CM 4710, Biochemical Processes **Fall 2007**
Homework #4 **Fri. 05 Oct., '07**

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1. Batch Growth of *Lactobacillus* on Glucose.

The following data were measured for the growth of *Lactobacillus* on glucose, starting with different initial substrate (Glucose (S)) at constant initial cell concentrations (X_o).

Time	Expt A		Expt B		Expt C	
(hr)	X (g/L)	S (g/L)	X (g/L)	S (g/L)	X (g/L)	S (g/L)
0	0.1	0.25	0.1	1	0.1	5
0.5	0.106	0.225	0.111	0.958	0.113	4.95
1.5	0.113	0.2	0.122	0.912	0.127	4.893
2	0.119	0.175	0.134	0.862	0.143	4.827
2.5	0.125	0.151	0.148	0.8	0.161	4.757
3	0.131	0.128	0.163	0.75	0.181	4.676
3.5	0.136	0.106	0.178	0.686	0.204	4.584
4	0.141	0.088	0.195	0.619	0.23	4.482
4.5	0.145	0.071	0.213	0.547	0.258	4.366
5	0.149	0.056	0.232	0.474	0.291	4.237

Plot each data set (A, B, and C) in an appropriate manner to determine whether any lag phase is present. From the appropriate data set, determine μ_{max}. Determine the value of K_S. Calculate the Yield over the entire batch time period for each data set.

2. Growth Parameters of *E. aerogenes* in a Chemostat.

Problem 6.7 of the text. Calculate $Y_{X/S}^{max}$ not $Y_{X/S}$

3. Production of Lactic Acid by Fermentation of *Streptococcus lactis* in a Chemostat.

Problem 6.19 of the text.

Due Fri. 12 Oct., '07

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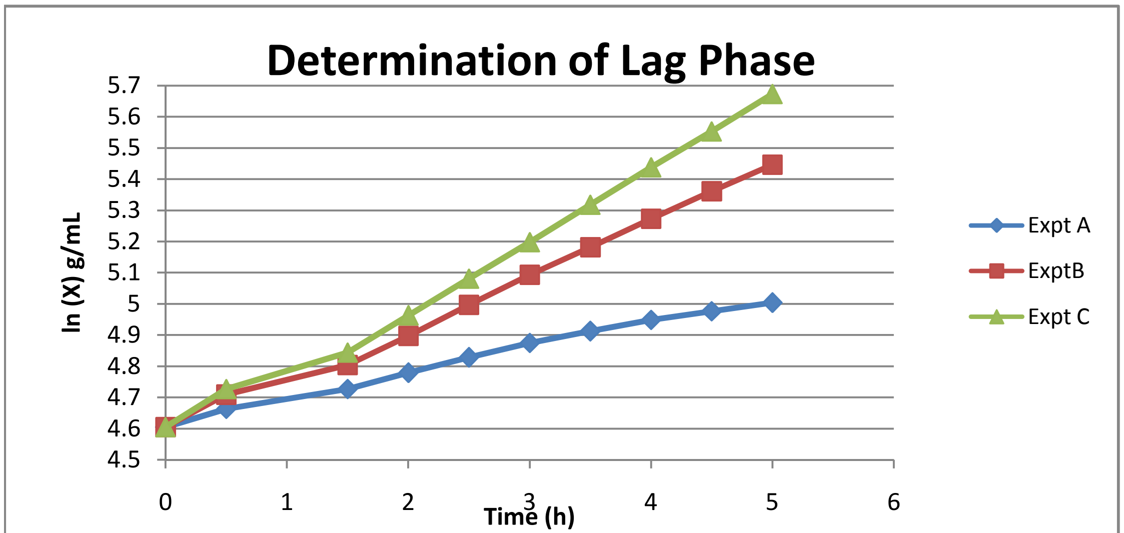
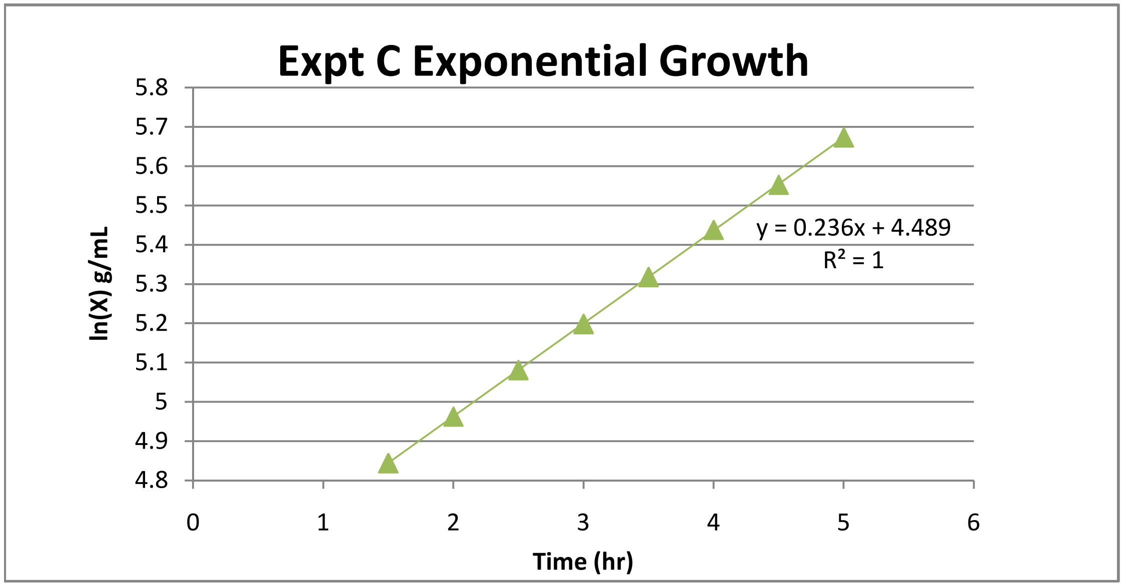
Fall 2007
Chapter 6 Homework
Problem 13 of Blanch and Clark, pg. 268
Growth of Lactobacillus (x) with Glucose (S)

