Material and Energy Balances
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Module No # 05
Lecture No # 22
Biochemical Reactions: Cell Growth

Hello everybody welcome to today's lecture on biochemical reaction with the focus being on cell growth in last lecture we talked about enzymatic reaction and how to perform material balances for systems where enzyme are taking place. Today we will talk about cell growth and fundamentals associated with it and perform some material balance calculations for systems where we have cell growth.

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Terminologies

- Growth rate – Rate of increase in cell mass concentration (X, g/L)

$$Growth\ rate = \frac{dX}{dt}$$

  - Extensive property
- Specific growth rate (μ_{net})

$$\mu_{net} = \frac{1}{X}\frac{dX}{dt}$$

  - Intensive property
- These can also be expressed in terms of cell number concentration (N, number of cells/L)
- Gross specific growth rate (μ_g) = μ_{net} + k_d
  - k_d – rate of cell mass loss due to cell death or endogenous metabolism

First let us learn some terminologies associated with cell growth. Growth rate is defined as the rate of change of cell mass concentration which is represented as X and the unit is usually grams per liter this is given as growth rate is DX DT so this is the mathematical representation.

So we need to understand that this is the extensive property which means it depends on the size of the system. So if you start with 1 gram per liter and doubling times is 10 minutes after 20 minutes you would have 4 grams per liter of cells. Whereas if you start with 10 grams per liter per cells initially after 20 minutes you would have 40 grams per liter and your growth rates are technically different this is because of initial inoculum size that has been used.

To eliminate this difference we use something called specific growth rate this is an intensive property and Mu net which is specific growth rate which is given as 1 /X DX DT. So this is an intensive property and we can compare culture without about inoculum size which was used for growing the cell. We can compare cultures without worrying about the inoculum size that was used for experiments.

These terms can also be represented in terms of cell number concentration instead of cell mass concentration the specific growth rate look at here is the net specific growth rate we should remember that there is another term called gross specific growth rate which is the net specific growth rate + KD where KD is the rate of cell mass loss due to cell dead or endogenous metabolism so this in addition to Mu net will gives you the gross specific growth rate.

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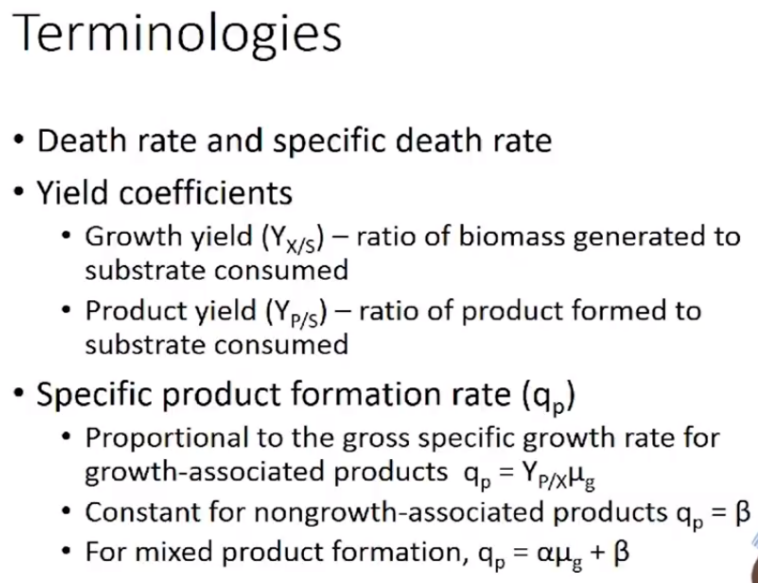

There is also death rate and specific death rate which can be define similar to growth rate and specific growth rate you have yield coefficient where growth yield would be ratio bio mass generated to the substrate consumed and product yield would be the ratio of product formed to substrate consumed. You also have a term called specific product formation rate this is directly proportional to the gross specific growth rate for growth associated products.

So this is usually represented as QP and this QP = YPX MU G for growth associated product formation YPX is the yield of product per unit bio mass which is produced constant value of specific product formation rate will be observed for non-growth associated product formation.

So that is why QP would be equal to beta a constant for non-growth associated product formation. In case you have mixed product formation you have some non-growth associated product and some growth associated product being formed simultaneously.

