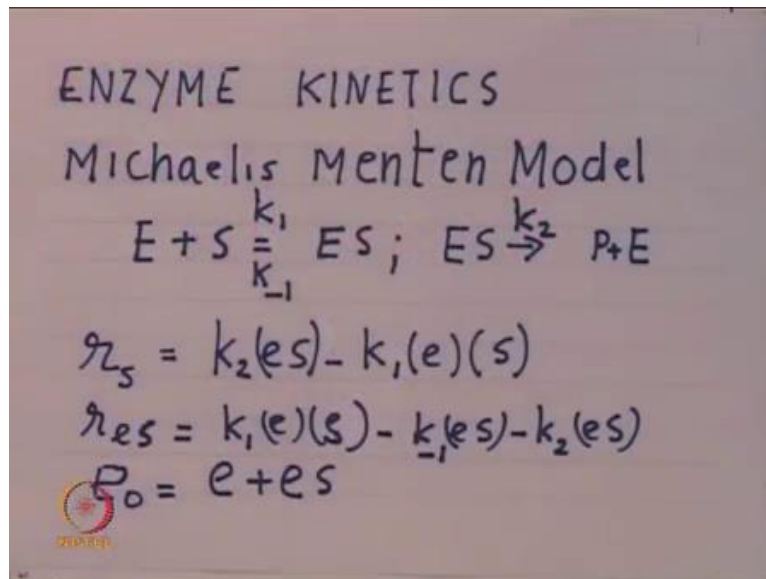


Advanced Chemical Reaction Engineering
Prof. H. S. Shankar
Department of Chemical Engineering
Indian Institute of Technology, Bombay

Lecture No- 38
Illustrative Examples 1) Enzyme Reaction 2) Microbial Reaction
3) Waste Treatment

Let us get going with what we said last time, just recap which you late.

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We started with Enzyme Kinetics and we said that enzymes of proteins they have active sides, and these are active sides get expressed by an appropriate use of with a co factor. At suitable P S the active sides get fully expressed and they are able to bind with a substrate to give you certain reaction rates. Michaelis Menten this Model E plus giving E s and E S going to products P plus E it is a very celebrated model early 190 and it gives us a relationship of these forms of which we are spoken already.

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$$r_s = k_{-1}(es) - k_1(e_0 - es)s$$

In Batch Equipment

$$\frac{d}{dt}(Vs) = r_s V$$
$$V \frac{d}{dt}(es) = r_{es} V = 0$$

QSSA: Quasi Steady State Approx

That most important thing in these that in enzyme kinetics is that the complexes are assumed to be stationary. Now, these complexes of course, there are a lot of measurements available in the literature, about how good are these assumptions, these complexes seem to be reasonably stationary in biology. But, in commercial systems like even in design, these could be a revelation or in other words that the constancy of s may not be as good as you would like it to be.

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$$r_{es} = 0 \text{ so}$$
$$0 = k_1(e_0 - es)s - k_{-1}(es) - k_2(es)$$
$$[es] = \frac{k_1 e_0 s}{k_1 s + k_{-1} + k_2}$$
$$r_p = \frac{k_2 k_1 e_0 s}{k_1 s + k_{-1} + k_2} = \frac{V_M s}{K_M + s}$$
$$K_M = \frac{k_{-1} + k_2}{k_1}$$

Based on these that the form of the rate function that we get is $V_M S$ divided by $K_M + S$, where V_M is the Michael's maxim velocity and K_M is the Michael's parameter. Notice here, coming back notice here, that this K_M is the rated which the complex gives you the product.

And the rate function V_M depends on the K_M , so typically what does happen is that we do an experiment using certain value of V_{naught} , but in another exercise we find that the V_{naught} is different, because the actual application requires you to a different quantity of enzyme.

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Department of Chemical Engineering @ IIT Bombay
CE 600 Chemical Reaction Engineering

Practice Problems : Enzymes Kinetics and Enzyme Reactors

Q1. MM parameter for enzyme urease are $V_m = 1.33 \text{ mol/s.L}$ at $E_0 = 5 \text{ g/L}$ and $K_m = 0.266 \text{ mol/L}$.

Q1.1. Estimate time needed to remove 95 % urea in a 0.5 lit batch vessel containing 0.001 g/L urease.

NPTEL

See here is an example of enzyme urease and the V_m is given as 1.33 and then at a given at some level and you are ask to estimate what happens if the quantity of the enzyme is different you understand. This is an exercise which tells you the experiment has been conducted at 5 grams per liter for which V_m is given as 1.33. But you are ask to calculate at different level of the enzyme, is this clear.

This is the exercise is parolee straight forward if you recognize that K_M is actually the parameter of the process and e_{naught} is what you can change from experiment to experiment. So, once you change e_{naught} V_M changes and therefore, the process the rated at which the process takes place changes.

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$$\begin{array}{l} \boxed{} \\ e_0 = 5 \frac{\text{g}}{\text{L}} \\ V_M = 1.33 \frac{\text{mol}}{\text{s} \cdot \text{L}} \\ V_M^1 = 0.266 \times 10^{-3} \frac{\text{mol}}{\text{L} \cdot \text{s}} \\ K_M = 0.266 \frac{\text{mol}}{\text{L}} \end{array} \quad \begin{array}{l} \boxed{} \\ e_0 = 0.001 \frac{\text{g}}{\text{L}} \\ V_M^1 = ? = \frac{1.33 \times 0.001}{5} \\ K_M = 0.266 \frac{\text{mol}}{\text{L}} \end{array}$$

So, with this quickly, let us see what happens to this problem it is parsley elementary what do we have, we have a batch equipment experiment 1 is done at e_0 equal to 5 grams per liter. And, experiment 2 is at 0.001 grams per liter that is that is only difference, and here V_M is given as 1.33 mole per second per liter. And, easy question what is the V_M here, when e_0 is 5 grams per liter V_M is 1.33. So, when e_0 is 0.001 what is the V_M directly, so V_M here is 1.33 divided by 5 multiplied by 0.001.

So, V_M for our case I will call it V_M dash just to distinguish is 0.21033×10^{-3} mole per liter per second. Now, K_M does not change K_M is given K_M is 0.266 mole per liter, so what is the time required for a given level of reaction in universal etcetera; so what we have to do is fell elementary.

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$$-\frac{ds}{dt} = k_s = \frac{V_M s}{K_M + s}$$
$$\int \frac{(K_M + s) ds}{s} = - \int_{s_0}^s V_M dt$$
$$\left[K_M \ln s + s \right]_{s_0}^s = -V_M t$$

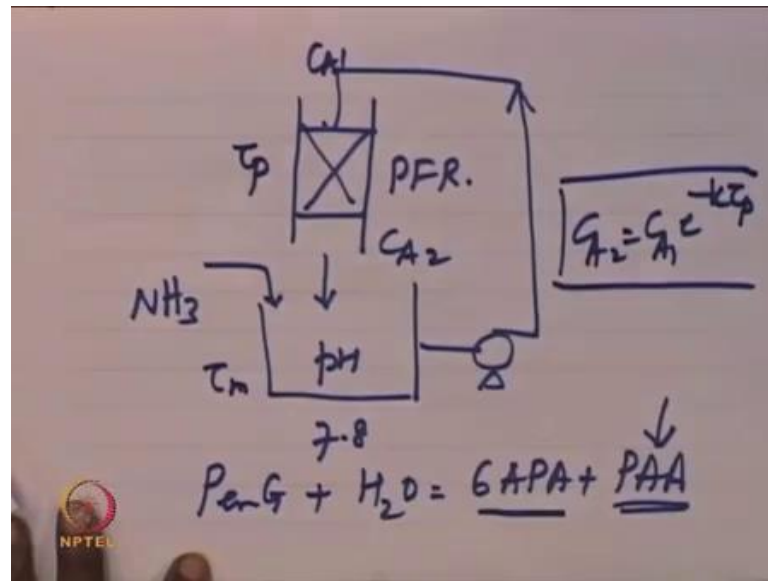
But, only tells you the weight it is ds times dt with a minus a sign which is rs that is equal to $V_M ds$ by $K_M + S$. So, all numbers are known therefore, we have ds divided by, multiplied by $K_M + S$ equal to minus $V_M dt$, this you have to integrate to give you whatever we want integrate and finished off. So, let me $K_M \ln s$ plus S , S naught to s equal to minus $V_M t$ is it, so we can integrate this finished off. So, let me let me complete this.

