# Bioreactors
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## Lecture – 06
## Solution to PP 2.1

Welcome to lecture 6 in this NPTEL online certification course on Bioreactors. In the last lecture, we had looked at enzyme kinetics, we derived the expression for the Michaelis-Menten enzyme kinetics and we also assigned a problem, a practice problem, 2.1. Let us solve that problem in this lecture. The problem read as; let me make it full screen here.

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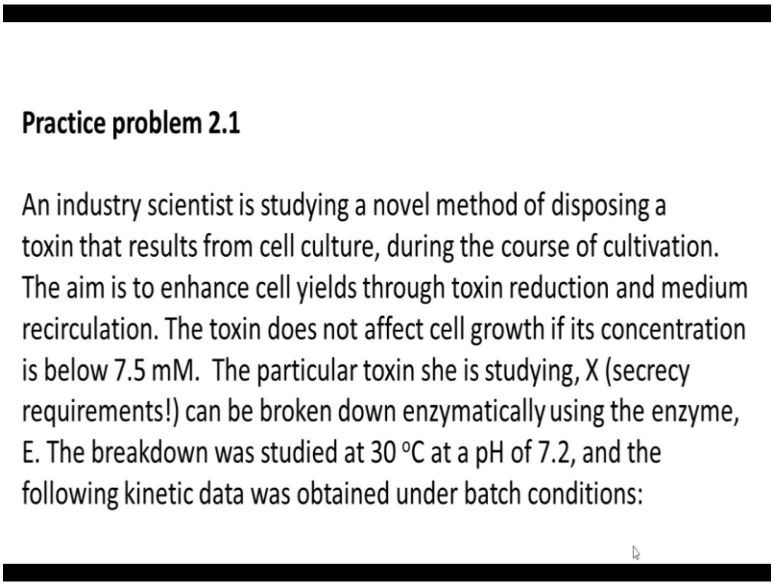

**Practice problem 2.1**

An industry scientist is studying a novel method of disposing a toxin that results from cell culture, during the course of cultivation. The aim is to enhance cell yields through toxin reduction and medium recirculation. The toxin does not affect cell growth if its concentration is below 7.5 mM. The particular toxin she is studying, X (secrecy requirements!) can be broken down enzymatically using the enzyme, E. The breakdown was studied at 30 °C at a pH of 7.2, and the following kinetic data was obtained under batch conditions:

An industry scientist is studying a novel method of disposing a toxin that results from cell culture during the course of cultivation. The aim is to enhance cell yields through toxin reduction and medium recirculation. The toxin does not affect cell growth if its concentration is below 7.5 millimolar. The particular toxin that she is studying, X can be broken down enzymatically using the enzyme, E. The breakdown was studied at 30-degree c at a p h of 7.2, and the following kinetic data was obtained under batch conditions.

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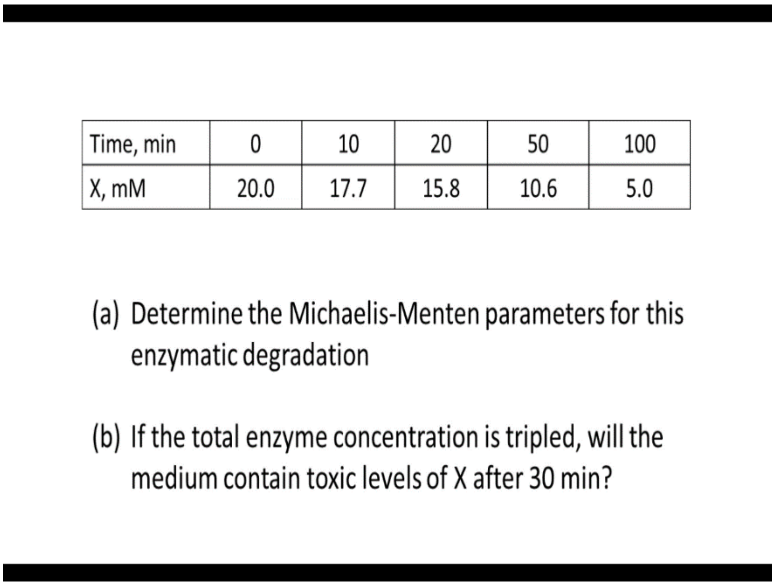

| Time, min | 0 | 10 | 20 | 50 | 100 |
|---|---|---|---|---|---|
| X, mM | 20.0 | 17.7 | 15.8 | 10.6 | 5.0 |

(a) Determine the Michaelis-Menten parameters for this enzymatic degradation

(b) If the total enzyme concentration is tripled, will the medium contain toxic levels of X after 30 min?