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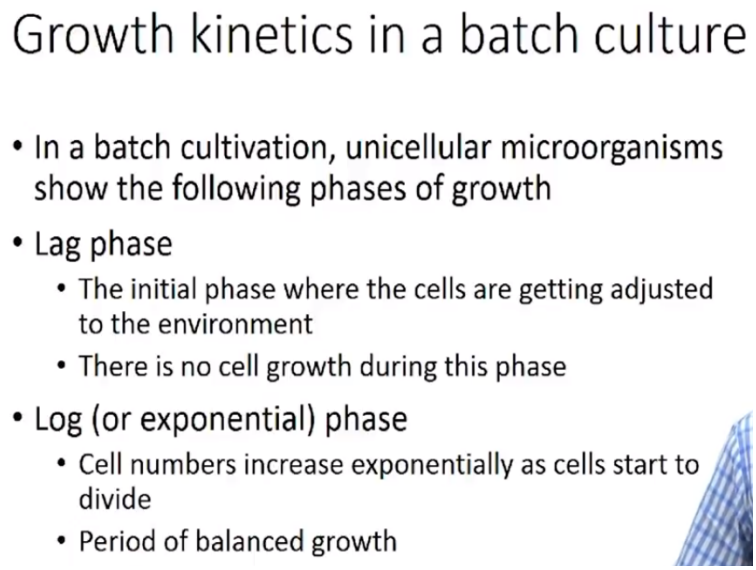
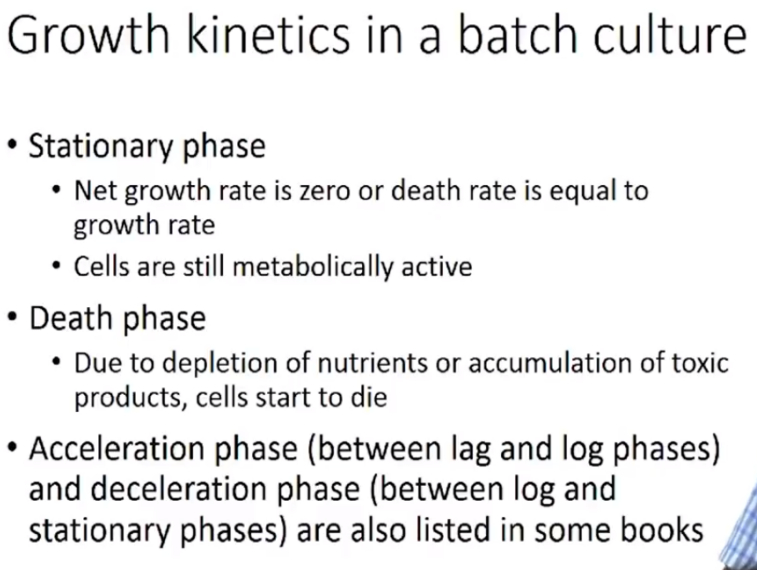


Then the specific product formation rate QP would be given as alpha Mu G + beta when you culture the cells in batch system what you see is unicellular micro organism go through different phases of growth. The first phase is the lag phase this is the initial phase where the cells are getting adjusted to environment and actually there is no cell growth during this period.

So during this time the cell are getting and understanding of what nutrient are available what are the conditions in which they are being cultured and they prepare the machinery they required for surviving in the environment and utilizing this nutrient to eventually grow and multiply. The next phase is the log phase or exponential phase here the cell number increase exponentially and the cells are dividing rapidly this is also a period of balanced growth and this during this period the cell growth follows first order kinetics.



The next phase is the stationery phase here the net growth rate is 0 is probably because death rate is equal to growth rate. Here the cells are metabolically active the growth associated product are actually produced during this stationery phase. So these non-growth associated products are also called us the secondary metabolize. The stationery base is followed by the death phase during this time due to depletion of nutrients and accumulation of toxic products the cells start to die.

Because of this the cell concentration start declining and this period is called as the death race in addition to this 4 distinct phases in some textbooks they also list acceleration and deceleration phases. Acceleration phase is the phase between lag and log phase and deceleration phase is the phase between the log and stationary phase.

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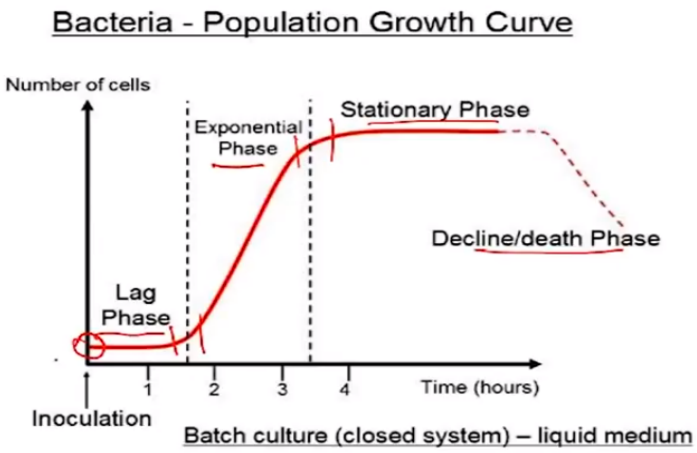
Growth curve



So what you see here is the growth curve so you have the lag phase initially where the cells getting used to new environment and the cell numbers are concentration is not increasing you then have a exponential phase where the cell are multiplying and the cell concentration is increasing rapidly. Then you have stationary phase where the cells are actually dividing and also dying thereby maintain the same cell concentration.