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$$K_M \ln \frac{s}{s_0} + (s - s_0) = -V_M t$$
$$\boxed{K_M \ln \frac{s_0}{s} + (s_0 - s) = V_M t}$$

We get let me just I am just taking it forward from here only so it is $K M l n S$ by S naught plus S minus of S naught equal to minus $V m t$ or $K M l n S$ naught by S plus S naught minus of S equal to $V m$ dash t . So, we can find out the time that is required for a given level of reaction. Now, the reason why I have done this is to draw attention to an exercise I had given you I want spend a little bit a time on that.

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See the exercise that was given to you little while ago was something like this, you had PFR, you have a huge tank here, it is a pump which does this. Now, this is the PFR which wholes enzyme, and now here you put ammonia addition, so that the PH is at 7.8. Now what happens in a process is that because of this reaction is the Pen G, the particular Pen G plus water giving you 6 APA plus phenyl acetic acid and because of this you find that the PH goes down and you have to adjust the PH.

Now, if you are allow this reaction to take place to complete extent in this equipment the PH would have been very low, an of the PH, because very low the enzyme gets completely denatured. The reason that is why people take the Pen G the large equipment and then pass this through this at a very you know very low velocity, so that the shear rates are not very large.

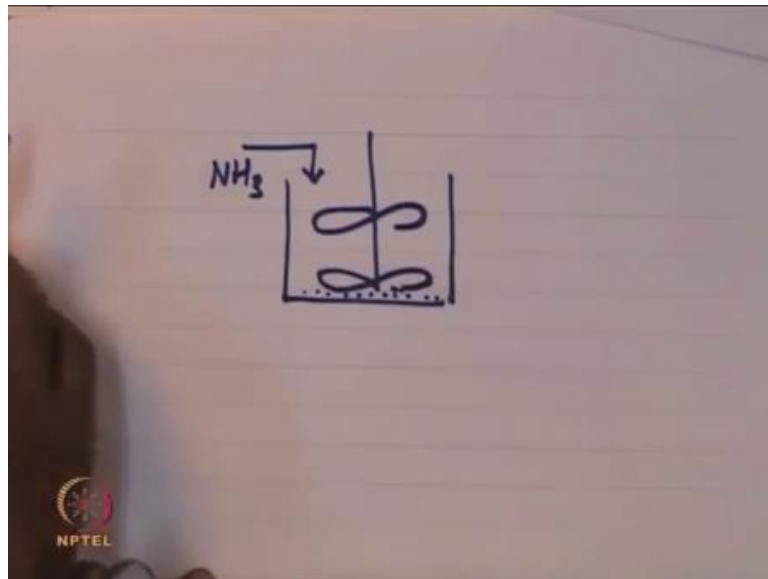
So, enzymes are very, very prezle molecule that is you are know, so if you put it a very high shear rates it denatures you are loss the enzyme costs are high. So, it is it is done appropriately low shear rates and therefore, the residence time so adjusted that the

amount of reaction is also very small per pass the conversions are very small. So, the PH changes are also very small which you adjust you continue this for a long time typically Pen G converts 6APA. A process in many industry, it will take 8 to 10 hours per batch. So, it is done very slowly.

Pen G is a fermentation product it is comes to anti biotic factories you hydrolyze this to make what is called a six amino penicillanic acid. This material is one of the very important pharmaceutical intermediate it goes a variety of semi synthetic penicillin's ampicillins are good example ampicillins made using 6APAas a raw material.

Now, in this hydrolysis you get phenylacetic acid as a product as a result of phenylacetic acid the PH becomes lower and lower as a reaction proceeds. And therefore in the reacting environment the PH is no longer 7.8 that is a appropriately desirable for the process, how do you ensure that this PH is maintained? One way is that you do not have this, you put the enzyme in a basket, I will draw it here there is also there in the industry.

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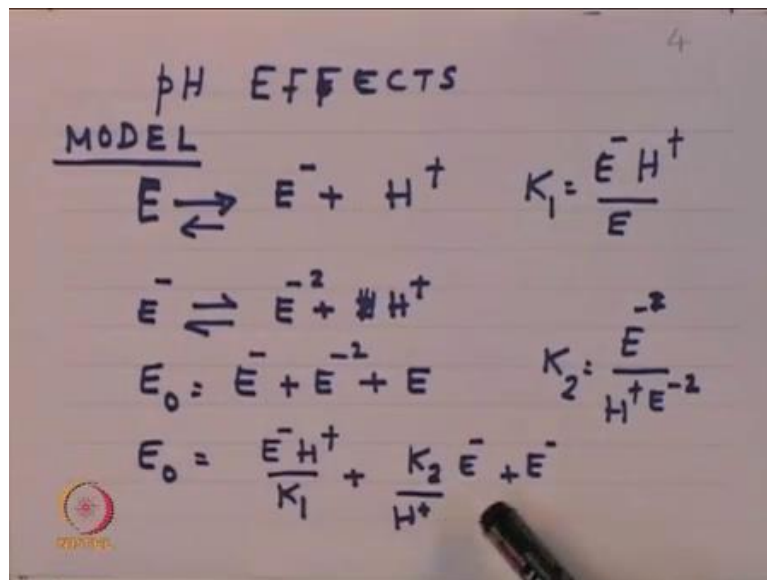
See in the industry, you also see this you have a batch equipment and very carefully designed stirrer in a this stirrer you know it is and then PH adjustment reactor is a batch reactor where you put ammonia add to adjust the PH. But here, what you have to do is that? You have to keep the agitation and then this enzyme at be sitting some like here and it is be done very carefully, most experience of process is that it is not easy to do it and therefore, people have thought of doing like this.

So, here the shear rates are lower than the shear rates you get here, that is why this is preferred, it is not that even this is available you will see it an industry also.

So, this whole thing takes about 6 to 8 hours, sometimes 10 hours, and then at the end of which you have 6 8PA and phenylacetic acid, which you said it for recovery, so this is their first problem that I had said for you take home. Therefore, you have to this residence time, if it is tau P and this residence time if it is tau m this is very large compare to this. Therefore, this CA if I call this is CA 1 and c a call this is CA 2, you can try it this CA 2 equal to CA one times exponential minus of K tau P, this is many not be a bad assumption.

Then, you write a material balance for this you can get the answers, so the learning aspects of this of this exercise is that in a commercial process where enzymes are very, very sensitive, this is a preferred configuration. And it how long you should run this reactor will come from solution of this along with the material balance solutions for this which you know how to write.

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See mentioned about PH f x last time, let just draw attention to this PH f x what I have saying is that enzyme ionizes and it ionizes further. And therefore, you have an E minus which is the active form of the enzyme and E minus 2 which is not an active form of the enzyme, and of course analyzed which is also not an active form of the enzyme; so based on the equilibrium for E minus and E minus 2.

