 1 by N dN by dt, so this with respect to number we can do that. Now as you know that any kind of microbial population or any kind of living population, always there will be a growth and there will be a death.

The actually the net specific growth rate of the cells should be equal to the growth rate of the cells and death rate of the cells, that is the specific growth rate minus specific death rate that actually should be equal to actual growth, because one thing we should remember that when we carry out any kind of microbial reaction only living cell, they participate in the reaction then the death cell will not participate in the reaction, so that we shall have to take into consideration.

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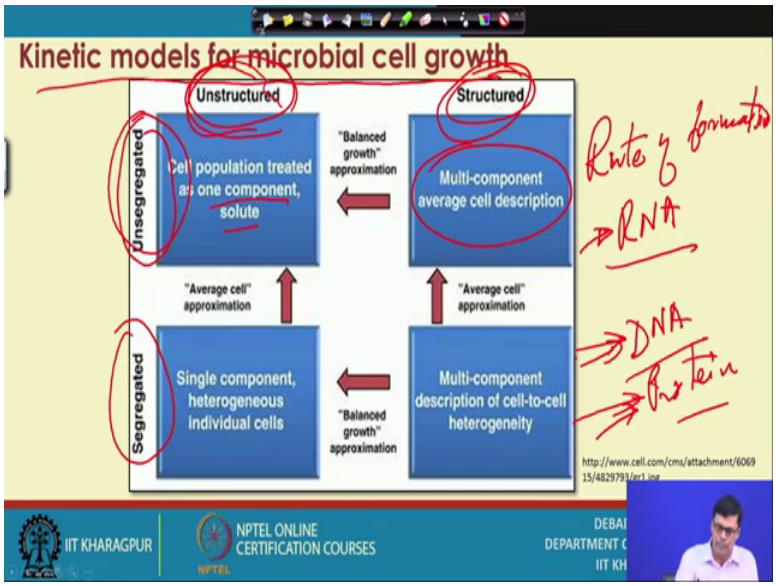

What topic I have given here, this is the kinetics of modeling for the microbial growth system. Though we have 4 different type of models one is structured model, unstructured model, un segregated model and segregated models. So, the mathematical model the when you use any kind of cell growth that you know we can have 4 different type of structure..

Now different type of models, then let us try to understand the what do you mean by segregated model or the un segregated model, structured model and unstructured model. Now what is segregated model? Suppose segregated model means in our society we are so many people's are here; now we find that some way that you know under no circumstances the one person is totally same as the other person, it is not possible even twin that we have; then twin children also there will be some kind of difference you will find.

So, what is the actual case as that all the human beings, all the human population their growth characteristics they differ from each other am I right? Now if it is so it is same is the applicable to the microbial system, even the microbes when grow there also different n number of cells are there, so growth characteristics that the individual cells will be different. Now if your mathematical model deals with the growth characteristics of the each cell individually, suppose we in our society how we identified the different people with respect to their name.

Now, in microbial population if we put a marker 1 2 3 4 5 6 7 8 and then we determine the growth characteristics of the individual, what is the growth characteristics or number 1 cell, what is the growth characters number 2 cell, what is the growth characters in number 3 cell and if the mathematical model deals with that, we call it segregated model and what do you call a un segregated model? When we assume the growth characteristics of all the cells 1 2 3 4 they are same, because you know this is the kind of idealist situation; because ideal situation means it is non existence, so real situation is that is really that present.

So, ideally when you make kind of ideal situation, then we assume the growth characteristics of the all the cells will be same so and then it will be un segregated model. Now what do you mean by structured model? Structured model means cell comprises of n number of bimolecular they have RNA, DNA protein and all these molecules they have. Now when cell grow this concentration of this RNA, DNA, protein and this may vary with respect to and with respect to time and if your model deals with the rate of formation of RNA am I right RNA. Then another model deals with rate of formation of DNA, another model may be they with respect to some kind of protein different type of protein.

So, you know then we call is structured model, but unstructured model is what? When we assume the rate of formation of RNA, rate of formation of DNA, rate of formation of different protein they are not differ from each other they are producing at the same, so that we call it the unstructured model. So, what you what I want to tell their unstructured and un segregated and unstructured model, when we consider the one component solute and this is called ideal model, ideal situation and this is structured model is considered as the real situation that we have.