And finally you have the decline phase or death phase where the cells are dying either due to nutrient depletion or accumulation of toxic materials. So the phase in between the lag and exponential phase where you have acceleration of cell growth however its cell does not follow the exponential curve is called as the acceleration phase and the phase between the log and the stationery phase where it is still does not follow the exponential curve but it is starting to slow own is called as the deceleration phase.

One thing you should understand and remember draw a growth curve is you see that at induction the value for cell number is not important whenever you draw growth curve you start with inoculum so therefore it is time point 0 when inoculation occurs you are going have a certain cell number which is then going to increase over the exponential phase. So please do not draw a growth curve with 0 as your origin.

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Modeling cell growth

- Unlike enzyme kinetics, cell growth is the result of various complicated reactions and networks
- At any given time, the cell population is a mixture of young and old cells
- Accurate mathematical modeling is impossible
- There will be too many parameters that can't be estimated
- Simplest model is the unstructured, non-segregated model

Having understood the growth curve let us move on to modeling cell growth unlike enzyme kinetics cell growth is actually a result of various complicated reactions and network. So it is almost impossible to draw any kind of model which will fully describe the system at any given times the cell population is actually a mixture of young and role cells and mathematical modeling would mean that there are going to be N number of parameters which are going to very difficult to estimate.

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Assumptions in modeling cell growth

- Cells can be represented as a single entity
  - E.g. – cell mass, cell number, concentration of protein or DNA or RNA
  - This assumption is true for balanced cell growth
- Cells are uniformly distributed in the culture
  - Cell suspensions are considered to be homogeneous solutions
  - The heterogeneity of the cells is ignored

So what people do instead is use a simple model which is a unstructured non segregated model we are going to look at one such model which is called the (()) (07:43) model before we do this we should understand what assumptions we have to be made for performing this type of an

unstructured non structured non segregated modeling. The first thing is we will assume that cells can be represented as single entity it could either be cell mass or numbers or concentration of proteins DNA or RNA.

So the use of concentration of protein DNA and RNA is acceptable for balance cell growth period which would be the exponential phase. Cells are assume to be uniformly distributed in the culture so cell suspension which are actually hydrogenous are considered to be homogenous solutions and the hydrogenate among the cells himself is also ignored when may be performed this type of modeling.

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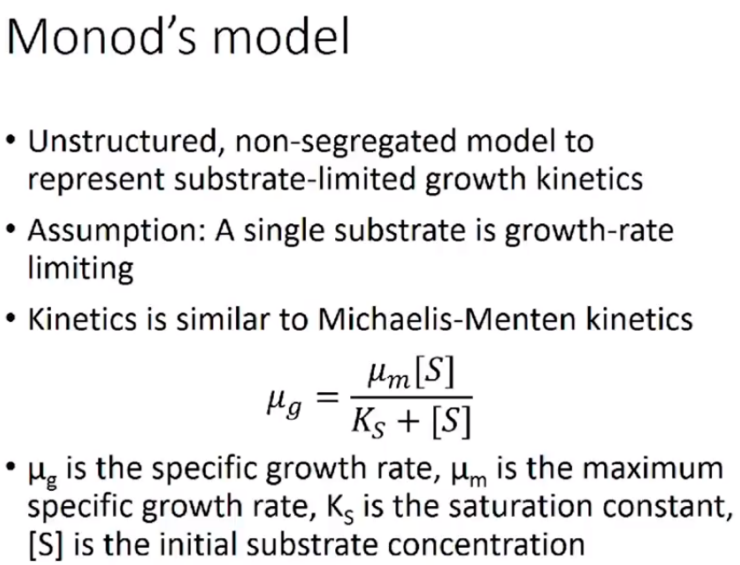

As I mentioned Monod;s model is once such model which is very popular and it is consistently used for describing cell cultures so this is an unstructured non segregated model which is used when have a substrate limited growth kinetics. You assume that a single substrate is actually the growth limiting substance and the modeling is performed based on that the kinetics itself is similar to the Michaelics- Menten kinetics and the equation for the gross specific growth rate Mu G given as Mu times S divided by KS + AS.