(Refer Slide Time: 11:42)

The image shows a handwritten derivation on a slide. At the top, the total enzyme concentration e_0 is given as $e_0 = e^- \left[1 + \frac{H^+}{K_1} + \frac{K_2}{H^+} \right]$. Below this, the fraction of free enzyme $y = \frac{e^-}{e_0}$ is derived as $y = \frac{1}{\left[1 + \frac{H^+}{K_1} + \frac{K_2}{H^+} \right]}$. At the bottom, the Michaelis-Menten equation is written as $(V_0)_m = k_3 e^- = k_3 y e_0 = k_3 y e_0$. A small logo is visible in the bottom left corner of the slide.

We have set up the final form, it is last time this is the final form which says that depending on the PH on the PH you will find the this ratio is affected. Therefore, the maximum velocity that you will observe will depend upon what is the extent to which the ionization gives you the value of y . So, this maxim velocity is affected by PH, so choice of PH ensures that you are operating at the highest velocity.

And, if you look at some of the enzymes it is available in the market this is very very sharp, I mean in the sense that is almost switch in the sense are if it is suppose to be at 7.8, if you are operating at 7.9 or 7.7, you would lose considerable amount of activity. This is like a switch it sort of it sort of collapses, but becomes very active at that particular enzyme. So, it is important to have very good PH regulation, you will find in all these processes good PH control is a part of the process requirements.

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$$v_m = k_3 e_0 \left[\frac{1}{1 + \frac{H^+}{K_1} + \frac{K_2}{H^+}} \right]$$

$$r_p = f[V_M, K_M, S]$$

Highest value of U_M at $H^+ = \sqrt{K_1 K_2}$

When we said all these things you also said that the best choice of PH is $K_1 K_2$, once you know K_1 and K_2 , we know what is the best weight run the process with this.

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Q6. Multiple steady states in bioreactors

Data below is obtained from a CSTR of volume 1000 lit. and feed enters at 3.2 lits.

Q6.1. Show that the data given is consistent with substrate inhibition model of enzyme kinetics.

S	4	8	1	1	20	2	2	32	3	40	4	48	52
r	0	0	0	0	0	0	0	0	0	0	0	0	0
s	1	1	1	0	07	0	0	05	0	04	0	03	03
	7	4	1	9	5	7	6	5	5	5	4	7	5

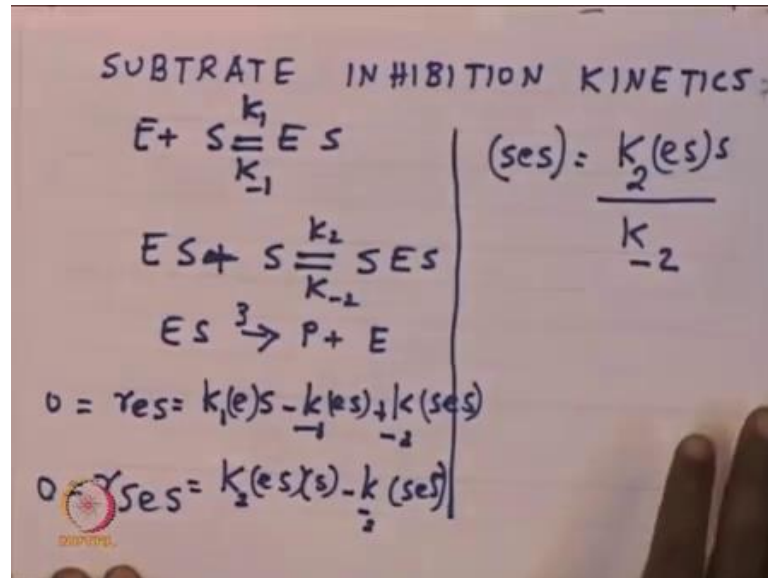
S mmol/L r s mmol/Lit.min Taken from P7- 14 Scott Fogler Elements of Chemical Reaction Engineering Prentice Hall 1986

Q6.2. If the reaction is carried out in a CSTR of volume 1000 lit. and feed enters at 3.2 lits. Find all the steady states. if possible specify which are stable. So = 50 mmol/L

Let us just go to this problem, so this is an interesting problem where substrate inhibition actually affects the process. So, we want to quickly understand, how so we get the form of the kinetic expression for substrate inhibition. So, these some data I have taken from Fogler's book 1986, and these are some kinetic data S is given r s is given and the values of S by different concentration and reaction rates at different concentrations are all given.

Notice that, the reaction rate goes up very high value and then start to come down, so you can somewhere here, the reaction rates takes the highest value and then starts to decrease afterwards 0.017 and it is starts to decrease.

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So, what the substrate inhibition model, see we said this and I was doing it in a hurry perhaps last times, I have done it again. So, you have r_s I mean this is the complex r_s and r_{ses} , so the model as is mention here, that enzyme complex it with substrate and then ES complex I mean complexes with substrate to give you this in active SES complex, this how the active sides of blocked, and this is the substrate inhibition model which is very popular you will find this in many many places there is many examples I have taken today also.

So, r_s equal to 0, r_{ses} equal to 0, then from this equation you can eliminate and find ses in terms of es and s . So, it is now very easy to find out what is the rate function I have done this.

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$$\begin{aligned}
 r_{es} &= 0 \\
 0 &= k_1 (e_0 - es - ses) - \frac{k_2}{k_1} (es) - k_3 (es) \\
 es \left\{ k_1 s + \frac{k_2}{k_1} + k_3 \right\} &= k_1 e_0 - \frac{k_1 k_2 (es)(s)^2}{k_2} \\
 es \left\{ k_1 s + \frac{k_2}{k_1} + k_3 + \frac{k_1 k_2 s^2}{k_2} \right\} &= k_1 e_0 s
 \end{aligned}$$

And it looks I mean you do all this manipulations and all that it. Finally looks something like this.

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$$\begin{aligned}
 r_p &= k_3 es \\
 &= \frac{k_3 k_1 e_0 s}{\left(k_1 s + \frac{k_2}{k_1} + k_3 + \frac{k_1 k_2 s^2}{k_2} \right)} \\
 r_p &= \frac{V_M s}{\left(K_2 + s + \frac{s^2}{K_1} \right)}
 \end{aligned}$$

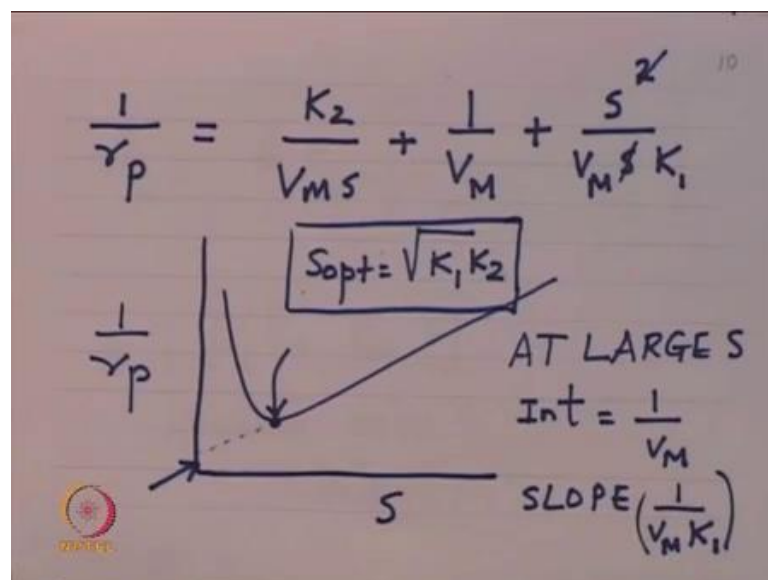
$$\begin{aligned}
 K_2 &= \frac{(k_2 + k_3)}{k_1} \\
 K_1 &= \frac{k_2}{k_1} \\
 V_M &= k_3 e_0
 \end{aligned}$$

So, the rate of formation of product has V_M , what is V_M is and then K_2 and K_1 are the two constants and they are given here V_M is $k_3 e_0$ naught notice here, that as you change e_0 naught the V_M will change K_2 and K_1 are the ionization constants K_2 by K_1 , all these constants and they come from your measurements on reaction rates.

