Homework #6

From the primary textbook (Shuler, et. al.) on cell growth, problems (6.3 or 6.5) and (6.10 or 6.14).


**PROBLEMS**

6.1. By using the following information, determine the cell number concentration in a fermenter containing 1 g dry weight/l biomass:

- Cell dimensions: $1 \times 2$ μm cylindrical cells
- Cell density: $1.05$ g/cm$^3$
- Water content of cells: 80%

6.2. Two different bacterial species with doubling times of 2 h and 4 h are growing in a batch nutrient-rich medium with their maximum growth rate. The initial concentrations are the same.
   a. What would be the ratio of $X_1/X_2$ after 10 h of batch growth?
   b. How long should the batch growth of the mixed cultures last in order to obtain $X_1/X_2 = 10$? Assume exponential growth.

6.3. A simple, batch fermentation of an aerobic bacterium growing on methanol gave the results shown in the following table. Calculate the following:
   a. Maximum growth rate ($\mu_{\text{max}}$)
   b. Yield on substrate ($Y_{XS}$)
   c. Mass doubling time ($t_d$)
   d. Saturation constant ($K_S$)
   e. Specific growth rate ($\mu_{\text{net}}$) at $t = 10$ h

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>$X$ (g/l)</th>
<th>$S$ (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.2</td>
<td>9.23</td>
</tr>
<tr>
<td>2</td>
<td>0.211</td>
<td>9.21</td>
</tr>
<tr>
<td>4</td>
<td>0.305</td>
<td>9.07</td>
</tr>
<tr>
<td>8</td>
<td>0.98</td>
<td>8.03</td>
</tr>
<tr>
<td>10</td>
<td>1.77</td>
<td>6.8</td>
</tr>
<tr>
<td>12</td>
<td>3.2</td>
<td>4.6</td>
</tr>
<tr>
<td>14</td>
<td>5.6</td>
<td>0.92</td>
</tr>
<tr>
<td>16</td>
<td>6.15</td>
<td>0.077</td>
</tr>
<tr>
<td>18</td>
<td>6.2</td>
<td>0</td>
</tr>
</tbody>
</table>

6.4. The growth of a microbial population is a function of pH and is given by the following equation:

$$\mu_g = \frac{1}{X} \frac{dX}{dt} = \frac{\mu_{\text{max}}S}{K_s \left(1 + \frac{H^*}{K_i}\right) + S}$$
a. With a given set of experimental data ($X$ and $S$ versus $t$), describe how you would determine the constants $\mu_m$, $K_s$, and $k_1$.

b. How would the double-reciprocal plot $1/\mu_g$ versus $1/S$ change with pH (or $H^+$) concentration?

6.5. The following data were obtained for the effect of temperature on the fermentative production of lactic acid by a strain of *Lactobacillus delbrueckii*. From these data, calculate the value of the activation energy for this process. Is the value of the activation energy typical of this sort of biological conversion? (See Chapter 3.)

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Rate Constant (mol/l-h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>40.4</td>
<td>0.0140</td>
</tr>
<tr>
<td>36.8</td>
<td>0.0112</td>
</tr>
<tr>
<td>33.1</td>
<td>0.0074</td>
</tr>
<tr>
<td>30.0</td>
<td>0.0051</td>
</tr>
<tr>
<td>25.1</td>
<td>0.0036</td>
</tr>
</tbody>
</table>


6.6. The logistic equation for batch microbial growth is given by the following equation:

$$ \frac{dX}{dt} = kX \left(1 - \frac{X}{X_m}\right) $$

a. Use the following approximations:

$$ X_m = Y X_s, S_0, X = Y X_s (S_0 - S) $$

Prove that the logistic equation can be expressed as follows:

$$ \frac{dX}{dt} = kX \frac{S}{S_0} $$

b. By using the following relationship, develop an expression describing the variation of substrate concentration with time according to the logistic equation:

$$ \frac{dX}{dt} = -Y X_s \frac{dS}{dt} $$

c. By comparing the form of logistic equation derived in part (a) with the Monod equation at low substrate concentrations (first-order kinetics), determine the logistic equation constant ($k$) in terms of Monod kinetic constants.

6.7. It is desired to model the growth of an *individual* bacterium. The cell transports $S_1$ into the cell enzymatically, and the permease is subject to product inhibition. $S_1$ is converted into precursors, $P$, that are converted finally into the macromolecular portion of the cell, $M$. The catalyst of all reactions is $M$.

a. $ S_1 \xrightarrow{M} S_1 \text{ (per unit surface area), where } S^* = \text{outside concentration of } S $  

b. $ S_1 \xrightarrow{M} P $
c1. Energy + $P \xrightarrow{M} M$
$S_1 \xrightarrow{M} \text{energy}$
\[
\begin{aligned}
\text{coupled reaction}
\end{aligned}
\]
c2. Or, $S_1 + P \xrightarrow{M} M$

d. The dry weight of the cell is $T$ and is equal to $T = S_1 + P + M_1 = \rho V$, where $\rho = \text{cell density}$ and $V = \text{cell volume}$

Write the mass balance equations and define all symbols necessary to describe the changes in $S_1$, $P$, $M$, and $T$ within the cell. Remember that the cell volume is always changing.