Where Mu g is the specific growth rate Mu M is the maximum growth rate KS is saturation constant and S is the initial substrate concentration.

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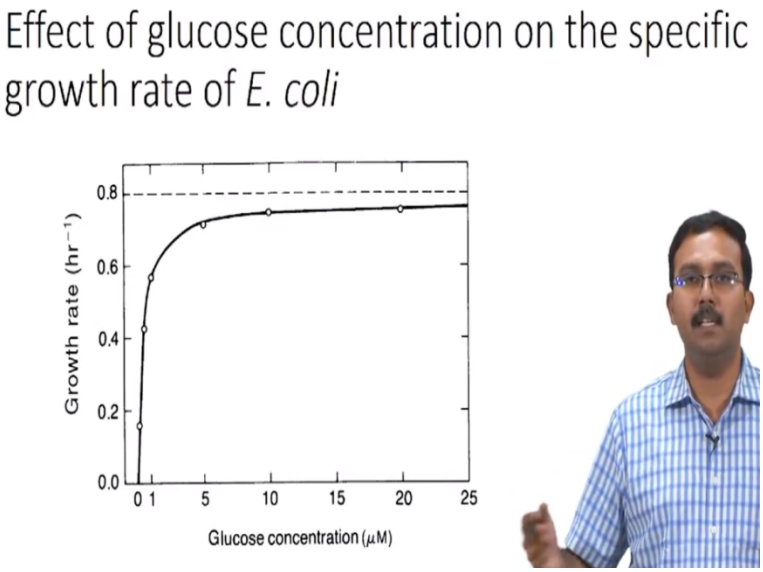

(Refer time: 09:17) So when you actually culture E.Coli with different subset concentration and measure the specific growth rate this is how the graph would look. Specific growth rate versus subset concentration graph looks very similar to what you would have observed with V versus S curve for enzyme kinetics. So this is a saturation kinetics and as you can see the specific growth the maximum value Mu max or Mu M when the subset concentration increases beyond he certain point.

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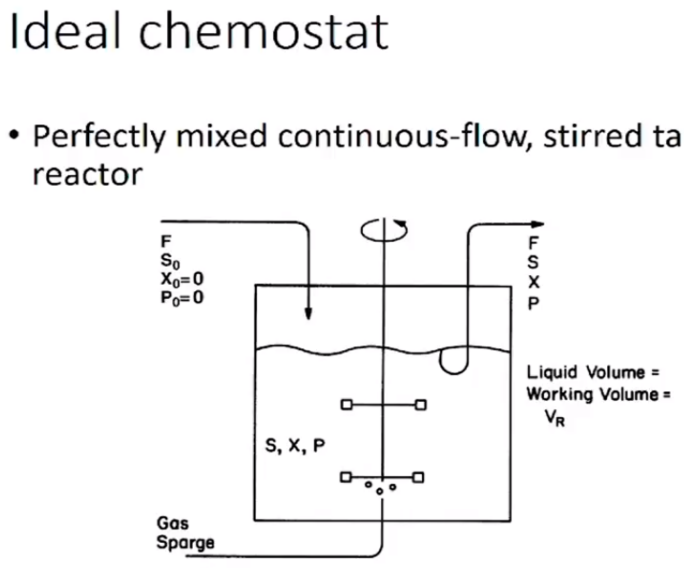

To perform material balances for cell culture systems let us take some ideal Chemostat and ideal chemostat is perfectly mixed continuous flow stirred tank reactor so this chematic is given here where you have F is the volumetric flow rate S naught is the initial subset concentration X

naught is the inlet bio mass concentration usually you have sterile feed and inoculum is just given as at time is 0 the reactor.

So if feed does not usually contains biomass so it would usually be a sterile feed giving X naught to be 0. P naught is the concentration of product inlet stream so which is also usually 0 F, X, S, P are the corresponding value in the output stream you have the working volume which is the liquid volume inside the reactor that is called the reactor volume also given as VR and you have SX and P as the concentration inside the reactor as they are the concentration in the exit stream.

Just like we discussed CSTR enzyme kinetics here also the concentration of components in the exit will be same as the concentration of component in the inlet stream. So you have a well-mixed system which means whatever is fed is immediately mixed and the concentration is uniform throughout the reactor. In addition to the mixing condition you also see something called gas sparge.

So this is usually present because bacterial cells need lot of oxygen to grow and this oxygen is supplied through gas sparge which is either pure oxygen is being supplied or air which si supplied to provide oxygen to the cells to grow.