The variation of X with time was given. In part a; you were asked to determine the Michaelis-Menten parameters for this enzymatic degradation and part b if the total enzyme concentration is tripled, will the medium contain toxic levels of X after 30 minutes. This was the problem that was faced, so let us go and solve this problem.

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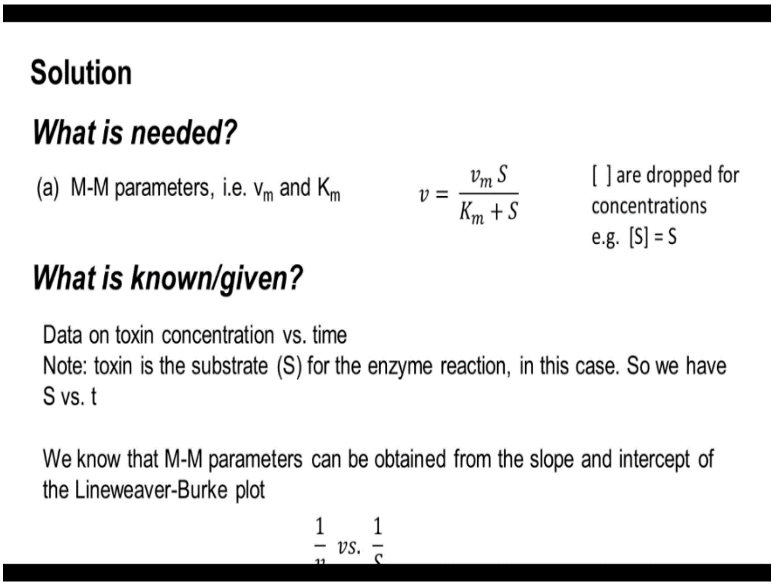

**Solution**

***What is needed?***

(a) M-M parameters, i.e. $v_m$ and $K_m$

$$v = \frac{v_m S}{K_m + S}$$

[ ] are dropped for concentrations e.g. [S] = S

***What is known/given?***

Data on toxin concentration vs. time

Note: toxin is the substrate (S) for the enzyme reaction, in this case. So we have S vs. t

We know that M-M parameters can be obtained from the slope and intercept of the Lineweaver-Burke plot

$$\frac{1}{v} \ vs. \ \frac{1}{S}$$

The first question, our usual first question, What is needed? Part a, Michaelis-Menten parameters which we know are v m and K m. In the Michaelis-Menten equation, v equals v m s by K m plus s. I have dropped this square bracket for the concentration term, in other words s without brackets, is the same as the concentration of s here, little cumbersome to write this square bracket every time, so I have dropped it. I am going to drop it from now onwards in this course. So, what is known or given? The data on toxin concentration versus time, the toxin is the substrate here. The toxin is the substrate for the enzyme reaction so we have s versus t. And we also know that the, from the lecture material in lecture 5, that the Michaelis-Menten parameters, the m m parameters can be obtained from the slope and intercept of the Lineweaver-burke plot, Lineweaver burke plot is one by v versus one by s.

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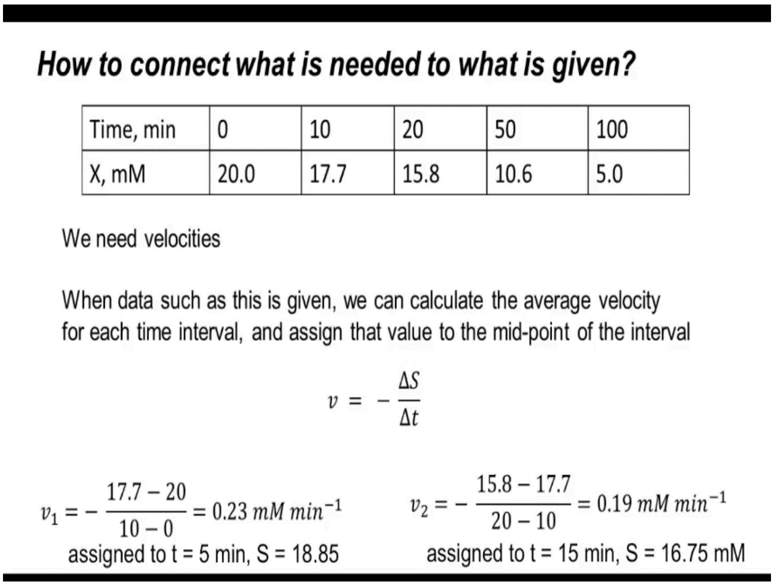