6.8. A biochemical engineer has determined in her lab that the optimal productivity of a valuable antibiotic is achieved when the carbon nutrient, in this case molasses, is metered into the fermenter at a rate proportional to the growth rate. However, she cannot implement her discovery in the antibiotic plant, since there is no reliable way to measure the growth rate ($dX/dt$) or biomass concentration ($X$) during the course of the fermentation. It is suggested that an oxygen analyzer be installed on the plant fermenters so that the OUR (oxygen uptake rate, g/l-h) may be measured.

a. Derive expressions that may be used to estimate $X$ and $dX/dt$ from OUR and time data, assuming that a simple yield and maintenance model may be used to describe the rate of oxygen consumption by the culture.

b. Calculate values for the yield ($Y_{x/o_2}$) and maintenance ($m_o$) parameters from the following data:

<table>
<thead>
<tr>
<th>Time</th>
<th>OUR (g/el-h)</th>
<th>$X$ (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.011</td>
<td>0.60</td>
</tr>
<tr>
<td>1</td>
<td>0.008</td>
<td>0.63</td>
</tr>
<tr>
<td>2</td>
<td>0.084</td>
<td>0.63</td>
</tr>
<tr>
<td>3</td>
<td>0.153</td>
<td>0.76</td>
</tr>
<tr>
<td>4</td>
<td>0.198</td>
<td>1.06</td>
</tr>
<tr>
<td>5</td>
<td>0.273</td>
<td>1.56</td>
</tr>
<tr>
<td>6</td>
<td>0.393</td>
<td>2.23</td>
</tr>
<tr>
<td>7</td>
<td>0.493</td>
<td>2.85</td>
</tr>
<tr>
<td>8</td>
<td>0.642</td>
<td>4.15</td>
</tr>
<tr>
<td>9</td>
<td>0.915</td>
<td>5.37</td>
</tr>
<tr>
<td>10</td>
<td>1.031</td>
<td>7.59</td>
</tr>
<tr>
<td>11</td>
<td>1.12</td>
<td>9.40</td>
</tr>
<tr>
<td>12</td>
<td>1.37</td>
<td>11.40</td>
</tr>
<tr>
<td>13</td>
<td>1.58</td>
<td>12.22</td>
</tr>
<tr>
<td>14</td>
<td>1.26</td>
<td>13.00</td>
</tr>
<tr>
<td>15</td>
<td>1.58</td>
<td>13.37</td>
</tr>
<tr>
<td>16</td>
<td>1.26</td>
<td>14.47</td>
</tr>
<tr>
<td>17</td>
<td>1.12</td>
<td>15.37</td>
</tr>
<tr>
<td>18</td>
<td>1.20</td>
<td>16.12</td>
</tr>
<tr>
<td>19</td>
<td>0.99</td>
<td>16.18</td>
</tr>
<tr>
<td>20</td>
<td>0.86</td>
<td>16.67</td>
</tr>
<tr>
<td>21</td>
<td>0.90</td>
<td>17.01</td>
</tr>
</tbody>
</table>

(Courtesy of D. Zabriskie from “Collected Coursework Problems in Biochemical Engineering,” compiled by H. W. Blanch for 1977 American Society Engineering Education Summer School.)
6.9. *Pseudomonas* sp. has a mass doubling time of 2.4 h when grown on acetate. The saturation constant using this substrate is 1.3 g/l (which is unusually high), and cell yield on acetate is 0.46 g cell/g acetate. If we operate a chemostat on a feed stream containing 38 g/l acetate, find the following:

a. Cell concentration when the dilution rate is one-half of the maximum
b. Substrate concentration when the dilution rate is 0.8 $D_{\text{max}}$
c. Maximum dilution rate
d. Cell productivity at 0.8 $D_{\text{max}}$


6.10. The following data were obtained in a chemostat for the growth of *E. aerogenes* on a glycerol-limited growth medium:

