ENZYME KINETICS (Michaeli's-Menten)

$$\underset{\text{ENZYME}}{E} + \underset{\text{SUBSTRATE}}{S} \underset{k_2}{\overset{\overset{\text{FAST}}{k_1}}{\rightleftharpoons}} \underset{\text{COMPLEX}}{[ES]} \underset{\text{SLOW}}{\xrightarrow{k_3}} \underset{\substack{\text{(RECYCLED)}\\ \text{ENZYME}}}{E} + \underset{\text{PRODUCT}}{P}$$

(DEF'N OF CATALYSIS: E "comes back")

Key to problem: Focus on [ES]

$$\frac{d[ES]}{dt} = \underbrace{k_1[E][S]}_{\substack{\rightarrow\\ (+)\\ \text{FORMATION}}} - \underbrace{k_2[ES]}_{\substack{\leftarrow\\ (-)\\ \text{"BACK"}\\ \text{LOSS}}} - \underbrace{k_3[ES]}_{\substack{\rightarrow\\ (-)\\ \text{"FORWARD"}\\ \text{LOSS}}}$$

And

$$\frac{d[P]}{dt} = +k_3[ES]$$

If formation of ES is "fast" then assume equl'n [ES]: $\frac{d[ES]}{dt} \cong 0$

If $\frac{d[ES]}{dt} = 0$ then

$$k_1[E][S] = \underbrace{k_2[ES] + k_3[ES]}$$

$$k_1[ES][S] = (k_2 + \cancel{k_3})[ES]$$

SMALL COMPARED TO k_2

$$\frac{k_2}{k1} = \frac{[E][S]}{[ES]} \equiv K_M$$

AN EQUILIBRIUM CONSTANT FOR THE FORMATI OF THE ES COMP

Use MASS BALANCE: $E_T = [E] + [ES]$

substitute for [E]:

$$[ES] = \frac{E_T[S]}{K_M + [S]}$$ and since $$\frac{d[P]}{dt} = k_3[ES]$$

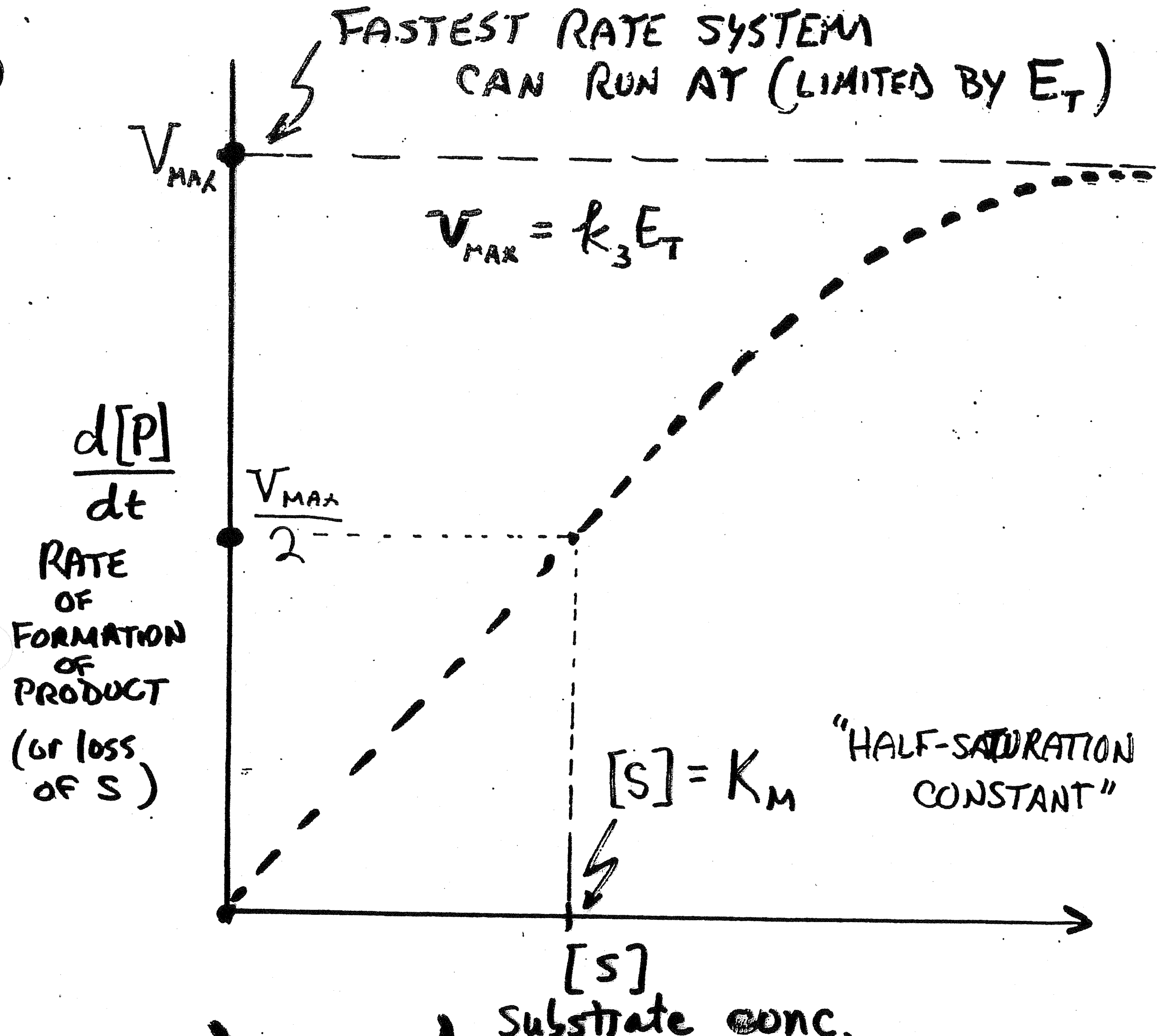
$$\frac{d[P]}{dt} = \frac{(k_3 E_T)[S]}{K_M + [S]}$$