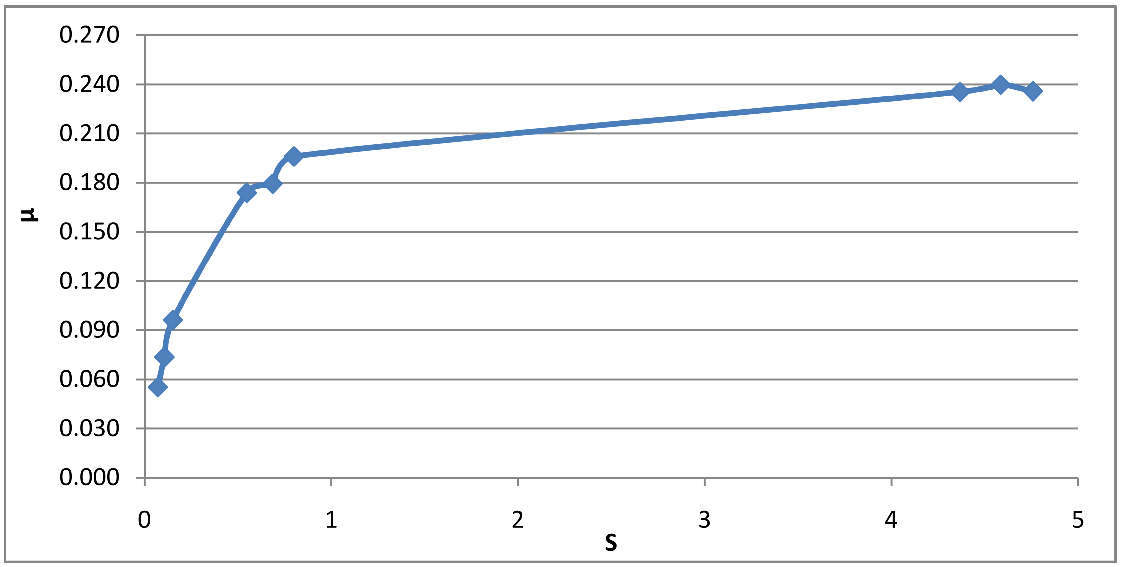
	Time	Δln(X)	Expt A			Expt B			Expt C		
	(hr)	Δt	X (g/L)	S (g/L)	ln(X) (g/mL)	X (g/L)	S (g/L)	ln(X) (g/mL)	X (g/L)	S (g/L)	ln(X) (g/mL)
	0		0.1	0.25	4.605170186	0.1	1	4.60517019	0.1	5	4.60517019
	0.5		0.106	0.225	4.663439094	0.111	0.958	4.7095302	0.113	4.95	4.72738782
	1.5		0.113	0.2	4.727387819	0.122	0.912	4.80402104	0.127	4.893	4.84418709
	2		0.119	0.175	4.779123493	0.134	0.862	4.8978398	0.143	4.827	4.96284463
point 1	2.5	0.236	0.125	0.151	4.828313737	0.148	0.8	4.99721227	0.161	4.757	5.08140436
	3		0.131	0.128	4.875197323	0.163	0.75	5.0937502	0.181	4.676	5.19849703
point 2	3.5	0.240	0.136	0.106	4.912654886	0.178	0.686	5.18178355	0.204	4.584	5.31811999
	4		0.141	0.088	4.94875989	0.195	0.619	5.27299956	0.23	4.482	5.43807931
point 3	4.5	0.235	0.145	0.071	4.976733742	0.213	0.547	5.36129217	0.258	4.366	5.55295958
	5		0.149	0.056	5.003946306	0.232	0.474	5.44673737	0.291	4.237	5.67332327