<table>
<thead>
<tr>
<th>$D$, h$^{-1}$</th>
<th>$1/D$</th>
<th>$S$, mg/ml, Glycerol</th>
<th>$1/S$</th>
<th>$X$, mg/ml, Cell Conc.</th>
<th>$\Delta S$</th>
<th>$\Delta S/X$</th>
<th>$\Delta S/X \cdot D$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.05</td>
<td>20</td>
<td>0.012</td>
<td>83.3</td>
<td>3.2</td>
<td>9.988</td>
<td>3.12</td>
<td>0.156</td>
</tr>
<tr>
<td>0.10</td>
<td>10</td>
<td>0.028</td>
<td>35.7</td>
<td>3.7</td>
<td>9.972</td>
<td>2.7</td>
<td>0.270</td>
</tr>
<tr>
<td>0.20</td>
<td>5.0</td>
<td>0.05</td>
<td>20</td>
<td>4.0</td>
<td>9.95</td>
<td>2.49</td>
<td>0.498</td>
</tr>
<tr>
<td>0.40</td>
<td>2.5</td>
<td>0.10</td>
<td>10</td>
<td>4.4</td>
<td>9.90</td>
<td>2.25</td>
<td>0.90</td>
</tr>
<tr>
<td>0.60</td>
<td>1.67</td>
<td>0.15</td>
<td>6.67</td>
<td>4.75</td>
<td>9.85</td>
<td>2.075</td>
<td>1.245</td>
</tr>
<tr>
<td>0.70</td>
<td>1.43</td>
<td>0.176</td>
<td>5.68</td>
<td>4.9</td>
<td>9.824</td>
<td>2.005</td>
<td>1.405</td>
</tr>
<tr>
<td>0.80</td>
<td>1.25</td>
<td>0.80</td>
<td>1.25</td>
<td>4.5</td>
<td>9.20</td>
<td>2.045</td>
<td>1.635</td>
</tr>
<tr>
<td>0.84</td>
<td>1.19</td>
<td>9.00</td>
<td>0.11</td>
<td>0.5</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>

Note: $S_0 = 10$ mg/ml

For this system, estimate the following values:

a. $K_s$, mg glycerol/ml
b. $\mu_m$, h$^{-1}$
c. $Y_{X/S}$, mg cells/mg glycerol
d. $m_i$, mg glycerol/mg cell-h


6.11. The kinetics of microbial growth, substrate consumption, and mixed-growth-associated product formation for a chemostat culture are given by the following equations:

\[
\frac{dX}{dt} = \frac{\mu_m S}{K_s + S} X
\]

\[
\frac{dS}{dt} = \frac{\mu_m S}{(K_s + S)Y_{X/S}} X
\]

\[
\frac{dP}{dt} = \alpha \frac{dX}{dt} + \beta X = (\alpha \mu_g + \beta) X
\]
The kinetic parameter values are $\mu_m = 0.7 \text{ h}^{-1}$, $K_s = 20 \text{ mg/l}$, $Y_{p/X} = 0.5 \text{ g} \text{ dw/g substrate}$, $Y_{x/P} = 0.15 \text{ gP/g} \cdot \text{ dw}$, $\alpha = 0.1$, and $S_0 = 1 \text{ g/l}$.

a. Determine the optimal dilution rate maximizing the productivity of product formation ($PD$) when $\beta = 0.02 \text{ h}^{-1}$ and $0.01 \text{ h}^{-1}$.

b. Determine the optimal dilution rate maximizing the productivity of cell (biomass) formation for the same value of $\beta$ as in part (a) ($DX$).

(Problem adapted from one suggested by L. Erickson and modified by C. Lee.)

6.12. Ethanol is to be used as a substrate for single-cell protein production in a chemostat. The available equipment can achieve an oxygen transfer rate of $10 \text{ g O}_2/\text{l of liquid}$ per hour. Assume the kinetics of cell growth on ethanol is of the Monod type, with $\mu_m = 0.5 \text{ h}^{-1}$, $K_s = 30 \text{ mg/l}$, $Y_{xRS} = 0.5 \text{ cells/g ethanol}$, and $Y_{O_2/RS} = 2 \text{ g O}_2/\text{g EtOH}$. We wish to operate the chemostat with an ethanol concentration in the feed of $22 \text{ g/l}$. We also wish to maximize the biomass productivity and minimize the loss of unused ethanol in the effluent. Determine the required dilution rate and whether sufficient oxygen can be provided.

6.13. Plot the response of a culture to diauxic growth on glucose and lactose based on the following: $\mu_{\text{glucose}} = 1.0 \text{ h}^{-1}$, $\mu_{\text{lactose}} = 0.6 \text{ h}^{-1}$, $Y_{\text{glucose}} = Y_{\text{lactose}} = 0.5$; enzyme induction requires $30 \text{ min}$ to complete. Plot cell mass, glucose, and lactose concentrations, assuming initial values of $2 \text{ g/l glucose}$, $3 \text{ g/l lactose}$, and $0.10 \text{ g/l cells}$.