### How to connect what is needed to what is given?

| Time, min | 0 | 10 | 20 | 50 | 100 |
|---|---|---|---|---|---|
| X, mM | 20.0 | 17.7 | 15.8 | 10.6 | 5.0 |

We need velocities

When data such as this is given, we can calculate the average velocity for each time interval, and assign that value to the mid-point of the interval

$$v = -\frac{\Delta S}{\Delta t}$$

$$v_1 = -\frac{17.7 - 20}{10 - 0} = 0.23\ mM\ min^{-1}$$

assigned to t = 5 min, S = 18.85

$$v_2 = -\frac{15.8 - 17.7}{20 - 10} = 0.19\ mM\ min^{-1}$$

assigned to t = 15 min, S = 16.75 mM

Now, how to connect what is needed to what is given? We need velocities because we need to plot 1 by v versus 1 by s, for that we first need v. When we are working with such a data, we can calculate the average velocity for each time interval. In this case between 0 and 10 minutes is the first time interval, 10 to 20 minutes is the second time interval, 20 to 50 minutes is the third time interval and 50 to 100 is the fourth time interval.

We can calculate velocities as delta X divided by delta t and we have times here corresponding to the concentration, but we do not have times corresponding to the rates. What is normally done is, the rate that is calculated is assigned to the mid point of the interval. In the first case, the interval is 0 to 10 and therefore, the average is 5, we calculate the rate and assign it to the time of 5 minutes. I'll show that to you, v is minus delta s delta t

$$v = \frac{-\Delta S}{\Delta t}$$

And in this case, the first case at 10 it is 17.7 at 0, it is 20. So, 17.7 minus 20 that is delta s and delta t is 10 minus 0, so that we have there, equals, this works out to be 17.7 minus 20 is minus 2.3; minus of minus 2.3 is plus 2.3, 2.3 by 10 is 0.23. 0.23 in millimole, this is been given in millimole, millimole per minute.

$v = \frac{-(17.7-20)}{(10-0)} = 0.23\, mMmin^{-1}$ assigned to t = 5 min, S = 18.85

So we need to assign it to the mid point of 0 and 10 that we saw was 5 minutes.So, at t equals 5 minutes, the substrate concentration, if we assume that it varies linearly in this time interval, that is an assumption that we are making - which turns out to be good if we have closely spaced points. Let us see, this will be some sort an estimate here. Some points are widely spaced, but any way this is the data that we need to work with, so let us do the best that we can with the data. So, the mid point, rather the substrate concentration that corresponds to the mid point t equals 5 minutes, is 18.85, which is the average of these two and that is what we need to assign. Then v 2 is between this and this, 15.8 minus 17.7, 20 minus 10, the negative of the whole thing that turns out to be 0.19 millimole per minute.

$v = \frac{-(15.8-17.7)}{(20-10)} = 0.19\, mMmin^{-1}$ assigned to t = 15 min, S = 16.75 mM

And we need to assign it to the mid point, the, even the substrate concentration there, because we are plotting 1 by v versus 1 by s here. So, the mid point of 10 and 20 minutes is 15 minutes and the mid point, assuming that the decreases linear in this region, 17.7 and 15.8 the average is 16.75. So, t equals 15 minutes, s equals 16.75 and so on and so forth.