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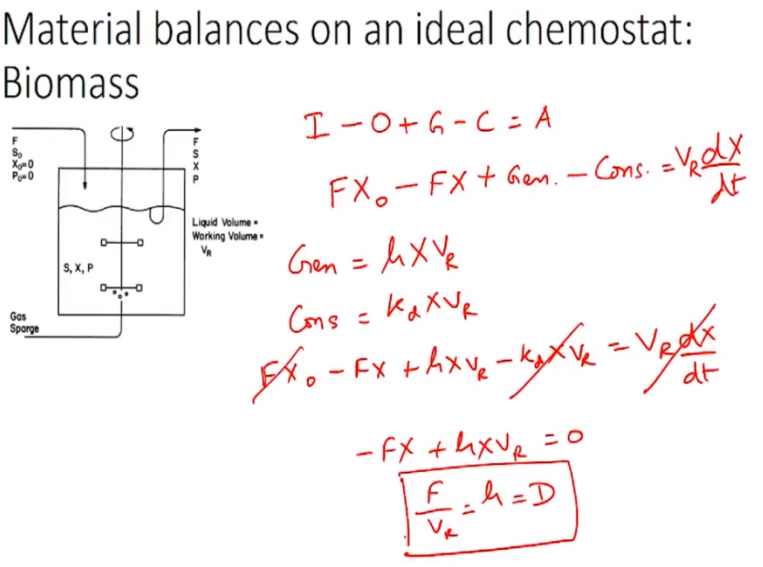

So let us now perform material balances for such an ideal chemostat so the first balances we are going to write is for biomass. Let us assume unsteady state and non-sterile feed for the time

being and then you cancel out term based on the assumption which is made. So let us start with the balance equation just like any balance equation your equation states with input – output + generation – consumption = accumulation.

So the inlet term would be F times X naught where F is the volumetric flow rate X naught is the bio mass concentration in the inlet stream – outlet being FX where F is the output flow rate and X is the bio mass concentration in the outlet stream + generation term will get what is the generation term would be – consumption let us also get the consumption later. So accumulation would be change in bio mass concentration within the system with respect to time.

So that is DX DT and because we wanted in terms of biomass total mass with respect to time we will multiple with VR or the working volume for the reactor generation for bio mass will depend on the growth of the cells so that would be based on the specific growth which is MU X which gives us the growth rate in terms of change in bio mass concentration with respect to time due to cell growth.

So we want to get change mass or bio mass of cell with respect to time so this again multiplied by VR which is the volume of the reactor will give us generation term. So similarly we can get the consumption term as KDXVR where KD is the specific death rate or the specific rate at which endogenous consumption of bio mass is happening. So this would be KDXVR so substituting all of these we get FX naught – FX + MU X VR – KDXVR = VRDX DT.

So you can also verify the dimension homogeneity of this equation just like we did for enzyme kinetics and you would be able to confirm that this equation is dimensionally homogenous when we consider a steady state operation this term would go to 0 and if we do not have any endogenous reactions or cell death your KD will also go to 0 if you have a sterile feed the X naught goes to 0 giving you an equation which is – FX + Mu X VR = 0.

From here we get F / VR = Mu so this is also equal to D which is the dilution rate is already know that F / VR is the dilution rate so what we have here is by altering the volumetric flow rate to the system we can alter the specific growth rate of the cells. So this makes chemostate a very useful tool for performing bio chemical reaction.

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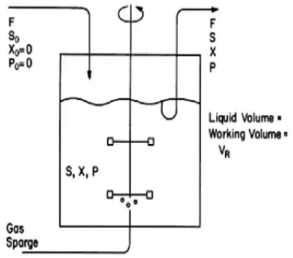
Material balance on an ideal chemostat: Substrate



$I - O + G - C = A$

$FS_0 - FS - Cons. = V_R \frac{dS}{dt}$

$Cons = Cons\,1 + Cons\,2$

$Cons\,1\,(Growth) = \frac{\mu_1 X V_R}{Y_{X|S}}$

$Cons\,2\,(Product) = \frac{q_P X V_R}{Y_{P|S}}$

In addition to writing the biomass balance we can also write a sub-state let us try to write the sub-state balance for ideal chemostat. So again we start with input – output + generation – consumption = accumulation so we will initially assume that it is not at steady state and then we will cancel of terms based on making the appropriate assumptions. So the input would be FS naught output would be – FS and here you will not have a generation term because sub-state is not going to get generated in the system is only being consumed.