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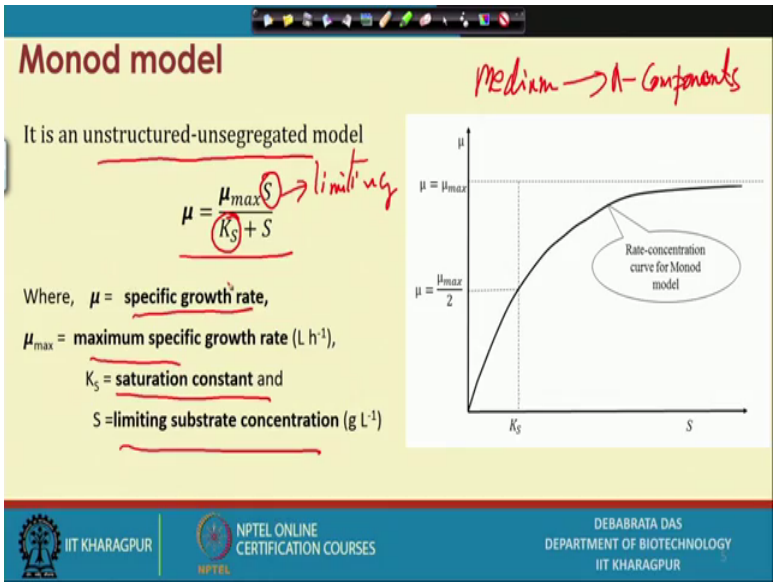

Now, Monod equation that is some kind of imbalance with the Michaels menten equation or enzymatic reaction and this is the actually on the basis of unstructured and un segregated model; that is the ideal model ideal condition that mu equal to mu max S Ks plus S and what is the S? S is called limiting substrate concentration limiting am I right.

Now, what do you mean by limiting substrate concentration? It is very interesting suppose we talk about media am I right, because of microorganism growth in a media and media comprises of n number of components, it may contain nitrogen source, it can contain carbon source, is can contain minerals, it can contain vitamin. Now suppose if you increase one particular component from 0 to infinity keeping other component, excess, then if you find that specific rate of growth is increases like this and with respect to change of that particular component, then we call it growth limiting substrate.

Now, it may be carbon source, it may be nitrogen source, it may be minerals it may be vitamin anything. But in case of enzymatic reaction I told you substrate is very specific with respect to the enzyme, that cannot be changed with a when you use glucose isomer is a enzyme then your substrate is the only glucose, you get cannot be fructose this can be only glucose is similar like this. So, mu is the specific growth rate of the cell and mu max this is the maximum, that you can see this is the maximum growth rate of the cell when it attains the Plato and when mu by mu 2 is there then Ks will be equal to S, Ks is the saturation constant.

Now, here I want to emphasize one thing that what is the significance of the value of Ks? Now if Ks value is low that means, you required low amount of substrate for getting a more amount of cell mass; if Ks is more you required more amount of substrate for getting the same amount of cell mass, so that is the significance of the Ks value.

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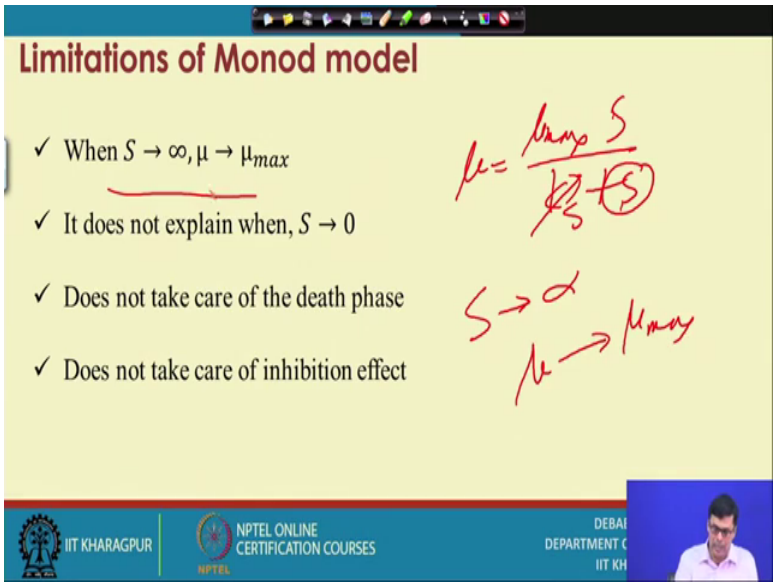

Now, this equation that if we look at that when this, what is that equation that we have? We have mu equal to mu max S k s plus S am I right.

Now, when S tends to infinity, if your this is the first situation if it is very high then we can neglect it, I mean if we neglect it then this S will cancel each other and then mu will be tends to mu max am I right; this is exactly what we have written here now.