Now, what is given here is r_p is given in this problem r_p is given, and you are asked to calculate what is the first part of the exercise is check whether it is constant with substrate inhibition kinetic model that is first part of the exercise. The second part of the exercise is that that it is actually you do get multiple steady states in these cases. So, you will have to see how many steady states you get, and of course you have to suppose to check whether there are stable or not and so on. So, you know how to do all that.

So, let us first see how best we can see whether it fits the multiple, if fits the substrate inhibition model.

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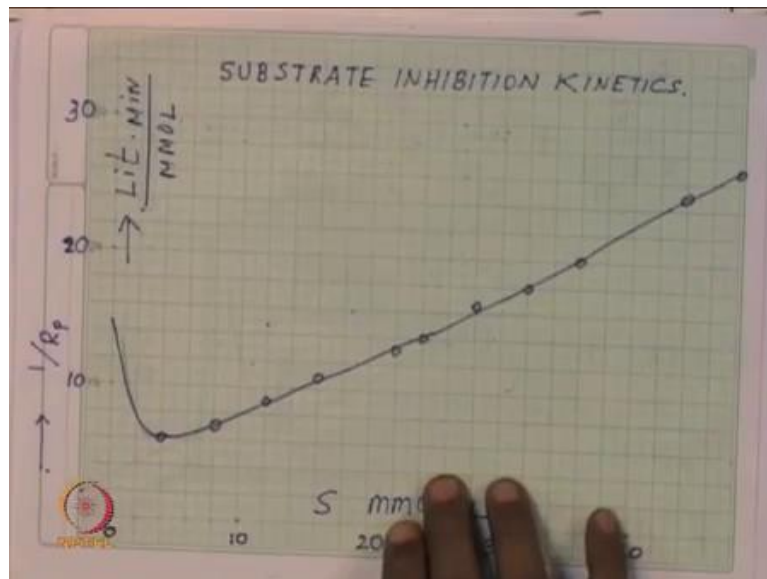
How do we do this? to do this, what you do is the following, you have to suppose you do this; that means, you do an inverse transformation you get one by r_p have you done. $1/r_p$ equal to $K_2/V_M S + 1/V_M + S^2/K_I$, this is fine. So, you do a linearization, so $1/r_p$ becomes $K_2/V_M S + 1/V_M + S^2/K_I$ by all these.

Now, notice here when S is very large, of course we do not know how large is large, only experiment will tell us, at large S the first term becomes unimportant, at large S first terms becomes unimportant. Therefore, if you plot this data as $1/r_p$ versus S , and then if you get something like this which we can extrapolate to 0, then slope intercept will give you the parameters correct you understand if what we are saying is right.

If you make $1/r_p$ versus S , and then if you get a behavior like this, then you can extrapolate this line and then this intercept what is this intercept value $1/V_M$ or in other words if our data is consistent with this kind of description only we can say this, and what is the slope? This is the slope V_M/K_1 , $1/V_M K_1$.

Now, what is this point, point of maximum reaction rate, therefore $S_{optimum}$ is root of $K_1 K_2$, so this also you know from the data correct, you understand what I am saying. So, you have you have three parameters and you have 3 numbers from where you can calculate, the three parameters is that clear can you quickly plot and then see how it looks like please all of you, I have plotted this.

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See you see here, see from 0 it goes to look at the data, it is 0 r_s is 0 goes to 0, 17, 0.17 it goes up and then comes down. So, the maximize somewhere very close to see actually there was some points I could not fit it in this data there are some more points which is 0 and 4 in Fogler's book, I have not taken it, this is clear.

Now, you plot this quickly please hurry up should not take too much time is a very very simple problem we have able to do it quickly $1/r_p$. So, minus actually this is minus of r_s , it is not r_s minus of r_s . See what I have written here is plus r_s that is not correct, minus of r_s is that clear,

It is rate of formation of product, so what is being said is that the rate at which substrate consume is the rate of formation of product under the quasi steady state of approximation that we have already proved. So, r_p equal to minus of r_s under the assumption that $r_e = 0$ that we have already proved is that is that clear it is a good question.

See under the quasi steady state approximation r_p equal to minus of r_s this is something that we have proved already. So, 1 by r_p we can write down all the numbers 0.17, see even if you point plot 2 3 points I would suggest that you know you plot few because you take point these around region and then extrapolate also you will get reasonably good answers you do not have to plot every things plots 4 or 5 points that is good enough. So, for example $c_0 = 4$ 0.5 is 20, so this is 1 point here, and inverse 0.004 is 25, so you have 2 points there actually you can just joint them, and extrapolate that will pretty good is that clear. So, take few points here, so it 0.4.

See this is 0.5 is 1 by 0.5 is 20 is 1 by 0.4 is 25 and 1 by 0.35 is about 33, 32 whatever. So, those point if you joint directly to 0 give you the intercept can you read out the numbers please you plot 1 by r_p versus S remember that r_p equal to minus of r_s under the quasi steady state approximation there is minus of r_s that minus sign is missing any numbers. I get $V_M S = 0.25$.

Intercept is I have taken intercept is 4, this 4 is what I have taken, this is line when I taken 4, this looking any answers what is a takes along V_M intercept is how much I have taken it as 4, no problem what is V_M inverse of that is V_M fine. I have got 0.25 very good what is K_1 slop is find 5.45 we have except all that, so what is K_1 I have got 8. So, V_M is 0.25 and K_1 is 8 is what I have got some of you got slightly different numbers that does not matter.

Now 0.25 is what I have taken and now the S optimum is 4 root of $K_1 K_2$ is 4 correct, root of $K_1 K_2$ is 4, you can see here, the highest reaction rate is somewhere here, so that gives K_2 as 2 K_1 is 8 K_2 is 2 and V_M is 0.25 this is what I have got.

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$$S_{opt} = \sqrt{K_1 K_2}$$

$$4 = \sqrt{K_1 K_2}$$

$$K_1 K_2 = 16$$

$$K_1 = 8 \text{ mmol/L}; K_2 = 2 \text{ mmol/L}$$

$$V_M = 0.25 \text{ mmol/L} \cdot \text{min}$$

So, my numbers are V_M 0.25 millimole per liter per minute these are the numbers. So, what I have got to top I have got as 0.5p fine, that is these are not. So, all the parameters are shall go forward. So, parameters I will read out I will get K_1 is 8 K_2 is 2 and V_M is 0.25 this is what I get.

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$$FS_0 - FS + r_S V = 0$$

$$F(S_0 - S) = -r_S V$$

$\underbrace{F(S_0 - S)}_{P(S)} = - \underbrace{r_S V}_{Q(S)}$

And this next part, if you want to do is you have $CSTR$, this is and this rate law applies which means r_S , so this rate law applies this is all right what we have saying. So, let us write the material balance $F S_0$ naught minus of $F S$ plus r_S times V is 0 or f into S

naught minus of S equal minus of r S times V this is I call this as P I call this as Q and both are functions of substrate concentration.