← Lag Phase is the period between the first three data points for all three expts

(also, Expt C is the only one with a linear fit.
Expt A and Expt B do not have linear fits)





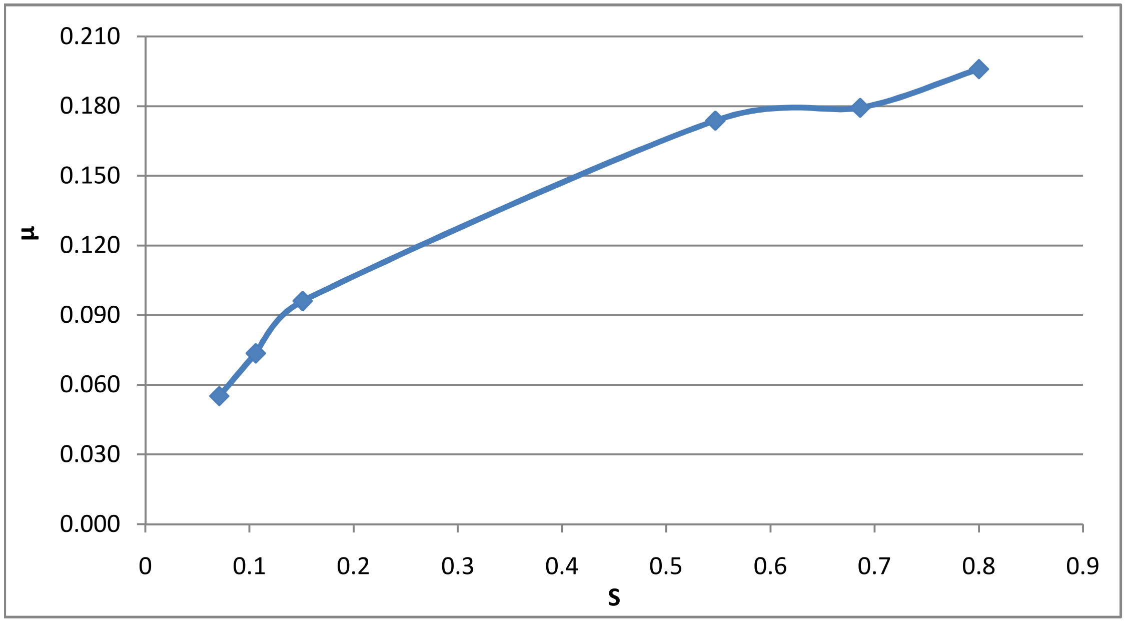
← µmax =0.24 h^{-1}

µ	S
0.055	0.071 (
0.074	0.106
0.096	0.151
0.174	0.547
0.179	0.686
0.196	0.8
0.235	4.366
0.240	4.584
0.236	4.757

when $\mu_g = 1/2\ \mu_{max}$: $K_s = S$

Yield = $-\Delta X/\Delta S$

	Expt A	Expt B	Expt C
$-\Delta X$	-0.049	-0.132	-0.191
ΔS	-0.194	-0.526	-0.763
Yield	**0.2526**	**0.2510**	**0.2503 gX/gS**



replot with only the values of

μ close to μ = 0.12 so Ks can be determined easier

Ks = 0.275 g/L

(graphical approximation)