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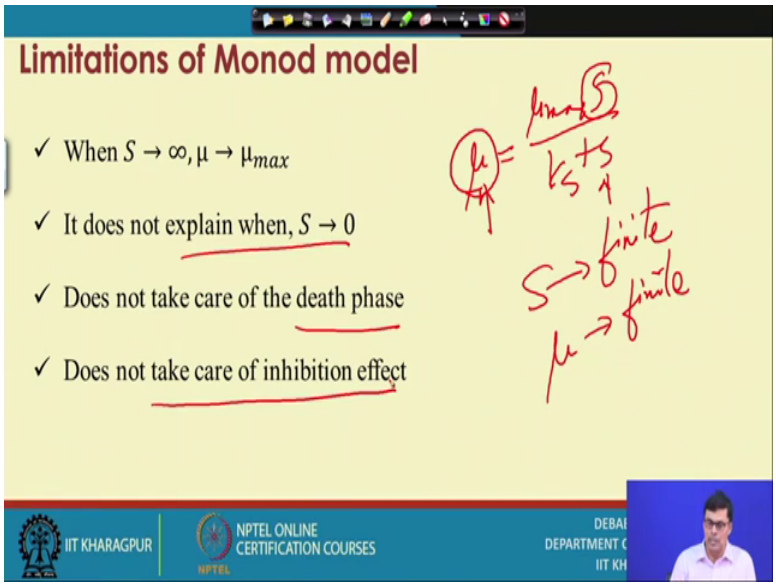

Now, another when you write this equation mu max S Ks plus S, now S will be finite when S is finite because, if S is finite then I can say mu should be finite am I right. So, this you can add here and another is that it does not explain when S is tends to 0, it does not explain what will happen when S tends to 0 and it does not take care the death of the cells, because the when you the mu that only that growth of the cells they happen, but in the actual practice when cell grow there is the some death of the cells and is does not take care the inhibition effect, there will be some substrate inhibition and product inhibition, that is not concentrating in the Monod equation.

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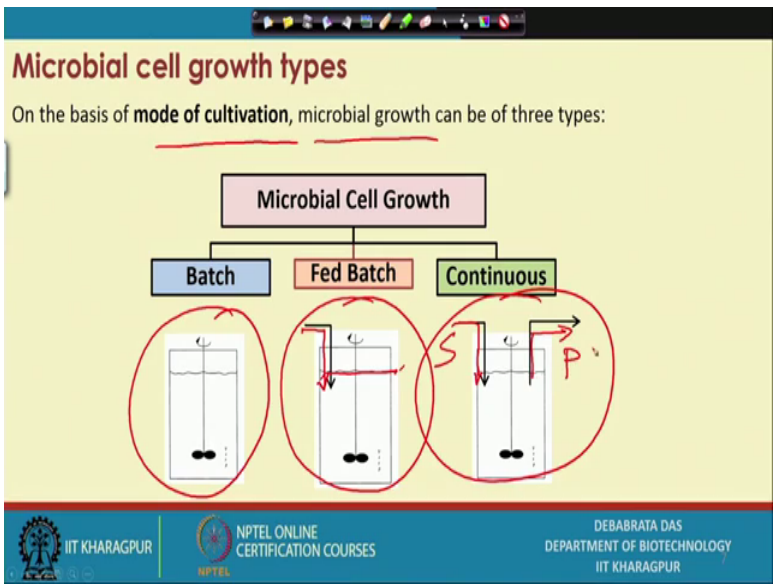

Now, if you look at the different processes that we have, how you do the cultivation of the mode of cultivation of the microbial cells. We have 3 different types of processes we have batch process, we have fed batch process, we have continuous process. Now let me explain the fed batch I have already explained you take the material at a time, let it react after the reaction is the where you take it out. In between you are not a adding anything to the reactor and taking out anything from the reactor, but what is fed batch reactor? Fed batch reactor means you add the substrate in different slowly.

So, initially let you take small volume of substrate, let the reaction take place and then when substrate concentration decreases again you add some substrate, so that you maintain the concentration below the inhibitor slowly. So, there is the input of substrate and there is no output of substrate. So, that is what exactly a we have shown here there is the input of substrate, but there is no output of substrate..

Now when it comes at the total volume then we stop the operation we take it out. In continuous system is something different way I told you whenever we operate any kind of continuous system, we first operate in a batch mode let the reaction take place when the react rate of reaction is maximum, then we feed the substrate like this and continuously and take the product from this; though here substrate is coming and products is coming out like this and this is a continuous process.