Q versus s has been plotted already just plot P versus S can you plot P versus S, so you have to plot this function F into S naught minus of S, F is given as 3.2 S naught is also given 50. So, you can plot this plot this and find out how many steady states you get see you have to plot Q versus S you already this data is given you have to plot and P versus S it is this function F into S naught minus of S, you have to plot points of intersection are the steady state, the equality points of intersection are the steady state.

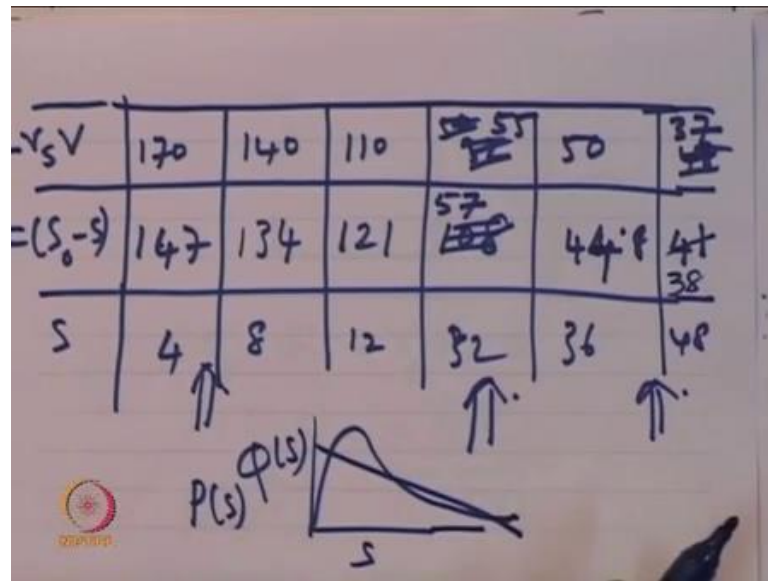
So, please plot and tell me hurry up is this clear what we are saying yes or no what we are saying is that this is material balance this is material balance F of S naught F of S r S V equal to 0 yes or no, this is this data is given already I call it as Q that Q versus S is already plotted you know you have to plot and find, but P versus S is what is this function has to be plotted. So, the equality is points of intersection wherever they equal is the steady states.

So, you find out plot and tell me how many steady states please plot Q versus S the data is actually given Q is actually r S minus of r S, so you plot that and then you plot this function F of S naught minus of S is a straight line F of S naught minus of S is a straight line F is 3.2 and V is 1000, V is given, V is 1000. You have to plot Q which is minus of r S V versus S; that means, you have to multiply r s by 1000, and then plot is that clear that is a right hand side. The left hand side is F is 3.2 S naught is 50 minus of S, so it is slop it is got a negative slop take 2 points in joint that it is a best just take 2 points in joint.

Same graph you want to see point of intersection I see you are not able to plot is it same on the same can you not plot what is a problem choose the different scale what is it mater? I say choose a different scale it is minus r s I told you negative sing is missing choose a different scale on the same plot please or other alternative is make a table and you can see from the table itself that might me even faster just make a table.

Now, you can read out the number I will write here.

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So, I will make two tables all right minus $r S V$ and then F times S naught minus of S and this is S . So, this read out a few numbers that see is they I will just write this number itself it is 170 140 110 this is at 4 8 12 some later while you let us take some 48 50.

And, all that you do not want too many no 48 and 32 please tell me sorry 32 and 48 and 36 tell me the numbers 32 is 50 no or 55 and 36 is 50 and 48 is 37 is it all right F of S naught minus of S 3.250. So, at 4 46 multiplied by 3.2 is how much just two points will do 1 47 and three point 2 multiplied by 14 will do 448 please plot. Now, at is you write down plotting may be difficult write down all the numbers, fill in all these numbers please what should I have write here 134 and then 121 how much 57.

Now, can we say why are the answers are somewhere here I think one answer is here, we get three answers, three steady states will come once you plotting the problem is best, three steady states will come what are the answers here, how much? 41. So, there is some answers somewhere here also 50 to 37, 44 to 41, so it will cross. So, similarly there is an answer here also somewhere is that clear, so when you plot you will get three answers since you do not do not have graph sheets today you are not able to plot it properly. So, this something that you can plot.

So, what is the highest conversion we will get highest conversion comes from where corresponding the lowest value of S somewhere here it 38 is it any way, so there is one answer here is it clear. All of you, when you plot it you will get three steady states three

point of intersection when you plotting Q versus S and then P versus S, and they will intersect is that clear they will intersect and you will get three steady states. So, you can do it at home, but the principle is what we have illustrated here and you can do this at home.

So, what we are trying to get across here is that substrate inhibition is quite common in biology and therefore, if you running a chemo stat by chemo stat we mean c s t r, you are likely to have problems of multiple steady states. When you are multiple steady states you have the issue of stability of those steady state we have done all that. You can determine from your stability analysis which of these steady states are stable that you will do at home and give it to me.

We go forward now answers we will not bother about answers now you can do it at home. Let us go forward see what we are trying to do in this exercise.

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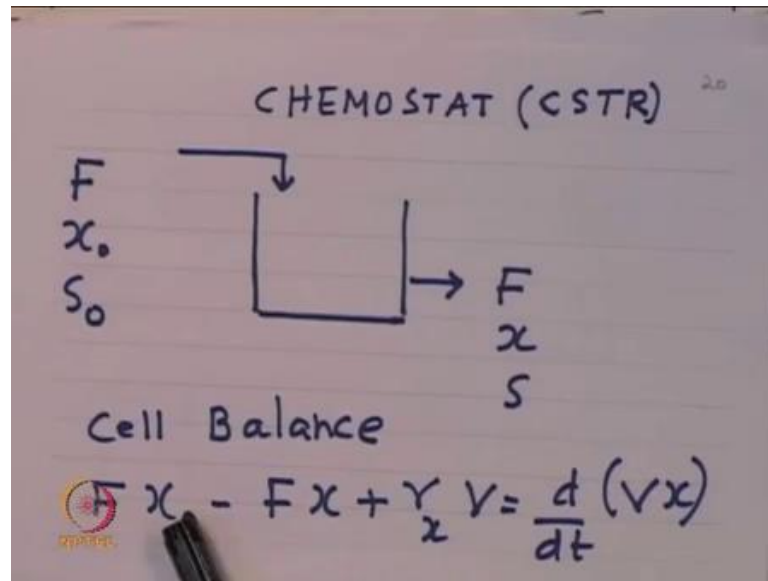
Q1 Waste Water Treatment;

6 million lit/d sewage of a campus of 20000 population water with COD of 200 mg/L is to be treated. The growth parameters are $\mu_m = 0.3/\text{day}$ and $K_s = 10 \text{ mg/L}$. River discharge norms desire near primary water quality for drinking purposes of COD less than 10 mg/L. Estimate the size of the vessel needed. Take B =

NIPTE
Q2 Natural Selection

We have taken an exercise 20000 there is population of 20000 and 6 million liters of water has to be treated, these numbers are something similar to what happens in the IIT campus. So, I put some numbers, so that we want to find out what is the size of the equipment that we require. So, this is the exercise we want to look at.

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So, let me quickly explain to you what is the fundamental that you have to understand. So, what is it that we have we have a chemo stat what is the chemo stat chemo stat is c s t r where feed comes in continuously feed goes out continuously, and because of the of the process that take place in this equipment. This S naught which is a pollution level comes down to S.

