Bioreactor Design and Analysis
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Lecture 08
Design of Fed Batch Bioreactors - Part 1

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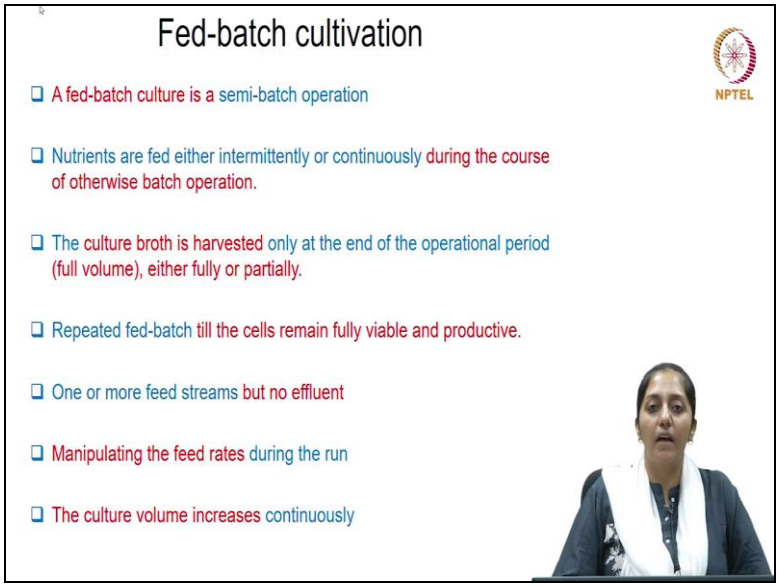

Welcome back students, so in the last class we were discussing design of batch reactors. Now in this class we are going to move ahead with the design of fed batch reactors. So if you remember in the introductory lectures I spoke about the different types of and modes of cultivations in which batch mode of cultivation was one which we call as batch reactors. And then there was fed batch mode of cultivation.

And the reactors where fed batch mode of cultivation is done are also termed as fed batch reactors. So let us see how to design a fed batch reactor. So just to reiterate a fed batch culture this is also called as a semi batch operation. Nutrients are fed either intermittently or continuously during the course of otherwise batch operation. So this means that the nutrients ah can be fed after an interval of time inside a reactor once it starts or it can be fed continuously as the reactor is running till its harvest.

So this kind of feeding is called as fed batch feeding. Now the culture broth is harvested only at the end of the operation and what is the end of the operation, once the volume reaches the maximum volume possible in a reactor which is nearly kept around 80% of the total volume

of the reactor. So why did I say that the time here is restricted to the time of harvested is restricted to the maximum volume a reactor can reach up to; this is because being. In a fed batch mode of cultivation because there is only inlet stream and there is no outlet stream the volume will keep rising.

And hence we cannot continue the fermentation. Now repeated fed batch so the fed batch process can be repeated till the cells remain viable or fully viable or productive. One or more feed streams can be used as the feed inlet feed while there is no outlet stream. There is a choice of manipulating the feed rate. So one has to determine at what rate the feed has to be sent inside the reactor.

Then one has also to determine what should be the feed concentration the substrate concentration in the inlet stream has to be determined. How long the feeding is to be continued which means in what way the feeding is done. Whether you would like to do it intermittently as pulse feeding or you would like to do continuously as step feeding once started or you would like to keep varying the flow rate throughout the feeding with following any trend like exponentially increasing feeding and so on.