OR

$$\frac{d[P]}{dt} = \frac{V_{max}[S]}{K_M + [S]}$$

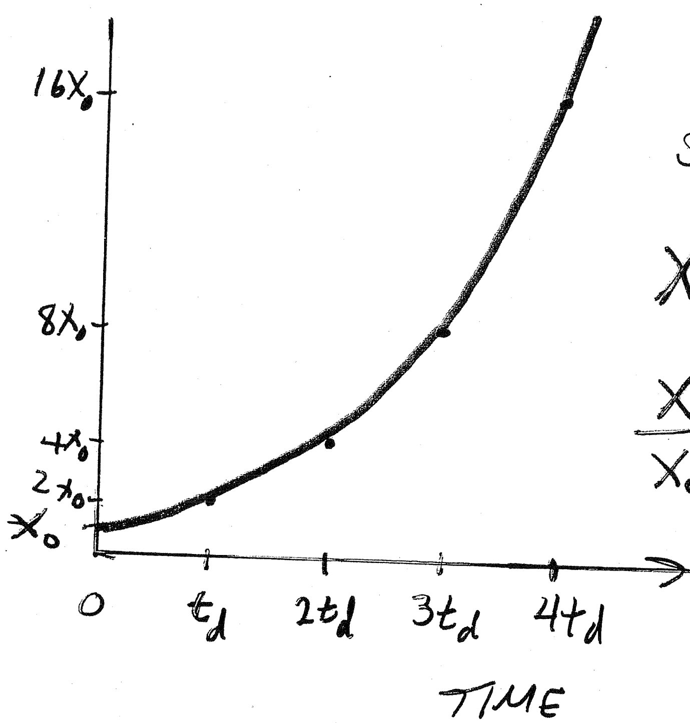
$$V \cdot \frac{c}{c + K_s}$$

MICHAELIS-MENTEN RATE EXPRESSION



$[S] \ll K_M$	$[S] \approx K_M$	$[S] \gg K_M$
$\frac{d[P]}{dt} \approx \frac{V_{MAX}}{K_M}[S]$	$\frac{d[P]}{dt} = \frac{V_{MAX}[S]}{K_M + [S]}$	$\frac{d[P]}{dt} \approx V$
RATE IS LINEAR WITH [S]	RATE IS HYPERBOLIC WITH [S]	RATE IS CONSTANT (INDEP. OF [S])