Now, we have various kinds of specifications on what is the value of S should be, for example, if you look at waste waters of communities say Bombay city for example, this S naught in Bombay city be around 600 to 650 milligrams per liter measured as oxygen demand, what is oxygen demand? It is the milligrams of oxygen that is required to oxidize the waste material to carbon dioxide and water. So, if it is carbohydrate like glucose $C_6H_{12}O_6$ you put oxygen it becomes carbon dioxide and water, how much is the oxygen requires is the stoichiometry will tell you

Now, what it says is that in Bombay city it is typically 600 milligrams per liter or more, but if you look at campus like our own where the consumption of water is quite large our concentrations are much less. Because, we consume a lot of water in our daily use compare to what happens to average citizen in the city of Bombay, but even then these 200 is considered quite large.

Because, what we are suppose to put into the environment is a near drinking water quality I mean this is what you would like to do, but we are not able to do for a variety of

reasons I hope fully in days to come we will have better and better technologies which people can afford. So, that we can reduce these two near drinking water quality.

So what has been specified here is that we should get it up to 10. Now this value of 10 what the reason the number 10 is given is that if you go around the world and find out what is the specification for primary water quality for drinking purpose. Or in other words, whenever the corporation of the world goes to supply of drinking water to the community.

They will look for source where the water quality is quite good must be good water quality it should be less than 10. That means, the level of pollution in that water must be less than 10 only that source they will take they will treat this and make it 0 and then give to you is it clear this is the process.

So, what we are supposed to do is that this 200 that comes out of this campus let us say it must be May 10. Before we let it out what happened in the city of Bombay they do variety of reasons the waste water of the city goes into the I mean into the ocean untreated in Bombay city that is the status all the water of the city is pump into the sea untreated.

So, the sea in bad shape for variety of reasons the fishes that we all consume is also not in great shape and so on various kinds of problems are associated with the fact that we interfering with the natural water quality of the sea. So, how do we do this, so the question here is what is it that what should be size of this equipment? So, that it is able to come from 200 to 10 as for as let us say this campus is concerned. You have going to the corporation of Bombay they are looking at 600 or 700 they want to make it less than 10.

This is a very tall order they are not able to do it variety of reasons, but may be in course of time they will have the money to do it, do this what I have done. Here is that I have written a cell balance how much cells are coming in how much cells are going out how much cells are generated how much cells are accumulating input output generation accumulation it is. So, now, let us see what happens at steady state we knock out this term the right hand side term we knock out.

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AT SS
 $F(x_0 - x) + \mu x V = 0.$
Let $F/V = D$ (1/TIME)
DILUTION RATE
 $D(x_0 - x) + \mu x = 0$
Sterile Feed
 $x(\mu - D) = 0.$

So, this is the steady state situation of the. So, $F x$ naught minus of x plus μ times $x V$ equal to 0, it is statement of material balance. Now, what we would expect to see in this tank depending upon the technology that you employ in this tank. If it is the aeration technology that happens around the world then because of the fact there is supplying lot of oxygen here the cells would grow and cells would multiplied enormously because you are supplying oxygen plus there is lot of food available. Previously, what happened is that food available, but there was no oxygen available therefore, the cell density was very small now because of the oxygen available cells are growing.

And, typically what seems to happened in biological processes that we want the cell to be of our choice. For example, if it is penicillin we want to have only pure culture of penicillin on other words in biology you are interested in growing the cell of our interest. So, this feed stream is typically sterilize, so that x naught is 0 this is what typically happens in a process. Now, therefore, sterile feed is a common need in biological process, therefore we look at the situation for this material balance for the case of sterile feed. Even if, you look at for example waste water, where the feed in the waste water this x naught is not 0, but it is very small considering the cell density here.

So, orders of magnitude is different, so to assume that x naught is 0 even a waste water is not a bad assumption in pure culture of course you will sterilize by thermal means or if a by $u D$ radiation or whatever. So, both cases whether it is waste water pure culture

biological process x naught equal to 0 is typically an assumption not an assumption is a requirement of the process.

So, what we have done here is that we just set F by V equal to D , this is what called a dilution rate, the word in biology word dilution rate is frequently used $1/D$ square is called dilution rate, F by V has dimensions of inverse of time, dilution rate you needs a inverse of time. So, what happens if I said X naught equal to 0 and the rate of growth is μx , so we end of this algebra equation X multiplied by μ minus of D equal to 0. Our cell balance leads to this algebra equation X multiplied by μ minus of D equal to 0.

Now, after all we are spending money constructing you knows all these equipments because you want to grow the cell correct. So, we are interested in not trivial solution to the problem we are not or X equal to 0 is really not of our interest may be look at it later, but as per the movement our interest is to grow the cell therefore, X naught equal to 0 or non trivial solution is what we are looking for is it.

(Refer Slide Time: 38:14)

The image shows a handwritten derivation on a slide. It starts with the equation $x(\mu - D) = 0$. Below it, it states $x \neq 0$, which leads to $\mu = D$. The Monod equation is then written as $\frac{\mu_m S}{K_s + S} = D$. Finally, the substrate concentration S is solved for, resulting in $S = \frac{DK_s}{\mu_m - D}$.

So, our non trivial solution is this when x is not 0 μ equal to D is our solution is this all right what we are saying when x naught equal to 0, the solution to this algebra equation is μ equal to D .

Now, we said that μ is described by this kind of Monod's model of which we talk about last time also, that μ is μ_m times S by K_s plus S , there varies other model this

is simplest form of the micro growth model, that it is available in the literature. So, we say that this μ must be equal to $\mu_m S$ by K_S plus S . Therefore, our steady state is describe by this equality, this is the equality which describe the steady state or the extent to which our substrate get consume from S_{naught} . It becomes S which is given by $D K_S$ by $d \mu_m$ minus of D just solving this.

So, what have we done what we have done is that we have an equipment in which S_{naught} K_S mean S went out and therefore, this S depends on growth parameters of the cells in the equipment and those growth parameters are μ_m and K_S , therefore the output concentration is given by D times K_S by μ_m minus of D . In other words if you chose the dilution rate at which you will operate then you can specify the extent to which you can clean the water is this clear simply choose dilution rate then you can determine the extent to which you can clean the water is it.

Now, what seems to happen? In biological process I mean if you got to any waste treatment plant they will tell you they listen you see my organisms have got washed out. This is the frequent complaint we will make you go to the plant you said plant is not running, you ask there, why it is not running? Our plant got washed out or in other words washout is a fairly serious problem in biological process, which is operating on a continuous mode.

And therefore, we have to protect the process against to washout therefore, we have to first understand what is washout then only we can protect let us look at what is the meaning of wash out meaning of washout is that you started your process with S_{naught} and what came out is also S_{naught} no treatment took place process the everything got washed out therefore, you put S_{naught} came out therefore, S_{naught} went into the sea or into the river whatever. Why did it happen? The answer is if you put S equal to S_{naught} here it will tell you the dilution rate at which wash out occurs correct.