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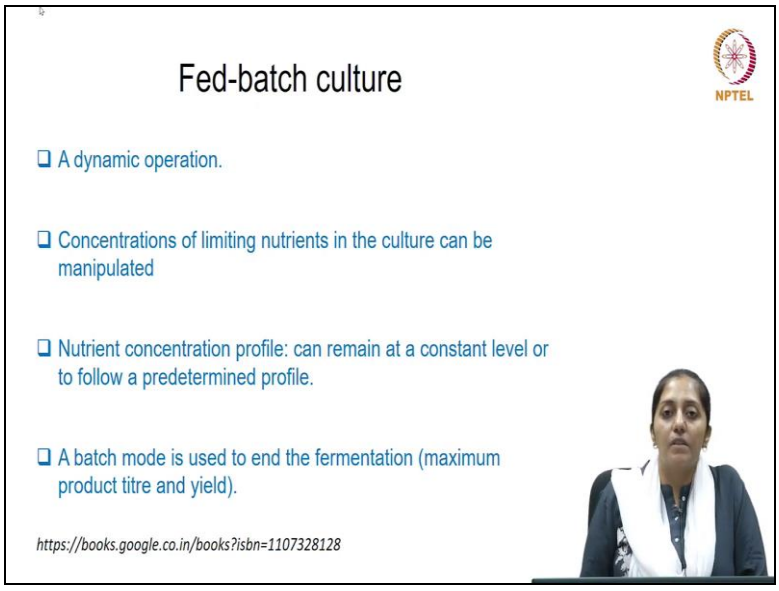

So because the inlet feed is continuously coming inside the reactor the volume is changing the concentrations of the variables therefore will be changing. So it is a dynamic system. Concentrations of the limiting nutrient in the culture therefore can be manipulated. Now the feeding can be controlled in a manner such that the nutrient concentration inside the reactor can be controlled at desired levels.

Generally a batch process is initiated and then it is converted to a fed batch process till the harvest to achieve maximum product yield and titers. Sometimes if it is a pulse feeding then one can even continue to run again after the maximum volume has reached run the reactor as a batch in order to ensure that any residual nutrient due to the inlet feed remaining inside the reactor also gets utilized.

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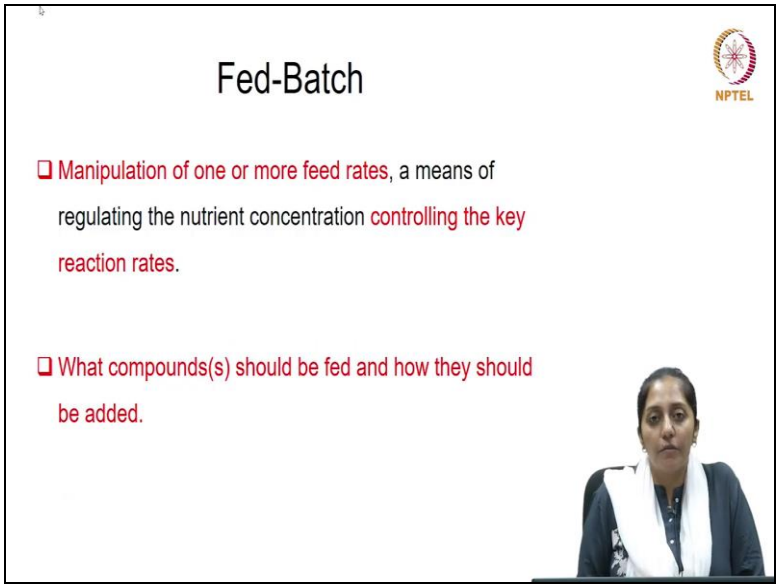

So what are the different permutations and combinations which can come out as a feeding strategy. While designing a feeding strategy in a fed batch this may involve which substrate are to be fed at what concentrations they are to be fed. How long they are to be fed inside the reactor and at what fashion they are to be fed which means at what flow rates whether constant flow rate or increasing flow rate so that is also to be determined.

There can be more than one feed stream inside the reactors because one or more nutrients may be required to be fed at different rates inside the reactor depending on the concentration which one wants to maintain inside the reactor. So the regulation of the nutrient concentration is carried out by manipulating one or more of these parameters in a fed batch operation.