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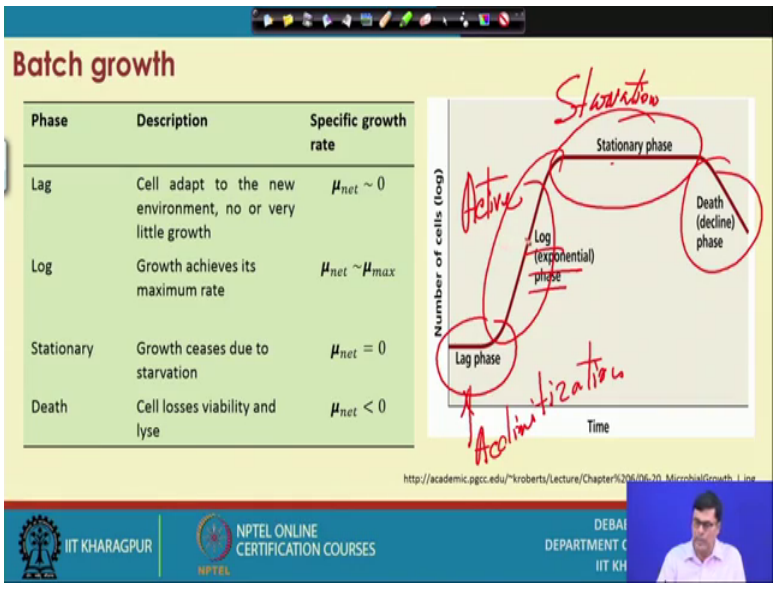

Now, whenever we handle any kind of microbial system, we should have very good idea about the life cycle of the cells because, when suppose I want to handle a new microorganism and try to find out this capability for producing certain product, the first you know that the when you take a microorganism is totally black box for me, because black box means we do not know how this organism is growing, when it will be growing maximum, when it will go to the stationary phase, that we do not know.

So, it is the whenever we handle any kind of new microorganism first our duty is to develop the lifecycle and during the life cycle what are the things you can have? You can have the lag phase, you can find out the stationary phase, then you can have the death phase. Now a phase has is it is significance, what is lag phase? Lag phase is considered as the acclimatization phase, so organism requires some time to acclimatize with new environment and what is the log phase log phase all or we call it exponential growth with this is means, here the organisms are very active this is very active am I right and this is the stationary phase, what is stationary phase?.

This is called starvation phase. What is starvation? Because, you are not giving the sufficient amount of substrate to the organism, so they starved for the survival the here rate of death is equal to rate of growth of the cell and death where the dying phase is the most of the organism there dying at this phase.

So, this are the, because, what is the significance? Significance is that whenever we do any kind of inoculation because, when you work with any kind of microorganism that we have to inoculate and when we inoculate you should be ensured your organism is very active and when you should inoculate? Usually this is consider mid log phase am I right and this is the late log phase.

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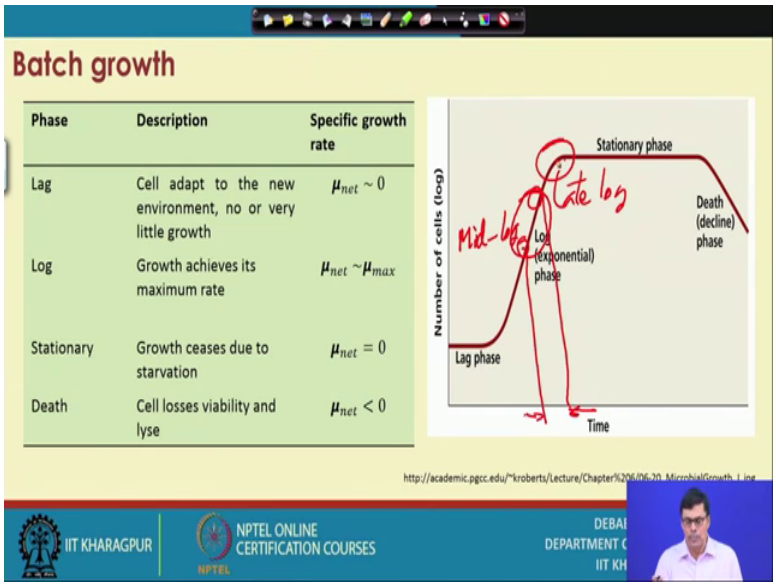

So, your inoculation should be you during this phases; that means, during this time this inoculation just to ensure that your organisms are very active at that time, because if you if you inoculate the cell here then every possibility that your population has lot of death cell. So, your rate of reaction is a problem, so this one has to keep it in mind.