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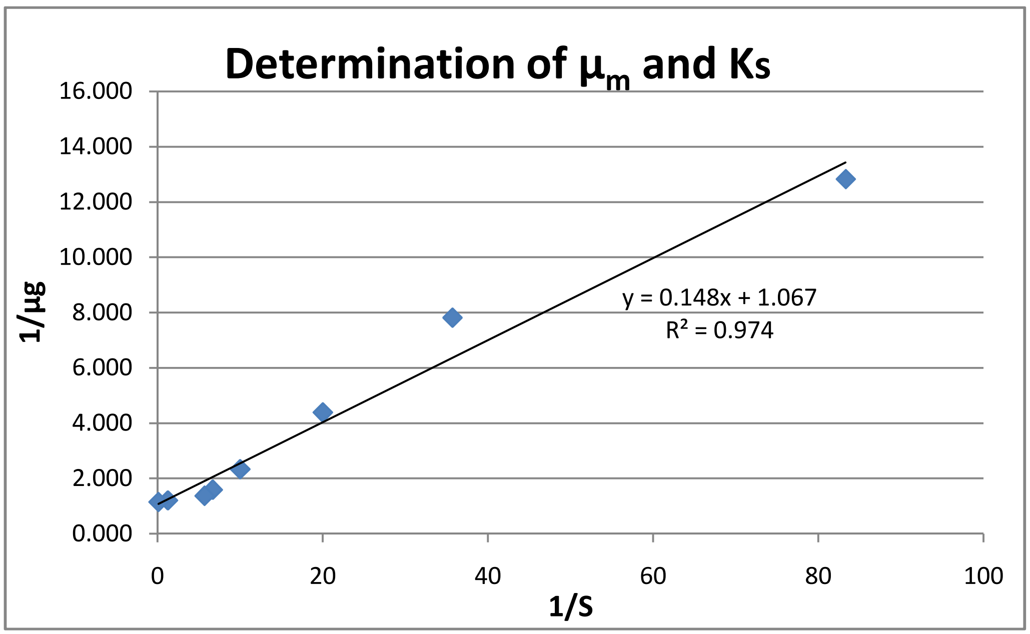
Chapter 6 Homework
Problem 6.7 of text

D, h^{-1} dilution rate	1/D h	S mg/ml glycerol	1/S ml/mg glycerol	X mg/ml cell conc.	ΔS mg/ml glycerol	ΔS/X mg/ml glycerol / mg/ml cell conc	ΔS/X*D mg/ml glycerol / mg/ml cell conc *h	kd h^{-1}	1/µg h^{-1}
0.05	20	0.012	83.3	3.2	9.988	3.12	0.156	0.028	12.826
0.1	10	0.028	35.7	3.7	9.972	2.7	0.27	0.028	7.814
0.2	5	0.05	20	4	9.95	2.49	0.498	0.028	4.387
0.4	2.5	0.1	10	4.4	9.9	2.25	0.9	0.028	2.337
0.6	1.67	0.15	6.67	4.75	9.85	2.075	1.245	0.028	1.592
0.7	1.43	0.176	5.68	4.9	9.824	2.005	1.405	0.028	1.374
0.8	1.25	0.8	1.25	4.5	9.2	2.045	1.635	0.028	1.208
0.84	1.19	9	0.11	0.5	---	---	---	0.028	1.152