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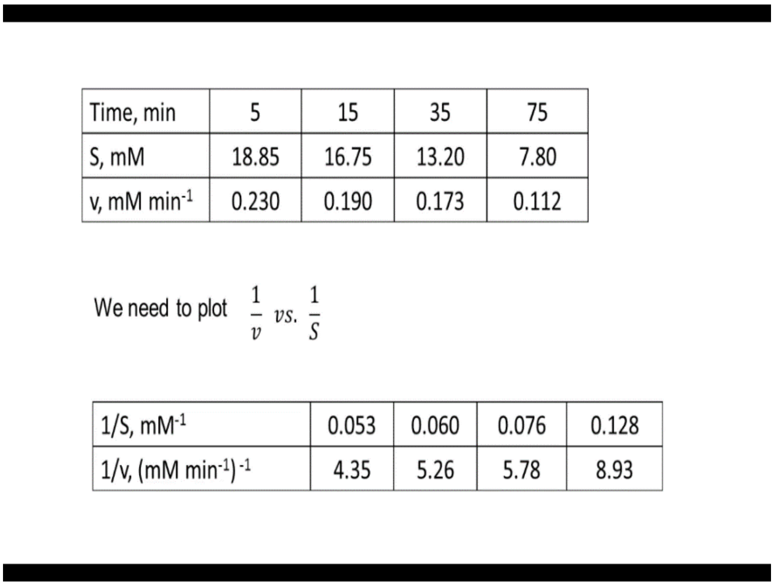

| Time, min | 5 | 15 | 35 | 75 |
|---|---|---|---|---|
| S, mM | 18.85 | 16.75 | 13.20 | 7.80 |
| v, mM min$^{-1}$ | 0.230 | 0.190 | 0.173 | 0.112 |

We need to plot $\frac{1}{v}$ $vs.$ $\frac{1}{S}$

| 1/S, mM$^{-1}$ | 0.053 | 0.060 | 0.076 | 0.128 |
|---|---|---|---|---|
| 1/v, (mM min$^{-1}$)$^{-1}$ | 4.35 | 5.26 | 5.78 | 8.93 |

To get these numbers 5, the v that we calculated was 0.23. The corresponding s at 5 minutes was 18.85, assuming linearity. At time t equals 15 minutes, s is 16.75, v equals 0.19 and the other these two calculations we had seen in detail, the same way we get these values for 35 minutes and 75 minutes, which are the mid points of those corresponding intervals, s mid points of the corresponding linear variations and v that was calculated, the average rate that was calculated in that interval.

| Time, min | 5 | 15 | 35 | 75 |
|---|---|---|---|---|
| S, mM | 18.85 | 16.75 | 13.2 | 7.8 |
| v, mM min$^{-1}$ | 0.23 | 0.19 | 0.173 | 0.112 |

Now that we have this, we need to plot 1 by v versus 1 by s. 1 by v versus 1 by s is what we need to plot. We have v and s, therefore, we need the inverse values of that. If we invert 18.85; 1 by 18.85 happens to be 0.053, you can verify the calculation, tell me if there has been a numerical error somewhere. The inverse of 16.75 is 0.06.

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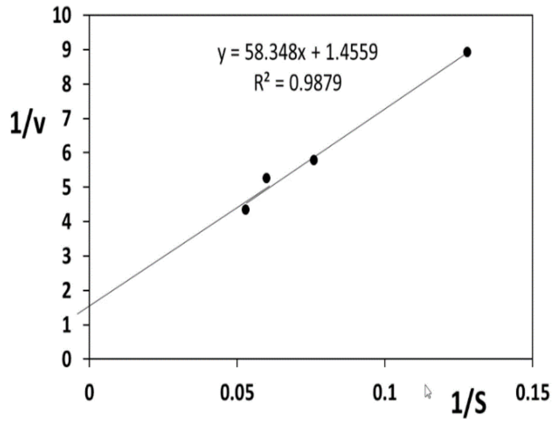

The inverse of 13.2 is 0.076, the inverse of 7.8 is 0.128 or 1 by that. Similarly, the inverses of v 1 by v is 1 by 0.23, 4.35, 1 by 0.19, 5.26; 1 by 0.173, 5.78 and 1 by 0.112, 8.93.

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|---|---|---|---|---|
| 1/v, (mM min$^{-1}$)$^{-1}$ | 4.35 | 5.26 | 5.78 | 8.93 |

Now it is best to use spread sheet to plot, you can use a graph sheet to plot, you can use a spread sheet to plot. Let me show it to you using a spread sheet. We have 1 by s, the values on this column and 1 by v, the values on this column. If we plot a graph between 1 by s and 1 by v, these are the same values that we saw in the table earlier.