Now, here what we have written the lag phase, the cell adapt to the new environment and no or very little cell growth takes place, the mu net equal to approximately equal to 0 log phase, the growth achieves is maximum this we consider is the active phase. When mu net equal to mu max; and stationary phase is the growth is this stationary phase; what you call starvation phase a due to starvation, where mu net equal to 0, rate of growth is equal to rate of death of the cell and the death phase this losses the viability and lyses take place mu net is less than 0.

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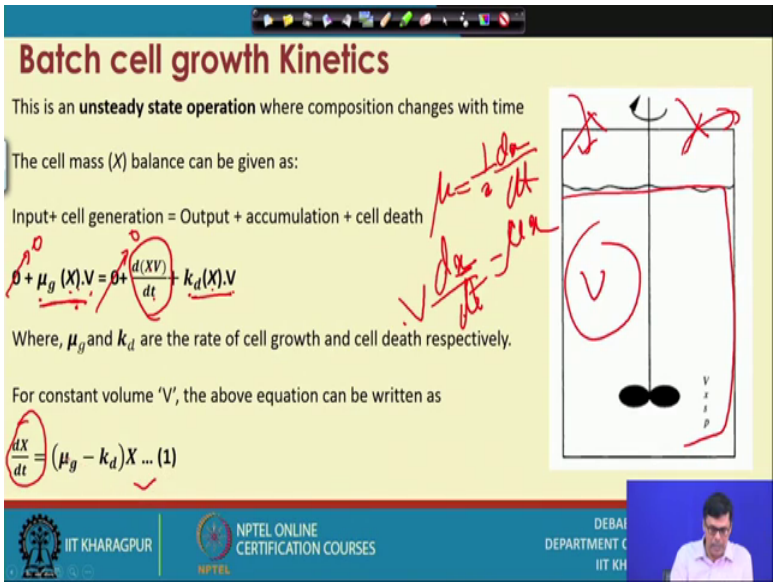

Now, if you do the cell mass balance across this reactor particularly in the batch process, then how we can do that? The rate of input of the cell because rate of input and output of the cell is are equal to 0 in a batch process, because you are not putting anything in the reactor continuously taking out this is not you are doing anything. So, this will be equal to 0. The rate of that the growths of the cell, that rate of input that rate of generation of the cells are generated.

So, there that will be mu g into X into V. So, we know that what is mu? Mu k equal to 1 by x dx by dt am I right. So, what is the dx by dt is the rate of growth of the cell that is equal to mu into x. So, what is dx by dt? X is mass per unit volume, so this is the volume of the liquid. So, growths take place and on the whole volume, so this is to be multiplied by V; the d dx by dt into V this we can write mu into X into V am I right.

Similarly in that you know that accumulation in the batch system, the cell mass the accumulation take place what we can write dXV into dt and cell mass depth we can write Kd into XV, kd is the specific death rate of the cell into X into V. So, this rate of growth actually rate of growth ultimately we can write mu max minus Kd into X, this is the net growth rate we can write like this.

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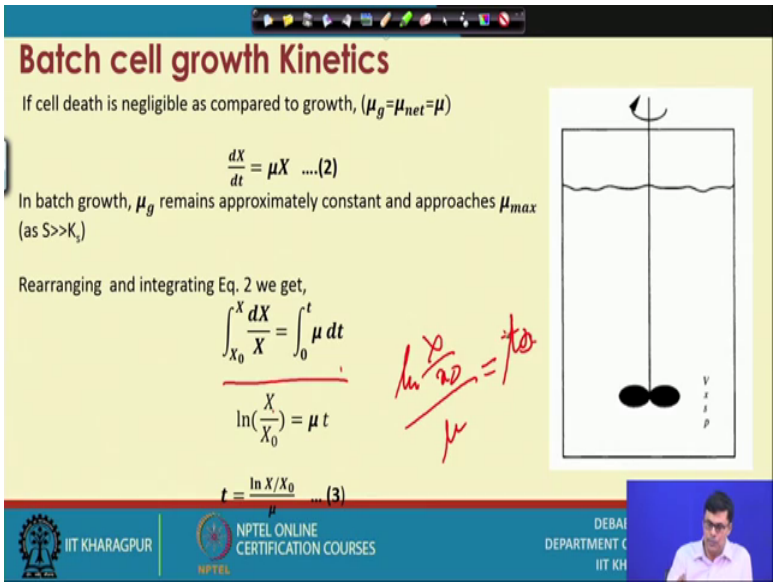

Now, how you can find out that time required for changing the cell mass from X take 0 to x. So, this is the very simple way we can do that, we know mu equal to 1 by X equal to dx by dt. So, this we can write in this form, then we can dx by this is the integrate X by X 0; the mu value or t value you can easily find out that ln though we can write ln x by x 0 divided by mu, this will be the time that required for a batch process; you know this is the batch process we can easily calculate.