Note: S_0 = 10 mg/ml



y-intercept = 1/µm = 1.067 h

µm = 0.937 h^{-1}

slope = Ks/µm = 0.236 mg glycerol*hr/ml

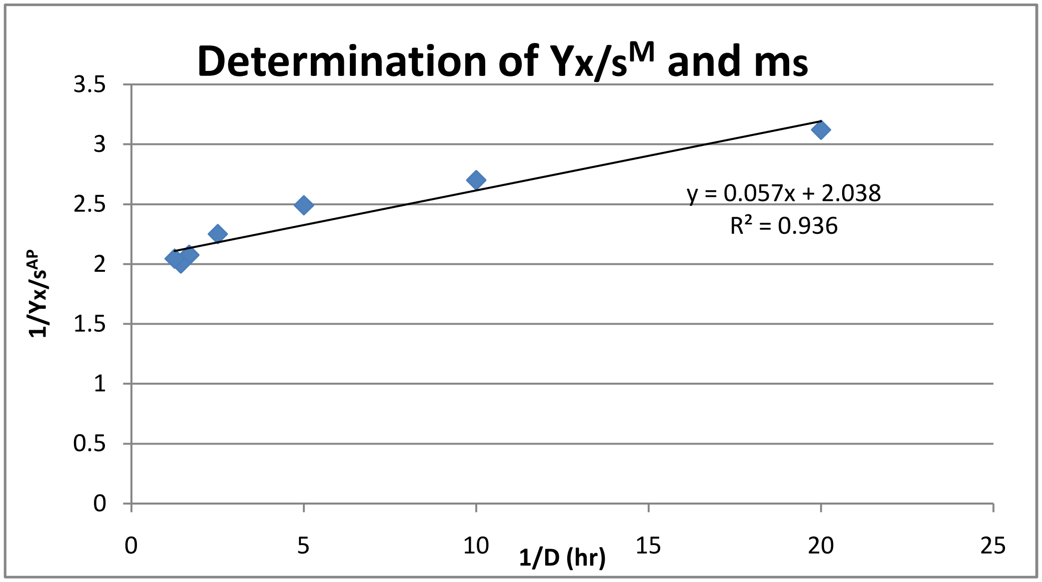
Ks = 0.221 mg glycerol/ml

Yield = -ΔX/ΔS

-ΔX = 3.2 (assuming Xo = 0)

ΔS = 9.988

Yield = 0.320 mg cells/ mg glycerol



slope = ms =	0.057	mg glycerol/mg cell-hr
ms =	0.057	mg glycerol/mg cell-hr
y-intercept = $1/(Yx/s^M)$ =	2.038	
Yx/s^M =	0.491	mg cells/mg glycerol
kd = ms*Yx/s^M =	0.028	hr^{-1}
kd =	0.028	hr^{-1}

6.7 Chemostat growth of E. aerogenes on glycerol substrate.

calc. K_s, μ_m, $Y^M_{X/S}$, and $m_s = \frac{k_d}{Y^M_{X/S}}$

c) & d)

for $k_d \neq 0$.

Cell mass balance leads to:

$$\mu = D + k_d \qquad (1)$$

Substrate mass balance leads to

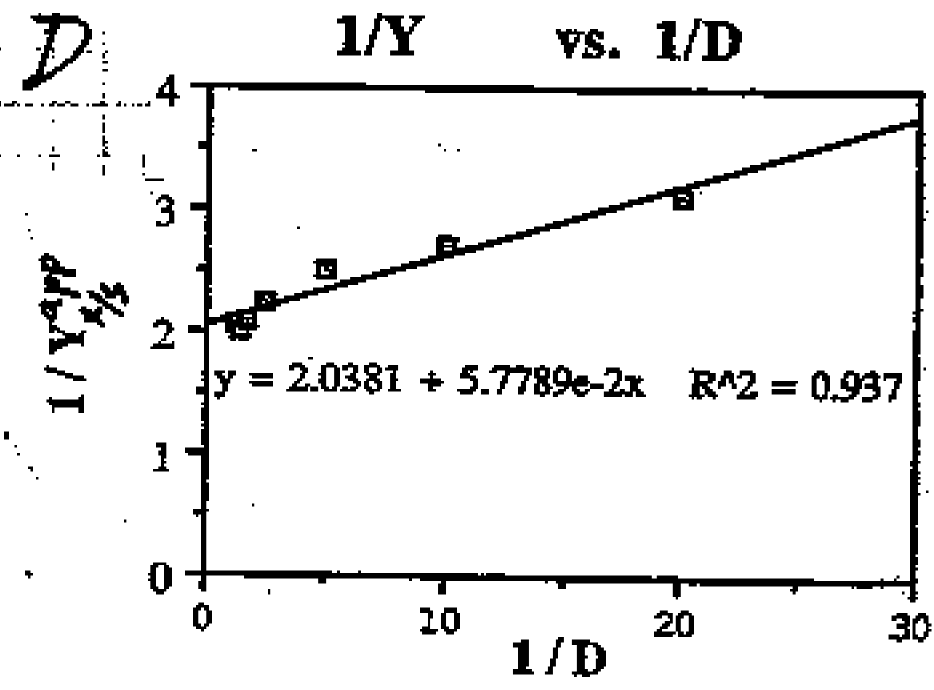
$$D(S_0 - S) = \frac{\mu X}{Y^M_{X/S}} \qquad q_p = 0 \qquad (2)$$