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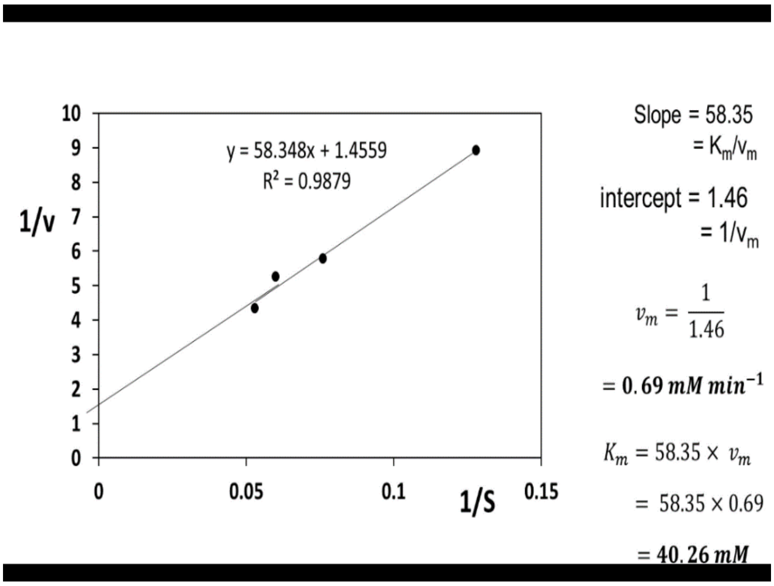


Slope = 58.35
$= K_m/v_m$

intercept = 1.46
$= 1/v_m$

$$v_m = \frac{1}{1.46}$$

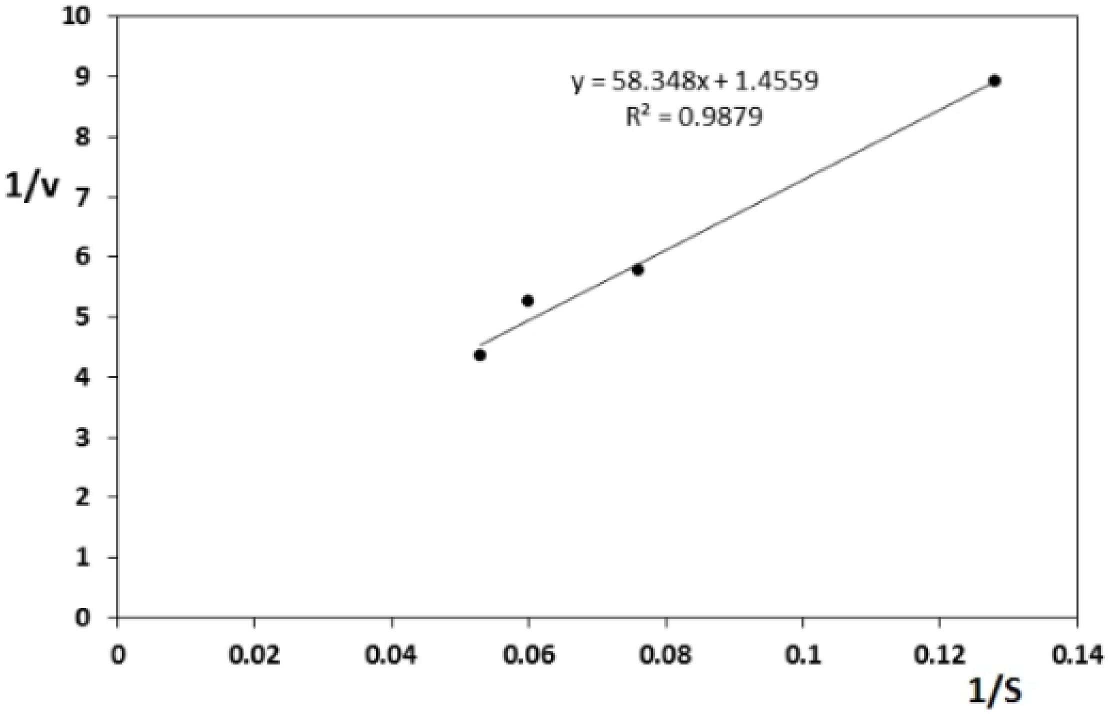
$$= \mathbf{0.69\ mM\ min^{-1}}$$

$$K_m = 58.35 \times v_m$$

$$= 58.35 \times 0.69$$

$$= \mathbf{40.26\ mM}$$

If we plot this 1 by v versus 1 by s, these are the points that we get. Now this spread sheet m s excel, allows us to use or allows us to draw a best line fit, the best line that fits to these points. This is the best line fit here with the least squares value of 0.9879 and the equation of the line which I have asked to display here is 58.348 x plus 1.4559, y is 1 by v, x is 1 by s. So, 1 by v is 58.348 into 1 by s plus 1.445.