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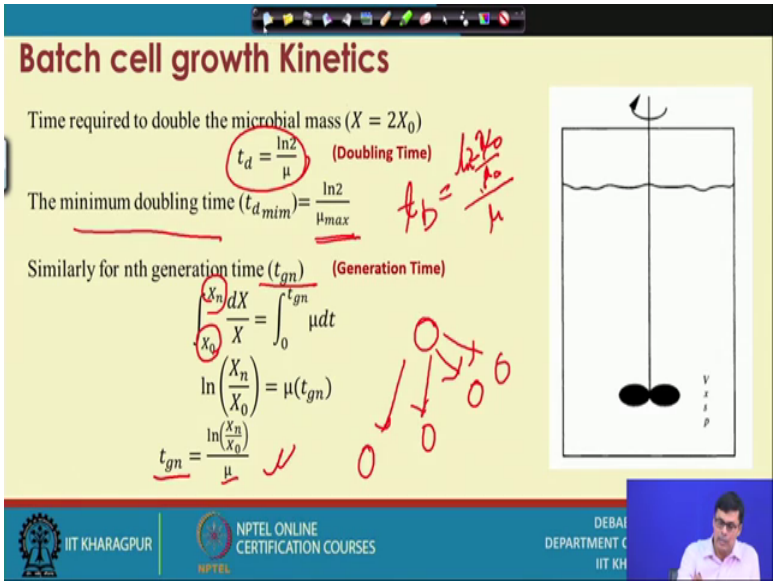

Now, in case of microbial population 2 things we come across 1one is called doubling time another is generation time. Doubling time means the time required to double the cell population and what is the generation time? Generation time means the time required for the cell division. Now during the cell division, the one cell may divide into 2 cell, it may divide into 3 cell also, it may divide into 4 cell also.

So, since the in case of generation time, during the cell division that whether 1 cell divided into 2 3 4 there they show it is different, the naturally not necessarily the doubling time is equal to generation time. Now if you are is a one cell divided into 2 cell, in that case generation time is equal to doubling time. So, here how we can calculate? Now what equation we have written in the previously t batch equal to what we can written ln x by x 0, no that you know that x 0 by mu am I right. Though this is 2 is doubling time means double the cell population..

So, this x 0 will cancel then this will be ln 2 by mu this is exactly this we have given the doubling time, this double the cell population that what is the minimum doubling time. This ln 2 by mu max, mu max is the maximum specific growth rate of the cells, so this is the minimum.

So, in a suppose in a problem, if I give you that minimum doubling time of the cell is this from that you can easily find out the mu max value. Now if you want to find out that what is the generation time, then X0 will be convert to Xn, let us assume the Xn is the we do not know that a part cell division how much cell is producing, let us assume for each cell division Xn cell is produce; then if then your generation time will be ln Xn by X 0 by mu, then we can calculate the generation time of the cells.

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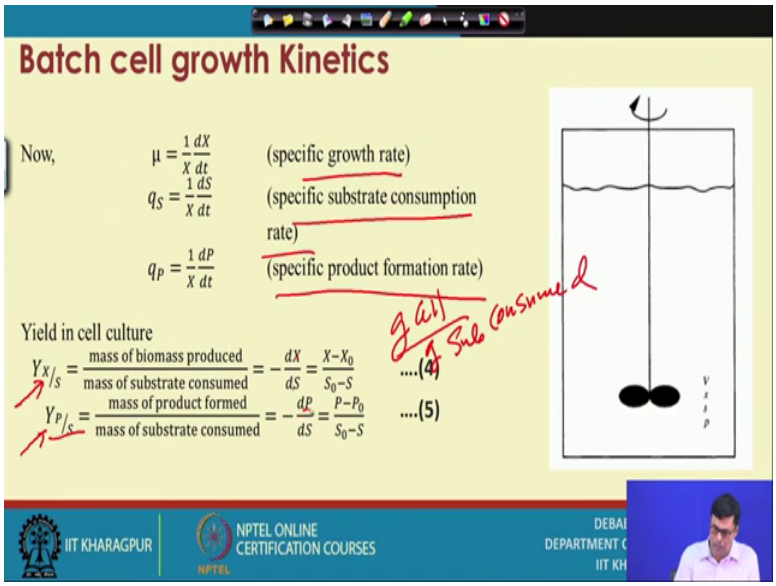