subst. (1) into (2).

$$D(S_0 - S) = \frac{(D + k_d) X}{Y^M_{X/S}}$$

$$\frac{(S_0 - S)}{X} = \frac{k_d}{Y^M_{X/S} D} + \frac{1}{Y^M_{X/S}}$$

$$\frac{1}{Y^{app}_{X/S}} = \frac{1}{Y^M_{X/S}} + \frac{m_s}{D}$$



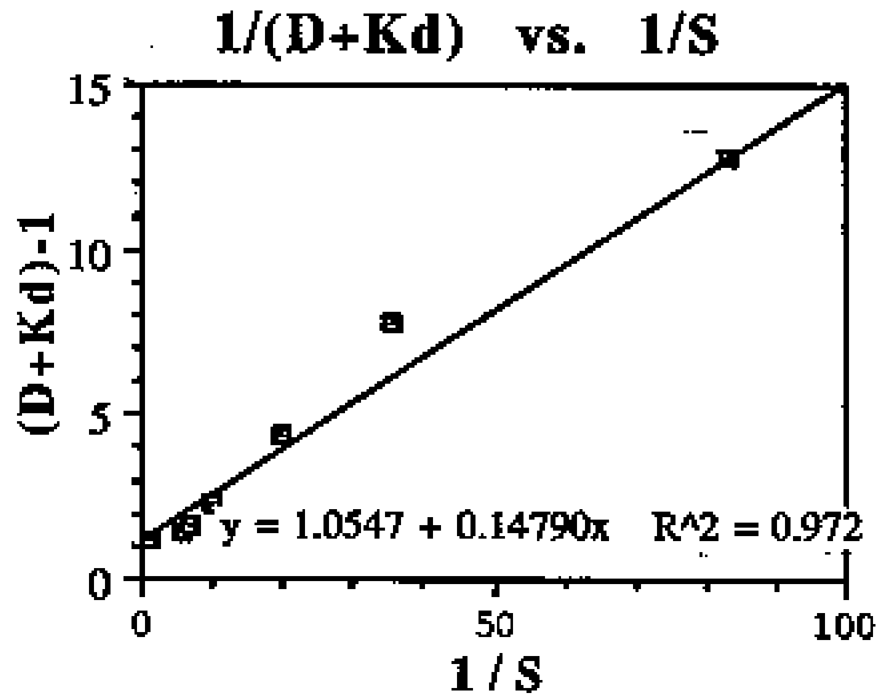
$Y^M_{X/S} = (\text{intercept})^{-1} = .491 \frac{gX}{gS}$

$m_s = \text{slope} = .0578 \frac{gS}{gX \cdot hr}$

$k_d = (.0578)(.491) = .028 h^{-1}$

Plot $\frac{1}{D+k_d}$ vs $\frac{1}{S}$.