You would have done this in your calculation in order to find that out, you could have also use a graph paper that is okay. If you do that, then you should end up here. 1 by, this is the same graph and this slope happens to be 58.35. The software would give you a large number of digits, but we need to choose the digits that are of relevance to us. Let us choose this, two digits here after the decimal point, slope is 58.35 and we know that in a Lineweaver-Burke plot, this slope equals K m by v m. Therefore, K m by v m is 58.35 and the intercept we know as 1 by v m in the Lineweaver-Burke plot that happens to be 1.46. I'm rounding of this large number of digits here to just to the two digits after the decimal point 1.46.

Slope = $\frac{K_m}{v_m} = 58.35$

Intercept = = $\frac{1}{v_m} = 1.46$

$\therefore\ v_m = 0.69\ mM\ min^{-1}$

$\therefore\ K_m = 58.35\ \times 0.69 = 40.26\ mM$

So, from this we can find out v m and k m, you know that simplest thing is the inverse of v 1 by 1 by, 1.46 would be v m and therefore, v m is 0.69 millimolar per minute and now that we know v m we can substitute at here and calculate a K m; K m happens to be 58.35 into v m, you know k m by v m is 58.35. Therefore, k m is 58.35 into v m, that turns out to be 58.35 into 0.69 or 40.26 millimolar. So, these are the Michaelis-Menten parameters v m which is 0.69 millimole per minute and k m which is 40.26, that was part a, we still have part b left.

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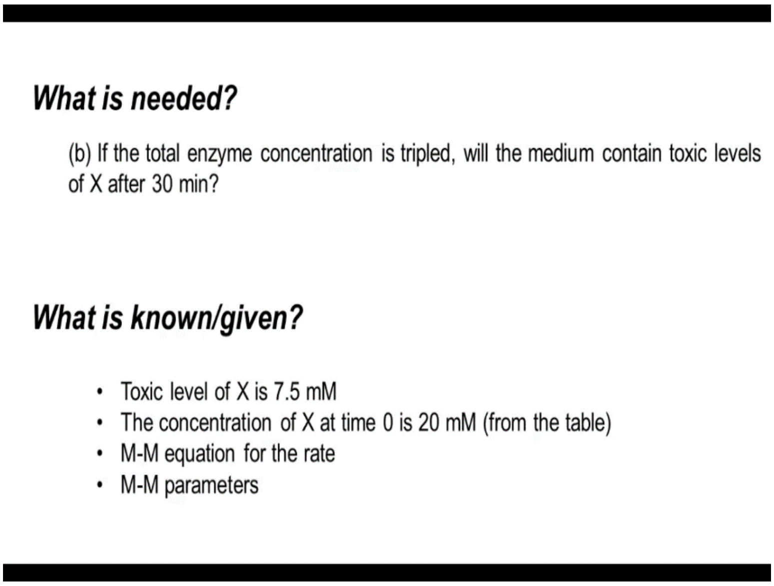

**_What is needed?_**

(b) If the total enzyme concentration is tripled, will the medium contain toxic levels of X after 30 min?

**_What is known/given?_**

- Toxic level of X is 7.5 mM
- The concentration of X at time 0 is 20 mM (from the table)
- M-M equation for the rate
- M-M parameters

Same questions for part b; what is needed? If the total enzyme concentration is tripled, will the medium contain toxic levels of X after 30 minutes, we need to find that out. What is known or given? The toxic level of X is 7.5 millimolar, the concentration of X at time 0 is 20 millimolar from the table. We know that the Michaelis-Menten equation governs the rate of this reaction, we know the Michaelis-Menten parameters from part a.