Now, now in this there are different terminology we use one is called specific growth rate of the cell, specific substrate consumption rate, specific product formation rate. I want to emphasize the here then when we use the term specific that is with respect to per unit cell mass concentration. As per examples specific product formation rate, rate of product formation per unit cell mass for cell mass per unit cell mass concentration, rate of substrate degradation and the specific rate of substrate degradation rate of substrate degradation per unit cell mass concentration.

Now, another term we come across that is the yield coefficient, this is the growth yield; that is the dx by ds the gram of cell produce gram of cell produce per gram of substrate consumed not added consumed, so that we should remember here. Also when Y p by S this will be gram of product form per gram of substrate consumed, so this we shall have to remember.

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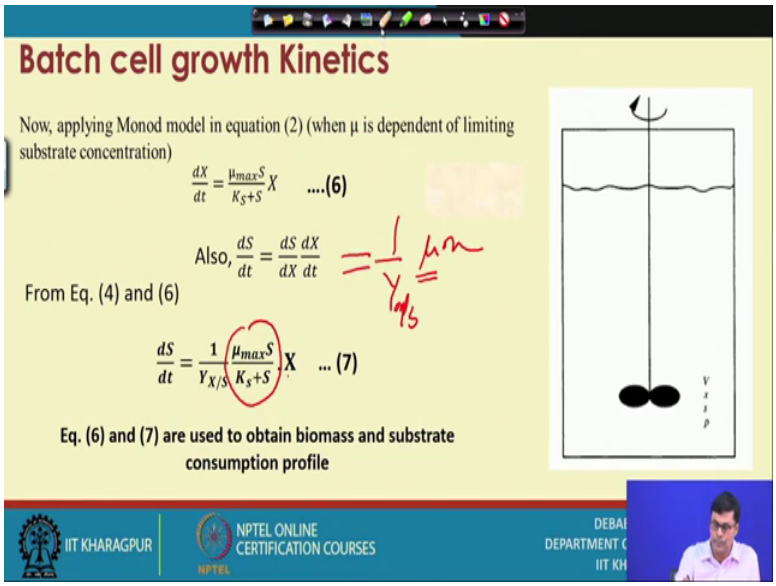

And this equation that we can write dX by dt is this that we can write it and then from this we can dS by dt. If you want to calculate this we can write dS by dx, dx by dt an we can put the value mu into x we can put and this is equal to 1 by this we can write, this is equal to into 1 by Yx by S dX by dt; d X by dt is the mu into x and what is mu equal to mu max S into x, so you can write it like this.

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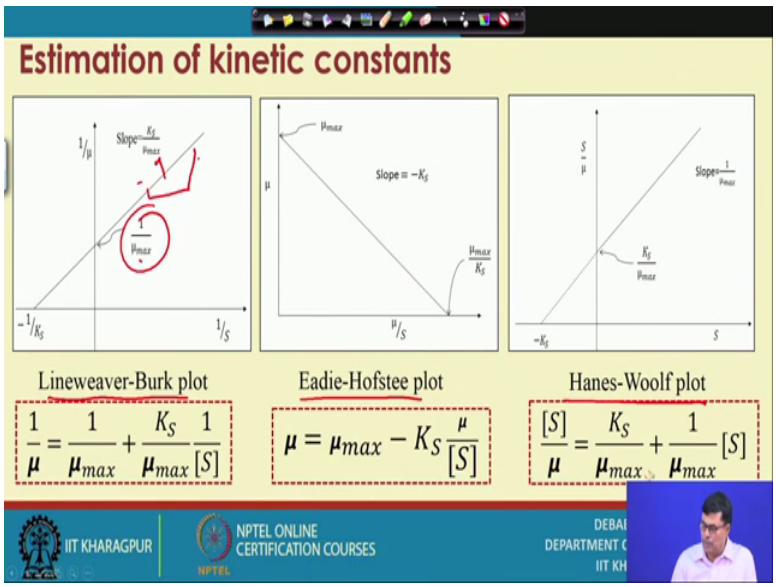

Now, how you can find out the kinetic constant we have shown in the enzymatic reaction 3 different plots we have, one is called a line weaver Burk plot another is the eadie

hofstee plot and Hanes Woolf plot, the similar way we can do that. If we plot line 1 by mu versus 1 by S we can find out from the intercepts 1 by mu x value slope, from this slope we can find out the value of Ks by mu max you put the value of mu max; here you will get the Ks. Similarly Eadie-Hofstee plot, we can find out the Ks and mu max value and hence and Woolf plot we can find out the Ks and mu max value.