EXPONENTIAL GROWTH



$$\frac{dx}{dt} = \mu X$$

SOLUTION:

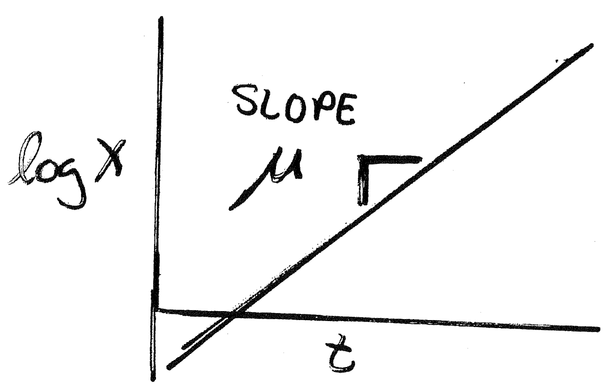
$$X = X_0 e^{\mu t}$$

$$\frac{X}{X_0} = X_0 e^{\mu t}$$

DOUBLING TIME OF POPULATION: $\frac{X}{X_0} = 2 = e^{\mu t_d}$

$$\ln 2 = \mu t_d$$

$$\boxed{t_d = \frac{\ln 2}{\mu} = \frac{0.69}{\mu}}$$



DEATH: Assume linear proportion of population

NET GROWTH: $\frac{dx}{dt} = \mu X - kX$

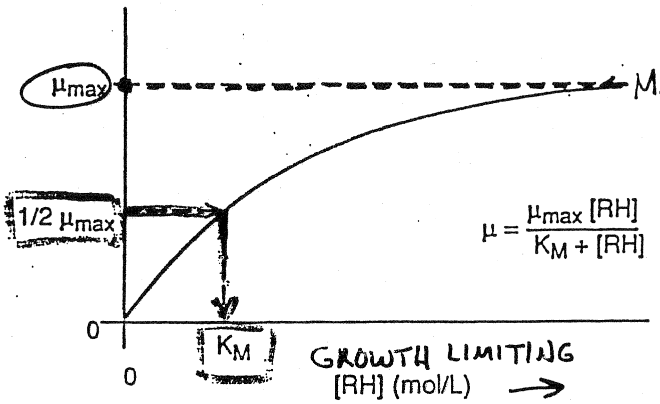
MONOD GROWTH & CORRESPONDING DECAY RATE

(a)

SPECIFIC GROWTH RATE

μ

(h^{-1})



MAX. GROWTH RATE

MONOD CELL GROWTH EQUATION

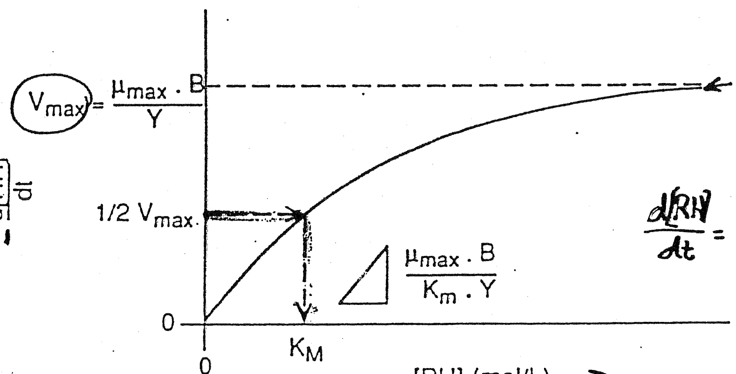
GROWTH LIMITING SUBSTRATE CONC.

["RH" just means some organic compound]

(b)

$\frac{d[RH]}{dt}$

RATE of CHANGE in [RH]



MAX. DEGRADATION RATE

$$\frac{d[RH]}{dt} = -\frac{V_{max}[RH]}{K_M + [RH]}$$

MICHAELIS-MENTEN EQN

SUBSTRATE CONC.