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Handwritten equations on a slide:

$$D = \frac{F}{V}$$

$$S = \frac{DK_S}{\mu_m - D}$$

$$D_W = \frac{\mu_m S_0}{K_S + S_0}$$

$$Dx = DY \left[S_0 - \frac{DK_S}{\mu_m - D} \right] \quad x = Y \left[S_0 - \frac{DK_S}{\mu_m - D} \right]$$

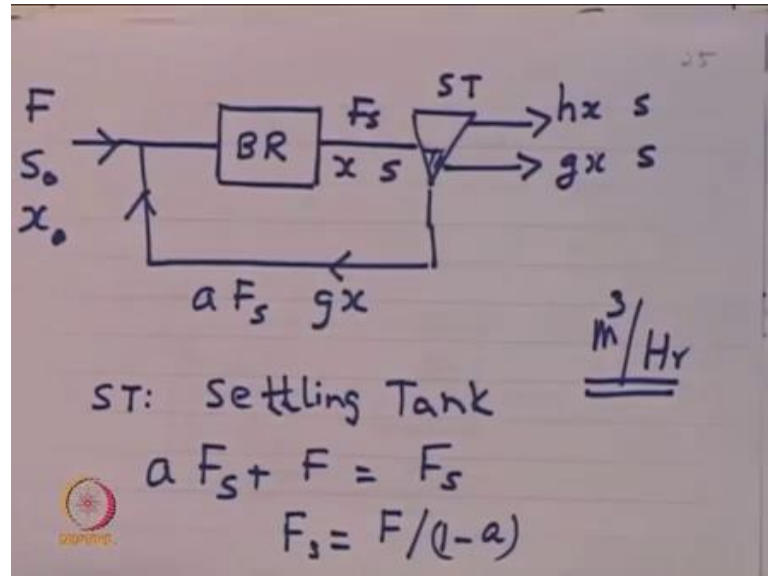
Let us do that put S equal to S naught here, so what is the washout dilution rate. So, what is D_W equal to $\mu_m S$ naught K_S plus S naught. So, what we are saying is that if you are given the Monod parameter and if you lose the concentrate in at which your waste water has coming, you can immediately tell dilution rate at which the washout will occur which means what to protect your plant against washout you should not a love the dilution rate to become as highest this. So, what is dilution, what is D_W ? D is simply F by V . The question is why does this dilution rate becomes as highest D_W is becomes very high, because if it is waste water treatment you find that between night and day there is great difference in consumption of water.

During the day the water consumption is f during the night the water consumption may be 0.2 or 0.3 F , you see therefore there is a great difference in the amount of water that flows into the plant during the day compare to during the night. Therefore, this variation if you have not properly accounted for in design you will find that the dilution rate during the day will be so large that your system will get washed out.

So, how do you protect your plant against washout? People say that, you know you build a huge tank it is called as the you know holding tank. So, large that you know even if there is great variation this tank is able to hold enough water, so that this does not affect your process. But if you are building a plant for the city of Bombay where we consume

3000 million liter per day, you cannot build a holding tank correct is not possible to build a holding tank we have to do something else, let us see what we can do.

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So, what is done is the following, you have a bioreactor then you have settling tank what is this tank? This is a settling tank, what settling tank do? You get settle layer which is got much higher cell density and clear water while the cell density is low correct or you can put a centrifuge here you can put a centrifuge.

So, that you get a very thick microbial biomass which you can recycle, and then clear water you can dispose you understand. This is what is done in the process industry you put a settling tank because they that cheaper and etcetera there are some places while the use centrifuge also. So, you have a settling tank which gives you dense biomass somewhere here somewhere here you will get dense biomass and part of that you put it back into the process is this clear.

Now, we want to see, how the effect of this recycle? how does it help us? what does it do? We would like we want to know so for that I have written a cell balance, what is this balance? See F is coming in at S naught and x naught, what goes out of the bioreactor F S x and S and after the settling tank what goes out is h x and g x and what recycled is a times F S and g x. I mean this is way of trying to represent, what our process is all about is that clear, this is the process that after the bioreactor whatever is the coming out a

fraction of that you recycle and in this settling tank the settle sludge which is got dense biomass part of that you recycle is that clear.

Now, what I have first written is a balance a times F S a times F S plus F equal to F S can I write this balance acceptable F is volumetric flow F is meter cubed per hour. Now, this volume balance is acceptable, if the density change is not significant volume balances are acceptable if density changes are not very significant in biology this cell here might be of the order of about 0.2 percent this is the kind of cell density here generally.

So, this is not essentially water we can say, so this is not great differences cell density therefore, we accept this volume balance as for as waste treatment is concerned. So, there if you get F S is F by 1 minus of a is it. Now let see how it helps us, so if done the cell balance in some detail.

(Refer Slide Time: 45:43)

The image shows a handwritten derivation on a slide titled "cell Balance". The equations are as follows:

$$F x_0 + a F_S g x - F_S x + \mu x V = 0$$

$$\frac{a g F x}{(1-a)} - \frac{F x}{1-a} + \mu x V = 0$$

$$\left[\mu - \left(\frac{1-a g}{1-a} \right) D \right] x = 0$$

Now, so what is the cell balance please look at this bioreactor some by here, this is the bioreactor you can see input F it has no cells have it knocked it off output is F S times, this a F S times g x input, output is F S times x plus mu x V equal to 0 is it, I will go through it.

Once again see this settling tank essentially separates a water into two fractions. One is a concentrated cell fraction which is g x, a light fraction which is h x, so this concentrated

fraction we recycle. So, how much we recycle fraction of F S? We recycle is that is the process clear to all of you.

So, this material balance now we can see here input I will knocked it off and there is input coming from here I have written it as a F S times g x, so output is F S times x and then the rate of generation cell equal to 0 is it. So, let us go forward now, so if we simplify what do you get a g F x F of x. So, this simplify like this do you all agree with this, so this things simplify like this, mu x common and then I have knocked out divided by V got dilution rate here f S has I have written as F by 1 minus of a is it, so all right with everybody. So, cell balance gives you mu minus of 1 minus of a g by 1 minus of a multiplied by D multiplied by x.

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The image shows handwritten mathematical derivations on a slide. At the top right, the number '27' is written. The main derivation consists of the following steps:

$$B = \frac{1 - ag}{1 - a}$$

$$(\mu - BD)x = 0.$$

Below this, it is noted that $x \neq 0$ and $\mu = BD$. A box is drawn around the Monod equation:

$$BD = \frac{\mu_m S}{K_s + S}$$

Finally, the substrate concentration S is given by:

$$S = \frac{BDK_s}{(\mu_m - BD)}$$

So, I have written this like this, where this 1 minus of a g by 1 minus of a I have called as B. I have called as concentration factor in biological literature B is this term 1 minus of a g by 1 minus of a I have just denoted it is B.

Now notice here, that this is all decision variables all these are decisions that u and I will take, so B is essentially in your hand you determine the value of B correct is this clear to all of us you will determine the value of B. Therefore, our material balance process our material balance now becomes this cell balance is mu times B d multiplied by x equal to 0.

Previously, we had only D , μ minus of d equal to now we have term B multiplied. Now, what is the value of B ? What is our estimate? What is B less than 1 greater than 1? What is it V is less than 1? Why is it less than 1? a is less than 1, g is greater than 1 is that clear, g is greater than 1, a is less than 1, that is why 1 minus of a g by 1 minus of a is less than 1 is it all right we agree.

So, B is less than 1, μ minus of $B D$ times x is 0, if $V = 1$ non trivial solution, because after all we are spending money to growth the cells anyway. Therefore, μ equal to $B D$ is our solution, therefore D this equality gives us the value of S is given by this relationship.

Previously, we had $D = K_S$ and $\mu = \mu_m$ actually previously this B was not there, on other words what happens is that the dilution rate that we had previously calculated that gets modified because of the recycle that is what it means. Now B is less than 1, therefore the value of S is given by this relationship is this with all of us.

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$$D_W = ? \quad S = S_0$$

$$B D_W = \frac{\mu_m S_0}{K_S + S_0}$$

$$D_W = \frac{1}{B} \left[\frac{\mu_m S_0}{K_S + S_0} \right]$$

Now, what is D ? if I ask you, what is D_W from here, if I ask you, what is D_W washout dilution rate what will you tell me? It is 1 by $B \mu_m S_0$ naught by K_S plus S_0 naught yes or no, what is the value of B less than 1. What have we done by choosing the B appropriately we have protected our system against washout is that clear? What we are saying, now we do not have to build a huge I mean equalization tank it is not possible when the when the flows are very large 100 200 500 million liters per day building a

storage tank is not possible. But this is possible you understand you can choose B appropriately to protect yourself from washout less than 1 yes, which means what that you have protected it against washout.