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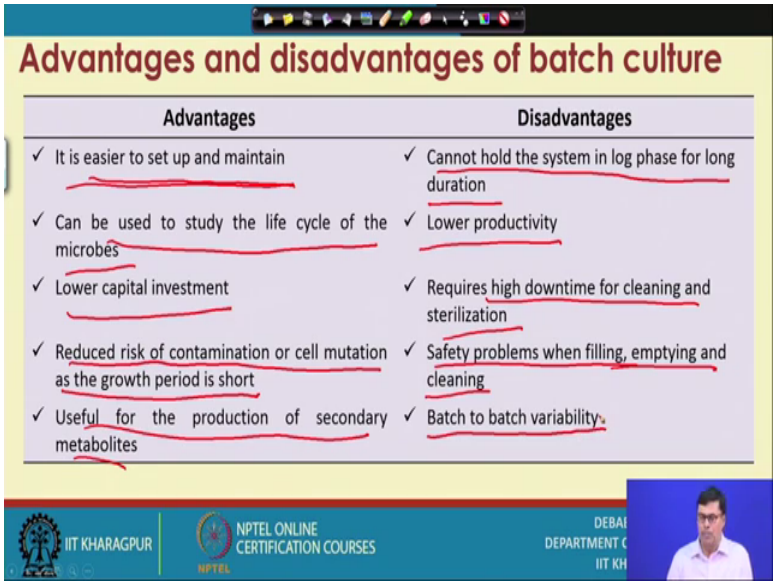

Now, what is the advantages and disadvantages of the batch process? In a batch process that easy to set up and maintain because if you look at our fermentation industry mostly their batch mode; as soon as the world trade organization we have joined treaty then all the we have free trade in the different countries, then all the foreign company they entered into India. Then our Indian companies they beam immediately they change over their technology to the advanced technology, then batch process they are convert to the continuous process, because continuous process the productivity is much more as compared to the batch process.

So, only the beauty of this process that is easy to set up and maintain, can use to study the life cycle of the cell that is very important to handle the cells, lower capital investment reduce the risk of contamination or cell mutation of the as the growth period is short and useful for the production of secondary metabolites, but these advantages is the cannot hold the log phase for longer period of time, because we have seen the life cycle. What is the life cycle? as the time passes on one phase which it over to other phase

because, log phase which would was the stationary phase that is the major drawback with the batch system, though if you want to suppose you want to have more cell productivity in the system.

So, you should operate the system in a log phase for longer period of time and that is not possible in case of batch process, lower productivity that is one of the problem, then required high downtime for cleaning and sterilization and safety problem when filling and emptying the cleaning the reactor and batch to batch variability, the batch to batch productivity may vary. So, this is the several problems that we have with the batch process.

So, in this lecture I try to tell the cell growth kinetics, cell growth kinetics is very important the reason is that lot of biochemical industries are operated by using the different type of microorganism; the beauty of this system is that one particular substrate can produce n number of different type of products, the first question is that how you monitored the cell mass concentration? The cell mass can be monitored if it is the unicellular cell, you can have either number of cells or the mass of the cells per unit volume, but in case of where multi cellular cell or the filamentous cells usual have be consider mass of the cell per unit volume.

Now, when you talk about the growth model it may or may be of 4 different types one is segregated model, un segregated model, structured model and unstructured model and then Monod equation is the similar to the Michaels menten equation can be use for explaining the growth kinetics. Now in the Monod model has the different drawbacks because it does not include the death of the cell, it does not include the inhibition of the cells and here they have assumed that when substrate is tends to infinity the mu tends to mu max, when S is finite mu is finite by this one explain what will happen when S tends to 0..

Then I try to find out that what is the application of life cycle on the cell growth because, when you handle any kind of organism life cycle plays very important role because when you do the inoculation, inoculation should be done in the log phase mid log phase to late log phase and then I try to find out the how you can are determine the growth kinetics constant. As for example, mu max and Ks by using line weaver Burk plot eadie hofstee

plot Hanes Woolf plot and also finally I discussed the display advantage and disadvantages of the batch process.

Thank you very much.