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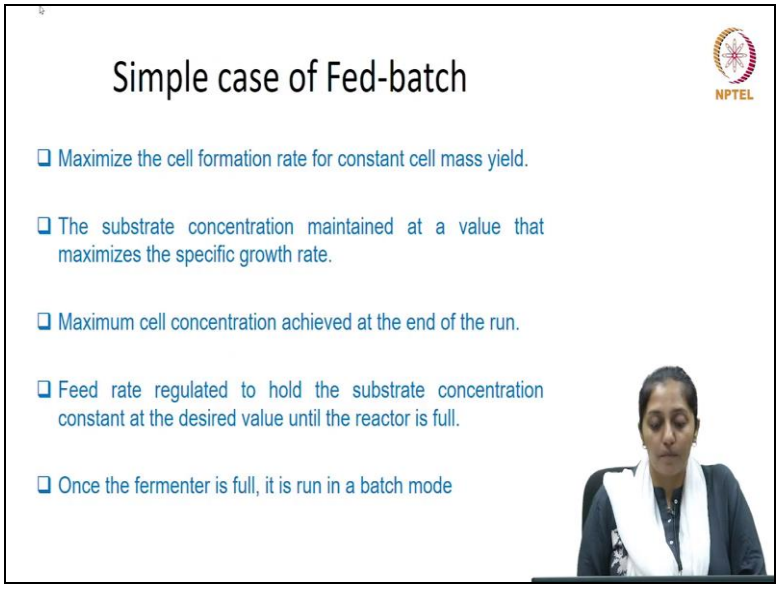

Let us take a simple case of fed batch and try to design the fed batch kinetics. Now the objective of running a fed batch operation is to maximize the cell formation rate for constant cell mass yield. So constant cell mass yield is your yx by s maximizing cell mass concentration is your x. The substrate concentration maintained at a value that maximizes the specific growth rate.

So which means that the substrate concentration is kept at a value where the specific growth rate are is maximum. The maximum cell concentration can then be achieved at the end of the run. So in order to ensure that you achieve maximum cell concentration in the end with maximum yield and productivities you maximize you keep the substrate concentration at a level where your specific growth rate is maximum and your cell formation rate which means growth rate is maximum at constant cell mass yield.

Then you can assume you will end up in maximum cell mass concentration. Now feed rate is regulated to hold the substrate concentration constant at the desired value until the reactor is full. We regulate the feed rate inside the reactor such that the substrate concentration can be held constant at the desired value till the reactor is full. Once the fermenter is full then as I said earlier the reactor is run in batch mode for maximum utilization of the residual substrate if present in the reactor.

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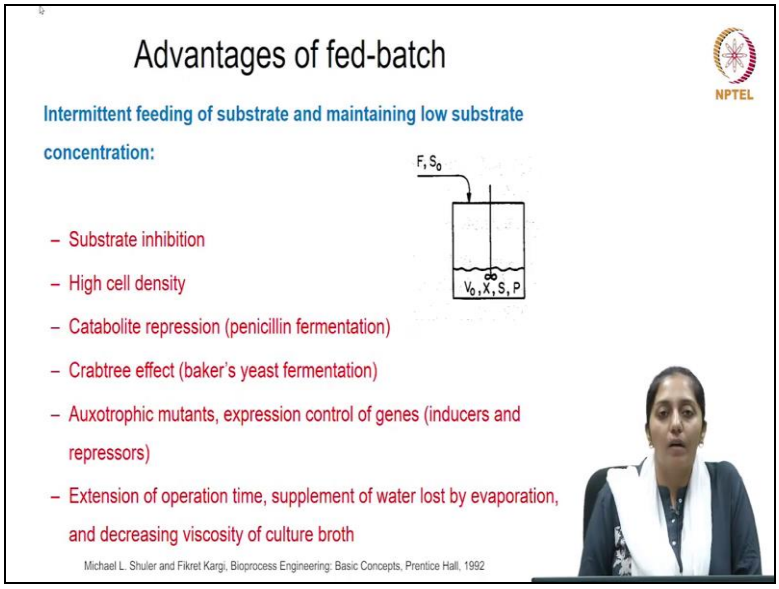

Now let us take some examples as advantage of a fed batch process. Intermittent feeding of substrate and maintaining low substrate concentration, now let us take some examples like substrate inhibition. Now imagine when inside a reactor there is only one feed stream or there is an inlet stream but there is no outlet stream then the substrate inhibition. If you remember the earlier classes in batch operation what was substrate inhibition?