Let me, previously let us say between night and day the values of f change by 3 times you choose B appropriately. So, that D W becomes 5 times the average flow, so that does not get washed out is it. Now, let us see, what is the effect of this on the productivity for cell.

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Handwritten equations on a whiteboard:

$$\text{Cell Productivity}$$

$$P = \left[Fx_0 - aF_S g x + F_S x \right] / V$$

$$P = \left[\frac{-agFx}{1-a} + \frac{Fx}{1-a} \right] / V$$

$$Dx \left[\frac{1-ag}{1-a} \right] = \underline{BDx}$$

Let us do cell productivity, what is cell productivity? can I say this is cell productivity input output, so much is produced, so much is coming in divided by volume, what is this term? I put a plus here minus here. So, it becomes 1 minus of a g by 1 minus of a, so this becomes B D x this is all right please tell me, productivity for cell is B D x; that means, in the recycle scenario the because B is less than 1, the amount of cell that is produced for unit volume per unit tank is less.

So, what we done we have lost in terms of productivity, but gained in terms of washout dilution rate or on other words what we compromise see whenever we have a problem we compromise we gain at some cost this is the cost and this is the gain. This is the gain and this is the cost is that clear, what we are saying is it, what we are saying that, what we have ensure is that, productivity we have a sacrifice and to safe guard the system against washout is this clear all of us.

Now, let us look at this problem now quickly all the data is given tell me what is the size of equipment, that is required for let us say a campus like IIT. So, IIT campus see I told you know we are we generate lot of waste water means consumption also very very large, we are 6 is more than 6 million now is much much more than 6 million. So, 6 million liters 20000 population and so on please do calculation quickly.

So, we have to look at answer is this, what is s required? 10, what is B ? here is point 3, K S is given, everything is given yes or no. So, we can calculate, what is the dilution rate at which we must operate? yes or no, tell me what is the answer, what is the dilution rate at which we must operate S is 10, B is given K , everything is given or you can calculate D from this expression is easy and easier is easier to calculate from here.

We can calculate from here μ m S is given K S plus S divided by so D is simply 1 by V times. So, this is what this is 10 10 20, so 0.3 by 10, 0.1 5 divided by 0.3, so 0.5, so shall we say that dilution rate is 0.5 per day. So, what is the volume flow? 6 million means what 6000 cubic meters per day. So, what is the volume of the equipment? V equal to F by V is D , therefore, V equal to F divided by d F is 6000 D is 0.5, therefore it is 12000 cubic meter, so we need an equipment volume of 12000 cubic meters is this clear.

So, you will see in waste treatment, the equipment sizes are very large, the reason why it is very large because our reaction rates are very small, why is reaction rate very small? It is a very small because, we want to clean it to very high level of purity, and we are operating a stirred tank which is operating at the exit concentrations. Do you appreciate this problem? Please this is the problem that we face this is part of the reason, why the causes so high, that we are not able to afford is this clear?

Because, you are operating at exit concentration of 10 milligrams per liter concentration reaction rates are very low plus we have to operate because of wash out and all that we have to recycle which makes it inverse and as the result for a 6 million liters per day plant you require a 12000 thousand cubic meters volume, 12000 thousand cubic meters volume means typically it is 1 liter deep.

So, you are talking about 1 point 2 hectares which is our foot ball field, we can see foot ball in one side gymkhana, there is foot ball field down one side of the actually the ground that is about 1 hectare. So, that is kind of size that is required and in a Bombay city you know the cost of a land is you know such that it is 1 hectare might cost 1000

crores enough, so it is cheaper to throw it away in to the sea. That is a status is unfortunate, we must find a way around it just I want to spend 1 two minutes on something else. Let me see, if I can get what I here it is.

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Q3. Alcohol via fermentation

Kinetics of alcohol production using *saccharomyces cerevisiae* can be given as

$$r_s = (-)\mu_m S \exp(-kP) / (K_s + S)$$

where $\mu_m = 0.5$ /hr , $k = 0.017$ lit/g , $K_s = 2.2$ g/L , $Y_x = 0.08$ g cell/g substrate , $Y_p = 0.4$ g alcohol/g substrate , $Y_{CO_2} = 0.48$ g CO₂ / substrate. Take $S_0 = 100$ g/L

Q3.1. Estimate time need to get 90 % conversion in a batch equipment

Q3.2. Whats the dilution rate needed for 90 conversion in a chemostat.

So, what it says see alcohol industry the context of alcohol industry is that government of India wants us to put at least 5 percent alcohol in gasoline and for a variety of reasons global warming and so on, inputs reducing inputs and so on.

So, we just some data on a alcohol fermentations that you will see around the world most of these numbers will apply to wherever you see it does not matter. It says the rate at which *saccharomyces* is able to consume the hexo substrate is given by this kind of expression where $\mu_m S$ is divided by $K_s + S$ that is the usual form of the Monod's law it is multiplied by this term which is the exponential $K P$ where K is a constant, and P is the products, P refers to alcohol.

So, what we are trying to say here is alcohol fermentation from hexo sugar that you will see around the world we will face this kind of rate function problem which they must factor in design. So, what that means, product inhibits the rated which the reaction can occur, here is an instance of product inhibiting the rated at which the reaction can occur is this clear?

Now, under this situation if you I ask you what is it that we can do from the point of view of a process design, what will you say, product is inhibiting the process is it not? Letting it go forward product accumulates in the equipment and therefore, as the reaction proceeds the rate becomes smaller and smaller if you are operating a stirred tank or chemo stat in the biological literature.

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Kinetics of alcohol production using *saccharomyces cerevisiae* can be given as

$$r_s = (-)\mu_m S \exp(-kP) / (K_s + S)$$

where $\mu_m = 0.5 \text{ /hr}$, $k = 0.017 \text{ lit/g}$, $K_s = 2.2 \text{ g/L}$, $Y_x = 0.08 \text{ g cell/g substrate}$, $Y_p = 0.4 \text{ g alcohol/g substrate}$, $Y_{co_2} = 0.48 \text{ g CO}_2 / \text{substrate}$. Take $S_0 = 100 \text{ g/L}$

Q3.1. Estimate time need to get 90 % conversion in a batch equipment

Q3.2. Whats the dilution rate needed for 90 conversion in a chemostat.

Q3.3. Whats the dilution rate needed for 90 % conversion

The output concentration if you are going let us say from some concentration 100 milligrams per liter 100 grams per liter to 10 grams per liter as an example c s t r operates at the exit concentration. Therefore, the concentration of sugar at the exit is only 10; that means, this S is 10 correct, this S is 10.

What is this P? What is the alcohol product? How much alcohol has been produced? If I ask you, what will you tell me, you have started at 100 and it says alcohol formation is 40 percent is per gram of substrate; that means, 40 percent of glucose consumed is alcohol. So, if you are gone from 100 to 10 which is 90, 90 is consume, so 40 percent of 90 is 36, so 36 grams per liter of alcohol is sitting in the broth and the effect of that 36 grams per liter is minus K multiplied by 36 grams per liter that is the effect which reduces the reaction rate is this clear.

So, this problem is what is significantly retarding the progress of alcohol industrial productions around the world. We do not know, how to handle this problem? We do not seem to know, how to handle this problem? There is a lot of history to this I will talk

about this when we meet next time there is very interesting history to this and is worth knowing this history because if some of you go into this research. This history is useful because in at least you do not make the same mistakes that our forefathers have made I will stop there.