$[B]$ = cells/L	$\frac{d[B]}{dt} = \mu[B]$	$[B] = [B]_0 e^{\mu t}$
CONC. of CELLS	GROWTH RATE LAW	EXPONENTIAL GROWTH

$$\mu = \frac{\mu_{max}[RH]}{K_M + [RH]}$$

$$\text{Yield} \equiv Y = \frac{d[B]}{d[RH]}$$ (#cells grown / mol. substrate used)

$$\frac{d[RH]}{dt} = \frac{-1}{Y}\frac{d[B]}{dt} = -\frac{\mu}{Y}[B]$$

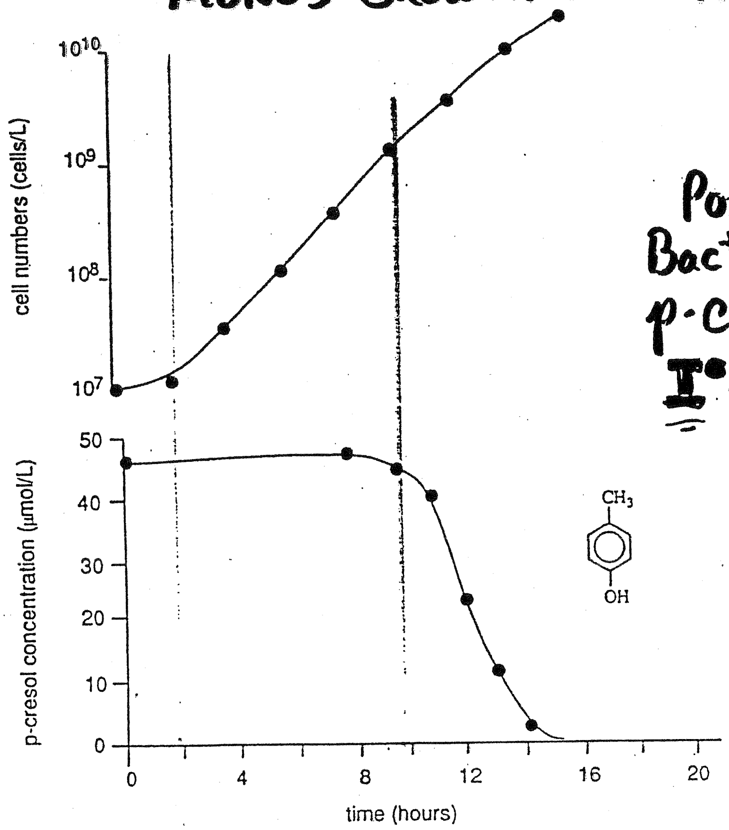
Now subst. Monod Law for μ ...

$$d[RH]/dt = -\frac{\{\mu_{max}[B]Y^{-1}\}[RH]}{K_M + [RH]}$$

MONOD GROWTH KINETICS

Cells/L (log scale)

p-cresol (μM)



Pond Bacteria using p-cresol as $\underline{\text{I}}°$-substrate

'e 14.15 Timecourses for cell numbers and *p*-cresol concentrations in a batch culture 'iment (Smith et al., 1978).

- When substrate does <u>NOT</u> limit growth: $\mu = \mu_{max}$

$$\underline{\mu_{max}} = \ln \frac{B(t_2)}{B(t_1)} \Big/ [t_2 - t_1] \quad \text{(SLOPE)}$$

$$\simeq \frac{\ln 100}{8h} \approx \underline{0.6\ h}$$

- After ~10 hr, cresol declines ~exponentially while cells keep growing expontentially, so can get Y!

$$Y = \frac{d[B]}{d[RH]} = \frac{d[B]/dt}{d[RH]/dt} = \frac{\Delta B}{\Delta RH} = \frac{B_{14h} - B_{10h}}{RH_{14} - RH_{10}} \approx \frac{(9.4E9) - (1.3E9)\ \frac{cel}{L}}{(4.4E\text{-}6) - (0.3E\text{-}6)\ M}$$

$$Y \approx 2 \times 10^{14}\ \text{cell/mol-cresol} \quad \left[\begin{array}{c}\sim 0.5 \times 10^{-12}\ \text{g/cell} \\ \text{TYPICAL CELL MASS}\end{array}\right] \Rightarrow \sim \frac{100\ \text{g-cells}}{100\ \text{g-cresol}}$$