Substrate inhibition is a phenomena where the substrate at inhibitory concentrations can lead to reduction in the specific growth rate of the culture. So if you want to avoid substrate inhibition you would like to keep the substrate concentration in the medium always below the inhibitory levels. So one can maintain this throughout the cultivation period if you can manipulate the feed rate or inlet feed rate.

So this is what is enabled in a fed batch process. Then high cell density, the limitation of a batch process is that it cannot go beyond that cell mass concentration once the substrate has completely depleted which means it has entered the stationary phase because now there is no more substrate to be consumed and the biomass to increase. But fed batch process because without allowing the cells to reach the stationary phase.

And thereby keep increasing the final biomass concentration and the cell density. Now catabolic repression is one example which can be overcome by using a fed batch process. In penicillin fermentation, penicillin is an antibiotic. So, industry is known to undergo catabolic repression because of the high amount of glucose present in the medium. So a fed batch process can help in maintaining the glucose concentration inside the reactor at levels which

do not allow catabolic repression to at levels which beyond which catabolic repression starts happening.

So when can maintain by manipulating the glucose feed rate inside the reactor. Then crab tree effect it is another limitation in a baker's yeast process where maximizing the biomass is the objective. However because of the large amount of glucose present it may start taking up substrate level phosphorylation for energy generation. So to avoid that the glucose concentration has to be kept desired under the concentration levels where the crabtree effects starts happening which is an undesirable effect.

Because if the substrate level phosphorylation starts happening then ethanol as a byproduct will start getting formed because of which there can be toxicity and other the pH might change the flavour might change and which is a disadvantage. So to avoid that the glucose concentration is kept under a limit by manipulating the inlet feed rate inside the reactor or in case of oxotrophic mutants where if one wants to control the expression of certain genes by manipulating the concentration of the inducers or repressors then that can be enabled using a fed batch process.

And I said earlier to get high cell density because using a fed batch operation you can extend the log phase or the operation time. Then it can also help because there is a continuous aqueous feed inside the reactor it can help to replenish the water lost due to evaporation during the fermentation process. And also helps in the decreasing viscosity of the broth due to higher cell densities.

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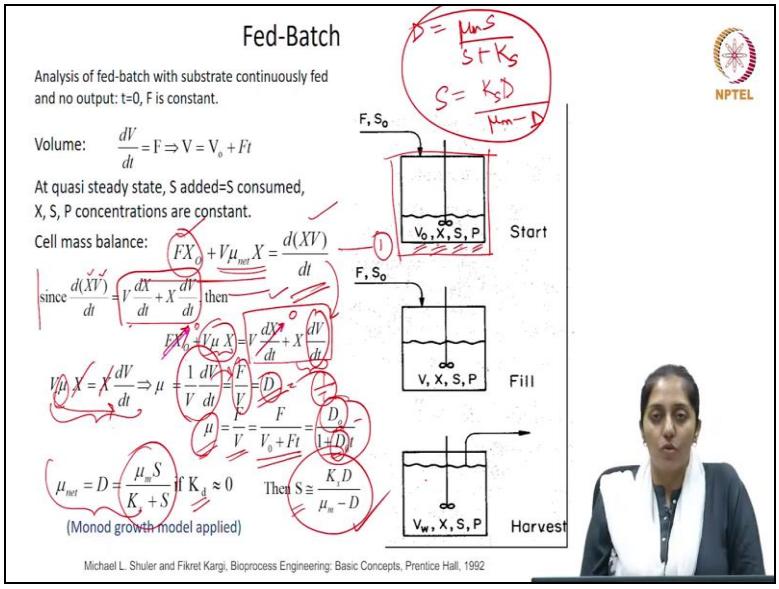

So now after discussing some of the advantages of fed batch process, let us see now how to design a simple fed batch process. Now here we will assume that in this fed batch process if you can see the schematic on the slide. We will make certain assumptions. We will assume that this fed batch process is a constant feed flow rate process which means there is a flow rate f of the inlet feed going inside the reactor and it is constant.