So we have consumptions so we will get to the consumption terms equals accumulation which should be similar to whatever we looked at with biomass. So it is VR DSDT so now for consumption terms there can actually be two different terms will call them consumption an consumption two consumption one would be consumption sub state for the cells to grow. So that would be the growth associated sub-state consumption.

In addition you can also have product formation which could results in consumption of sub state so consumption which is associated with growth of the cells would be given as Mu X VR / YXS what you see here is Mu X VR is the bio mass which is generated and YXS is the yield of he bio mass with respect to per unit of sub state condition. SO Mu X VR divided by YXS will actually give you the amount of sub state which is consumed per unit bio mess which is produced.

So the second factor so second consumption which is product formation can again be written in similar fashion which will be QPX VR / YPS. Where QP is the specific product formation rate

and X is the bio mass QP times X will give you the product formation for the growth associated proteins and growth associated products and you have VR multiplied to get the total amount of such product being formed and YPS is the yield of the product per unit sub state is being consumed.

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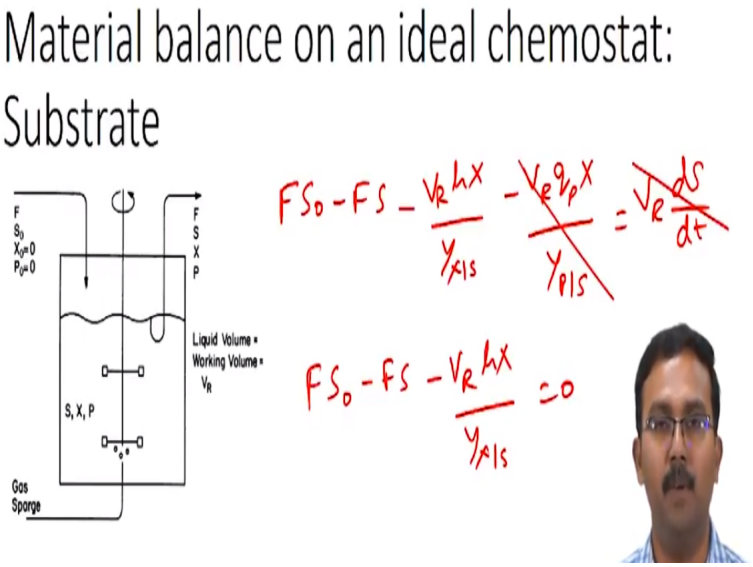

So thereby we can convert this into the amount of sub state which is actually consumed during the product formation so substituting all these back into the equation we get FS naught - FS – VR Mu X divided by YXS – VR QPX divided by YPS = VR DS DT. Now at steady state this term goes to 0 and product formation if it is 0 that would also go to 0 and your equation can be simplified to FS naught – FS – VR Mu X / YX = 0.

So this is a balance equation for a sub state while we perform for equation one thing you would have seen is the accumulation term for bio mass was also given as DX / DT and we had earlier defined the growth rate as DX by DT. But this is to be understood that these two terms are not equal except for bath reaction. In reality the change bio mass concentration due to many other factors other than growth can also happen it could be because of change in flow rate it could be because of accumulation of components and so on.

So that term is the accumulation term the change in bio mass concentration with respect to time due to cells dividing which is called also growth rate which is also DX/DY. So these two DX/DT's are not the same they are numerically equal only for a batch reactor condition now that

we have clarity on this let us move on to performing some example problems to fully understand how to apply these balance principles to bio chemical systems where cell cultures are happening.

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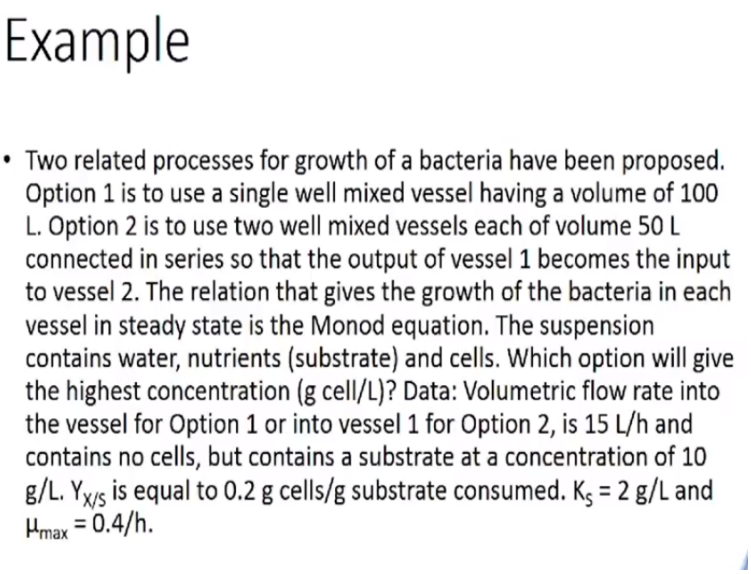

Here is an example problem two related processors for growth of the bacteria has been proposed option 1 is to use single well mix vessel having a volume of 100 liters. Option 2 is used to well mix vessels use to volume of 50 liter connected in series. So that the output for vessel 1 becomes the input to vessel 2 the relation that gives the growth of the bacteria in each vessel in steady state is the monod equation.