$\frac{1}{D+k_d}$	$\frac{1}{S}$
12.76	83.3
7.79	35.7
4.38	20
2.33	10
1.59	6.67
1.37	5.68
1.21	1.25



b. $\mu_m = (\text{intercept})^{-1}$

" $= 0.95\ h^{-1}$

a. $K_s = (\text{slope})\,\mu_m = (0.148)(.95) = 0.141\ (g\ S/L)$

Problem 4

6.19

$$S = \frac{K_s (D + k_d)}{\mu_m - D - k_d}$$

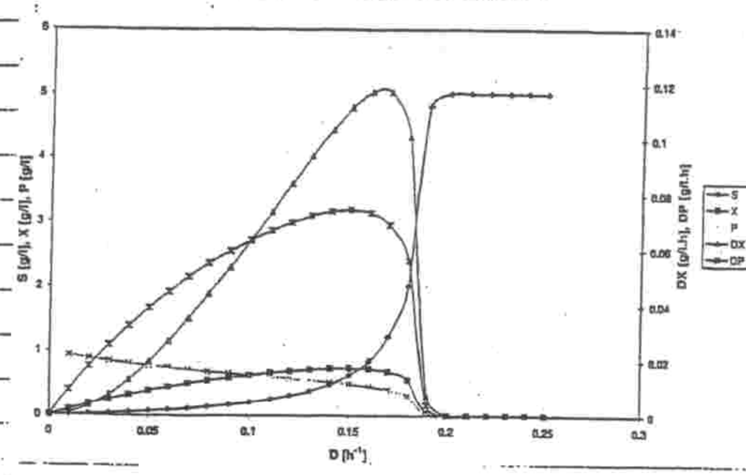
$$X = Y^M_{X/S} (S_o - S) \frac{D}{D + k_d + q_p Y^M_{X/S} / Y_{P/S}}$$

$$DP = q_p \cdot X \qquad P = \frac{q_p \cdot X}{D}$$

For $D > (\mu_m - k_d)$, $S = S_o$, $X = 0$, $P = 0$ (wash-out)

S, X, P, DX, DP as functions of dilution rate

a)



b) Maximize productivity of product (DP).

set $\frac{d(DP)}{dD} = 0 \Rightarrow \frac{dX}{dD} = 0$

$$X = Y^M_{X/S} \cdot \left(S_o - \frac{K_s (D + k_d)}{\mu_m - D - k_d} \right) \cdot \frac{D}{D + k_d + q_p (Y^M_{X/S} / Y_{P/S})}$$

$$\frac{dX}{dD} = Y^M_{X/S} \left[\frac{D}{D + k_d + q_p (Y^M_{X/S} / Y_{P/S})} \cdot - \frac{K_s (\mu_m - D - k_d) + K_s (D + k_d)}{(\mu_m - D - k_d)^2} \right.$$

$$\left. + \left(S_o - \frac{K_s (D + k_d)}{\mu_m - D - k_d} \right) \frac{D + k_d - q_p Y^M_{X/S} / Y_{P/S} - D}{(D + k_d + q_p (Y^M_{X/S} / Y_{P/S}))} \right]$$

setting this to zero brings us, after some algebra, to:

6.19 Problem 4

cont'd

$$D^2 \underbrace{\left(\frac{-K_s \mu_m}{k_d + q_p (Y^n_{x/s} / Y_{p/s})} + K_s + S_o \right)}_{a}$$

$$+ D \underbrace{\left(\frac{-k_d K_s \mu_m}{k_d + q_p (Y^n_{x/s} / Y_{p/s})} - \frac{q_p (Y^n_{x/s} / Y_{p/s}) K_s \mu_m}{k_d + q_p (Y^n_{x/s} / Y_{p/s})} - \mu_m K_s - 2\mu_m S_o + 2 k_d S_o + 2 k_d K_s + k_d S_o \right)}_{b}$$

$$+ \underbrace{\left(-\mu_m K_s k_d - 2\mu_m k_d S_o + \mu_m^2 S_o - K_s k_d^2 - k_d^2 S_o \right)}_{c} = 0$$

$a = 5.00$

$b = -2.00$

$c = 0.196$

$\Rightarrow \underbrace{D = 0.149 \ [h^{-1}]}_{\text{sensible answer}}$ or $\underbrace{D = 0.263 \ [h^{-1}]}_{\text{not a sensible answer}}$ since for $D > (\mu_m - k_d)$ there is a wash-out

Maximize productivity of biomass (DX):

set $\frac{d(DX)}{dD} = 0$

It is a considerable time saving to use a computer to solve the latter equation and find $D = 0.165 \ [h^{-1}]$

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