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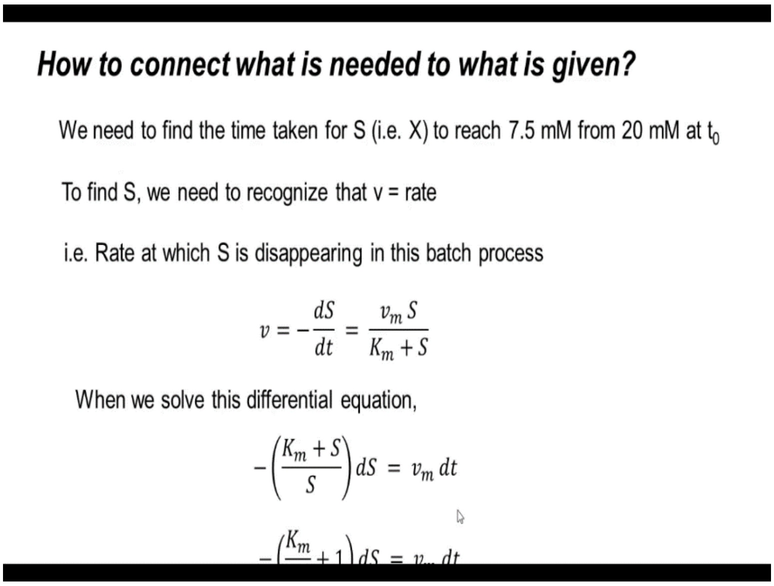

**How to connect what is needed to what is given?**

We need to find the time taken for S (i.e. X) to reach 7.5 mM from 20 mM at $t_0$

To find S, we need to recognize that v = rate

i.e. Rate at which S is disappearing in this batch process

$$v = -\frac{dS}{dt} = \frac{v_m S}{K_m + S}$$

When we solve this differential equation,

$$-\left(\frac{K_m + S}{S}\right)dS = v_m\,dt$$

$$-\left(\frac{K_m}{\phantom{S}} + 1\right)dS = v_m\,dt$$

All this we know and now we need to connect what is needed to what is given. How do we do that? We need to find the time taken for S or X or toxin is S here, toxin X is S here to reach 7.5 millimolars from 20 millimolars at initial time (Refer Time: 13:21). If that time happens to be more than 30 minutes, yes, there will be 7.5 millimolar or more when 30 minutes are up, if the time happens to be less in 30 minutes no, the medium will not contain toxic amounts. To find s we need to recognize that v is the rate, rate at which s is disappearing in this batch process, v equals minus d s d t, you know the accumulation rate of the substrate, the negative accumulation here equals v m s k m, divided by K m plus s.

$$v = -\frac{dS}{dt} = \frac{v_m S}{K_m + S}$$

If we solve this differential equation minus K m plus s by s; d s equals v m d t.

$$-(\frac{K_m + S}{S})dS = v_m dt$$

We are considering slight you know the solution of the differential equation. We need to get all the variables, appropriate variables to one side, all the s, is on this side, we have done that. K m by s plus 1, minus of that d s is v m d t.

$$-(\frac{K_m}{S} + 1)dS = v_m dt$$

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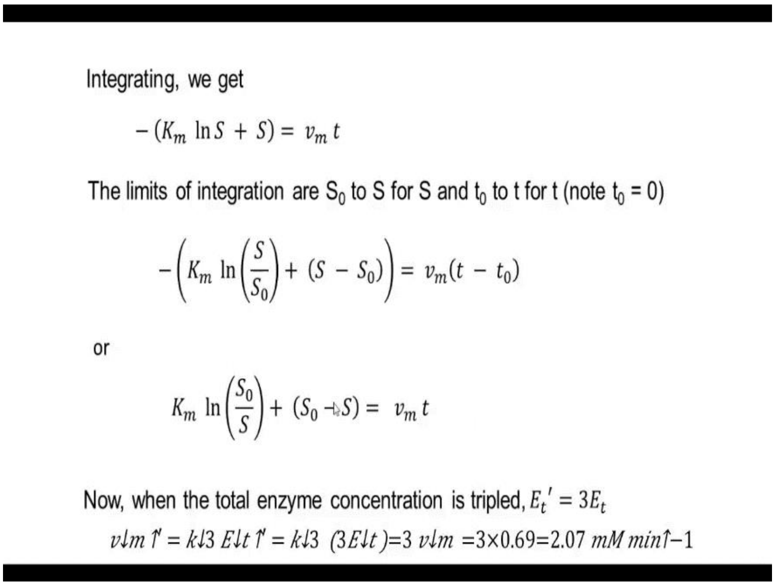