The suspension contains water nutrients substrate and cells which option will give the higher concentration of cells in terms of grams of cells per liter. You have been given that the volumetric flow rate into vessel 1 for option 1 and into vessel 1 for option 2 is 15 liters per hour and contains now cells but it contains substrate concentration of 10 grams per liter and Y axis is given as 0.2 grams of cells per gram of substrate consumed med and KS is 2 grams per liter and Mu max is 0.4 hour with all these information let us perform the material balance calculation to identify which of the two system will give us the higher bio mass concentration.

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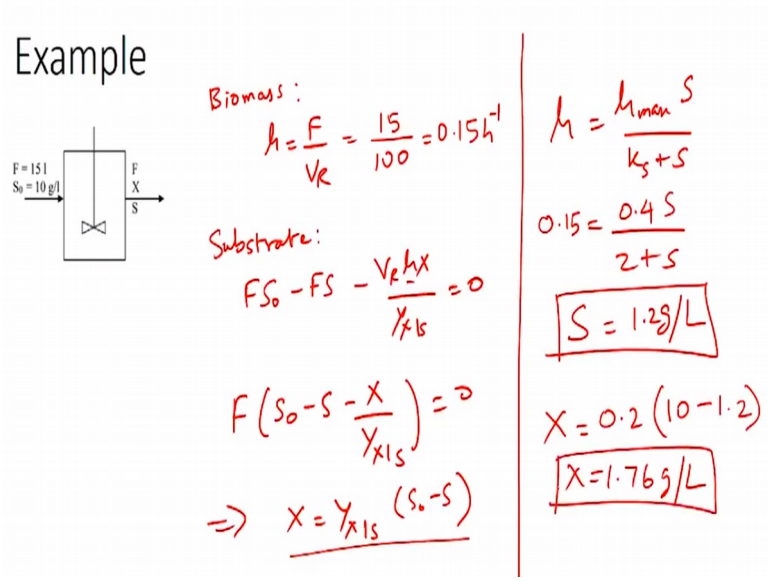

We will first start with the option 1 as we have been told that the system is operating at steady state and you have a sterile feed which does not contain any of the bacteria we would have the bio mass balance or the cell balance simplified to the equation Mu = F by VR this is based on the derivation we did earlier. So we can also write the substrate balance as we got earlier it would be FS naught – FS – VR Mu X divided by YXS = 0.

As we know that Mu = S / VR we can substitute he value for Mu here and we end up with F times S naught – S – X divided by YXS = 0. So this implies X = YXS times S naught – S from the values given in the problem we can calculate the Mu as 15 liters per hour divided by 100 liter which is volume of the reactor for 1 giving us a Mu of 0.15 hour universe. So using Monad equation we know that Mu = Mu max S divided KS / S given Mu max as 0.4 and KS as KS as 2. So using these values and substitute this as 0.4S / 2 + S = 0.15 which is Mu.

From here we can calculate the substrate concentration which is S giving a value of 1.2 per liter now that we know that outlet sub concentration we can substitute it back to equation for X to calculate the value for power mass concentration in the output stream which should be Y axis which is 0.2 times inlet subset concentrating 10 grams per liter – outlet concentration 1.2 concentration per liter giving us 1.76 grams per liter. So the outlet bio mass concentration is 1.76 bio mass grams per liter.