There is no change in the flow rate with time. Can you see the first line it is a volumetric flow rate. So it is dv by dt as given here. Now if you integrate this and assume that the initial volume inside the reactor is V 0; then because of the continuous feeding which is happening entering inside the reactor your volume will keep changing as a function of the flow rate and time. Now this f here because it is a constant feed flow rate system, so f is a constant here.

So now it will keep changing increasing rather with time. Now in a fed batch process we will try to design for simplicity of solving and designing the process. We will assume that the system is trying to achieve quasi steady state and is running at a quasi steady state. Now what does this mean? Quasi steady state would mean that steady state in general means that the variables are no more changing with time.

It is a homogeneous system so obviously we are not taking any special variations here. So the variables were only changing with time as in case of a batch process. But in a quasi steady state fed batch process you can assume that the variables are no more changing with time. So let us do a mass balance across this reactor which is shown in the on the slide. So as given

inside the reactors let us assume these variables volume is V 0 in the beginning X, S and P stand for biomass, substrate and product respectively inside the reactor.

Where in the inlet feed it is flowing at a constant flow rate f and the substrate in this concentration in this inlet field is S 0. So if you do a cell mass balance and let us assume that X 0 is the biomass. Now if you do a cell mass balance FX 0 is the inlet, so it is the rate at which the biomass is coming inside the reactor. The second term can you guess what is that what does it talk about right.

So it talks about can you see the term mu. So it is talking about the rate at which the biomass is growing inside the reactor with the volume V at a time and this is equal to the rate of accumulation inside the reactor. Now the outlet term is zero there is no consumption so consumption term is also zero. So therefore we only have accumulation growth and inlet in the continuity equation.

Now if you see the expansion of the RHS because X and V in a fed batch both are variables. So we cannot as in case of a batch we cannot bring V out of the bracket. So we will keep it inside. So now if you expand the differential then you get V dX by dt + X dV by dt I hope this is clear now then if you substitute this in the previous equation is copied FX 0 + V mu X and we have copied the second form in place of dX V by dt.

Now FX 0, X 0 I said is the biomass concentration in the inlet stream. Now if we assume that it is a sterile feed this term will go out because X 0 is zero. The second term remains; which demonstrates the rate at which the biomass is growing inside the reactor. Now here we are assuming quasi steady state. Steady state would mean that X is also a variable biomass. So we can assume that X is not changing with time.

So at quasi steady state this term will also go to 0 and the second term remains which is X dV by dt. Now you know dV by dt was equal to F. So now we will substitute F in place of dV by dt here. So this equation and also V mu X which is here so let us see let us substitute. So this becomes X times F which is equal to V mu X. So what they have done in this equation now V has been brought here.

So it becomes 1 by V dV by dt dV by dt has been substituted as F so it becomes F by V equals to mu X and X gets cancelled. So F by V is nothing but the dilution rate inside the reactor which is an inverse of the resonance time mu becomes equal to D which is the dilution rate. Now V can further be expanded in terms of F as given here. And let us assume the initial dilution rate as D 0 which will be F by V 0, V 0 we know.

So further substitution has been done and this has been brought as a function of D 0 which is known. So then this is how the mu will change with respect to time and D 0. Now if you remember this equation, equation 1 I will just clear it. It was written here mu net. Mu net is given in case if the culture is undergoing endogenous metabolism. So if you remember I was talking in during the batch process mu net was equal to mu minus k d where k d stands for the rate constant for the endogenous metabolism.

Now if this is following Monod's kinetics then mu is equals to D at quasi steady state the mu can be substitute as Monod's model in terms of S if we are assuming that and there is no endogenous metabolism present. And if you do the rearrangement of this equation you will become you will see S is equal to K S D mu m –D. So let see how here mu is equal to D which is further if it is following Monod's kinetics then mu m S upon S + K.

So if we do the rearrangement then your S will become equal to K S D upon mu m – D. This is what is given here. So under quasi steady state two things the specific growth rate of the culture is equal to the dilution rate of the system this is what we have gathered from here. The second key point; that if there is quasi steady state then if you want to determine how S is going to change it becomes a function of the dilution rate as this.