Integrating, we get

$$-(K_m \ln S + S) = v_m t$$

The limits of integration are $S_0$ to S for S and $t_0$ to t for t (note $t_0 = 0$)

$$-\left(K_m \ln\left(\frac{S}{S_0}\right) + (S - S_0)\right) = v_m(t - t_0)$$

or

$$K_m \ln\left(\frac{S_0}{S}\right) + (S_0 - S) = v_m t$$

Now, when the total enzyme concentration is tripled, $E_t{}' = 3E_t$

$v{\downarrow}m\,{\uparrow}' = k{\downarrow}3\ E{\downarrow}t\,{\uparrow}' = k{\downarrow}3\ (3E{\downarrow}t\ )=3\ v{\downarrow}m\ =3\times0.69=2.07\ mM\ min{\uparrow}{-1}$

Integrating, we get minus K m ln S plus S is v m t.

$$-(K_m\, lnS + S) = v_m t$$

And the limits of integration are $S_0$ to S for S and $t_0$ to t for t and $t_0$ = 0.Therefore, if we integrate that

$$-(K_m\, ln\frac{S}{S_0} + (S - S_0)) = v_m(t - t_0)$$

Or $K_m\, ln\frac{S_0}{S} + (S_0 - S) = v_m t$

When the total enzyme concentration has been tripled, what essentially happens? The total enzyme concentration, the new total enzyme concentration is three times the old total enzyme concentration, but what does total enzyme concentration affect? That is of relevance to us, it is the v m dash or the v m; the maximum rate.

We know that $v_m \; = \; k_3 \, [E_t]$

So $v_m{}' \; = \; k_3 \, [E_t]'$

$v_m{}' \; = \; k_3 \times 3 \times \; [E_t]$ since $[E_t]' = 3 \, [E_t]$

$\therefore v_m' \; = \; 3 \times k_3 \times \; [E_t] = 3v_m = 3 \times 0.69 = 2.07 mMmin^{-1}$

The rest is straight forward, we have the variation of substrate with time here, given here that we derived, we just have to substitute into that expression.

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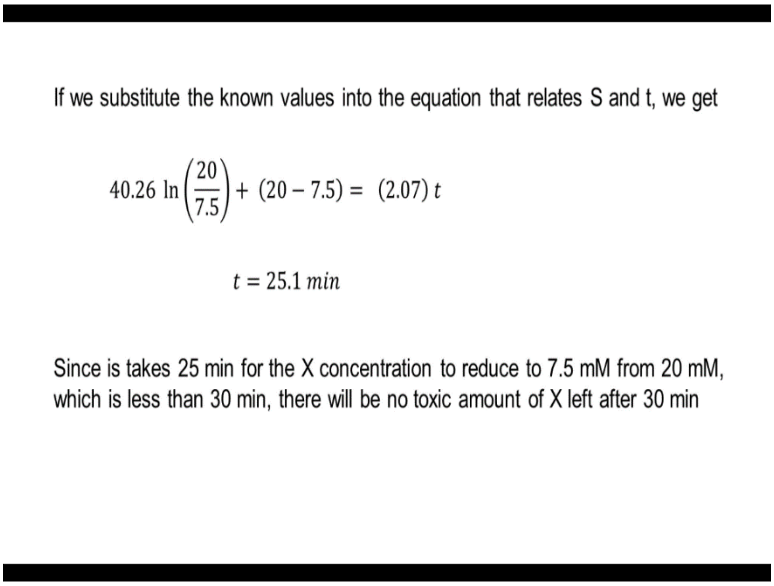

If we substitute the known values into the equation that relates S and t, we get

$$40.26 \ln\left(\frac{20}{7.5}\right) + (20 - 7.5) = (2.07)\, t$$

$$t = 25.1\, min$$

Since is takes 25 min for the X concentration to reduce to 7.5 mM from 20 mM, which is less than 30 min, there will be no toxic amount of X left after 30 min

If we substitute,

$$40.26\, ln\frac{20}{7.5} + (20 - 7.5) = \ 2.07t$$

t = 25.1 min

Thus it takes about 25 minutes for X concentration to reduce to 7.5 millimolar, starting from 20 millimolar, and 25 minutes is less than 30 minutes and therefore, at 30 minutes there will be no toxic amount of X left.