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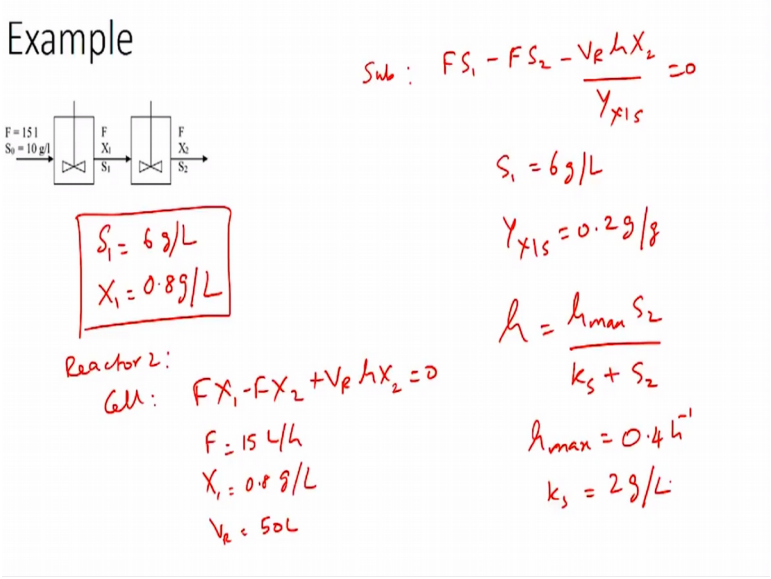

Now let us the second option here the second option there are two reactors are connected in series and the reactors volume are 50 liters. So the calculation which have to be performed for the first reaction first reactor will always be same as what we had done in previous example the only difference is the reactor volume of half. So we calculate the Mu appropriately and we will perform all these calculation which we did earlier values for S and H.

So if you were to perform the calculations you would get the values as S as 6 grams per liter which is S1 leaving the first reactor and your X1 would be 0.8 grams per liter I am not going to perform these calculation here I expect you to perform whatever we have done till now and get these values and verify if you are able to get the same value. Now let us write the balance equation for the second system.

So the second system the reactor 2 we can write a cell balance the cell balance would be FX1 – FX2 + VR Mu X = 0 so here we are assuming there are no cell or accumulation because it is steady state so we have an inlet bio mass concentration coming in in terms of X1 we have FX1 as the bio mass entering the system FS2 is bio mass leaving the system and VR Mu X and bio mass which is being generated.

So this would be S2 in this equation we know F = 15 liters per hour and you have calculate S1 as grams per liter and we know that VR = 50 liters. So this leaves only X2 and Mu as two variables which are unknown but what you see here is it is an non-linear equation so we might have to

solve non-linear equation to get the final values. So we can similarly write the balance for the substrate balance would be FS1 – FS2 – VR Mu X / YX S= 0.

Again we are assuming that there is no formation and there is no accumulation so using this we get this equation here again we already calculated S1 as 6 grams per liter we know F and S has been given to us so F and VR which are given us again this would be X2 so we now again have two variables which are unknown Y axis is again known to be 0.2 grams per gram.

So now with all the variables known we are left with Mu and X2 so we can also calculate Mu from the Monad's equation which should be Mu max S2 / KS +S2 where we have been given to what Mu max is given as 4 our inverse and KS is given as 2 grams per liter. So now that we have all the parameters we just have non-linear equations that need to be solved simultaneously by solving this non-linear equation we are able to get the values for the bio mass concentration which will be leaving the system.

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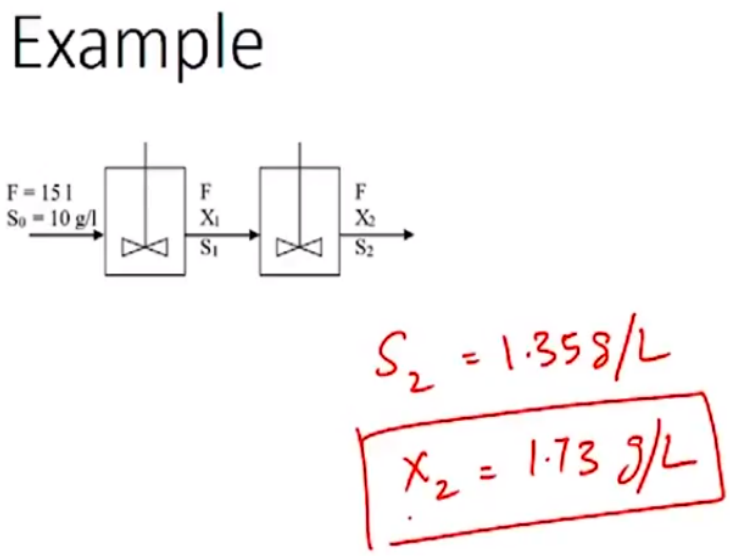

If you were to perform this equation you will end with the outlet sub set concentration as 1.35 grams per liter and your bio mass concentration as 1.73 grams per liter. So based on the system which we have looked at the reactor 110 liters volume and a single system actually gives a higher bio mass concentration compared to two smaller reactors in series. So with this I hope you have full understanding of how to perform material balance calculation for systems where cell culture is happening thank you.