6.14. The following data are obtained in oxidation of pesticides present in wastewater by a mixed culture of microorganisms in a continuously operating aeration tank:

<table>
<thead>
<tr>
<th>$D$ (h$^{-1}$)</th>
<th>$S$ (Pesticides), mg/l</th>
<th>$X$ (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.05</td>
<td>15</td>
<td>162</td>
</tr>
<tr>
<td>0.11</td>
<td>25</td>
<td>210</td>
</tr>
<tr>
<td>0.24</td>
<td>50</td>
<td>250</td>
</tr>
<tr>
<td>0.39</td>
<td>100</td>
<td>235</td>
</tr>
<tr>
<td>0.52</td>
<td>140</td>
<td>220</td>
</tr>
<tr>
<td>0.7</td>
<td>180</td>
<td>205</td>
</tr>
<tr>
<td>0.82</td>
<td>240</td>
<td>170</td>
</tr>
</tbody>
</table>

Assuming the pesticide concentration in the feed wastewater stream as $S_0 = 500 \text{ mg/l}$, determine $Y_{x/S}$, $k_d$, $\mu_m$, and $K_s$.

6.15. Wastewater is treated in a completely mixed aeration tank operated in continuous mode. The rate of chemical oxygen demand (COD) removal is given by the following equation:

$$R_s = \frac{Q(S_0 - S)}{V} = D(S_0 - S) = D(S_0 - \frac{K_s D}{\mu_m - D})$$

Here $\mu_m$ is the maximum specific growth rate constant (h$^{-1}$), $D$ is the dilution rate (h$^{-1}$), $K_s$ is saturation constant (mg/L), and $S$ is the effluent COD concentration (mg/l).

a. Determine the optimum dilution rate or hydraulic residence time (HRT) maximizing COD removal rate.

b. Determine the optimal HRT, the maximum $R_s$ and the biomass concentration for aerobic treatment where $\mu_m = 0.1 \text{ h}^{-1}$, $K_s = 0.1 \text{ g/l}$, $Y = 0.4 \text{ gX/g S}$, and $S_0 = 1 \text{ g/l}$. 
c. Determine the optimal HRT, the maximum \( R \), and biomass concentration for anaerobic treatment where \( \mu_m = 0.01 \text{ h}^{-1}, K_s = 0.25 \text{ g/l}, Y = 0.06 \text{ gX/g S}, \text{ and } S_0 = 1 \text{ g/l.} \)

6.16. *Pseudomonas putida* is used for fermentation of lactose present in cheese whey in a continuously operating aeration tank at a dilution rate of \( D = 0.28 \text{ h}^{-1} \). The lactose concentration in the feed and the effluent are \( S_0 = 2 \text{ g/L} \) and \( S_e = 0.1 \text{ g/l} \). The growth rate and lactose removal is limited by oxygen transfer. The following information is available:

\[
Y_{x/s} = 0.45 \frac{\text{gX}}{\text{g S}}, \quad Y_{x/O_2} = 0.25 \frac{\text{gX}}{\text{g O}_2} \quad \text{and} \quad C^* = 8 \text{ mg/l (saturation DO)}.
\]

a. Determine the steady-state biomass concentration (\( X \)) and the specific rate of oxygen consumption (\( q_{O_2} \)).

b. What should be the oxygen transfer coefficient (\( K_{L,a} \)) to overcome oxygen transfer limitations if the desired DO concentration in the fermentation medium is 2 mg/l?

6.17. In a chemostat, you know that if a culture obeys the Monod equation, the residual substrate is independent of the feed substrate concentration. You observe that in your chemostat, an increase in \( S_0 \) causes an increase in the residual substrate concentration. Your friend suggests that you consider whether the Contois equation may describe the situation better. The Contois equation (equation 6.38) follows:

\[
\mu = \frac{\mu_m S}{K_{ss} X + S}
\]

a. Derive an expression for \( S \) in terms of \( D, \mu_m, K_{ss} \), and \( X \) for a steady-state CFSTR (chemostat).

b. Derive an equation for \( S \) as a function of \( S_0, D, K_{ss}, Y_{x/s} \), and \( \mu_m \).

c. If \( S_0 \) increases twofold, by how much will \( S \) increase?

6.18. The maximum growth yield coefficient for *Bacillus subtilis* growing on methanol is 0.4 g X/g S. The heat of combustion of cells is 21 kJ/g cells, and for substrate it is 7.3 kcal/g. Determine the metabolic heat generated by the cells per unit mass of methanol consumption.

6.19. Calculate the productivity (i.e., DP) of a chemostat under the following conditions:

a. Assume Monod kinetics applies. Assume that negligible amounts of biomass (<1%) must be converted to product.

b. Assume the Luedeking–Piret equation for product formation (equation 6.20) applies.

c. Assume steady state:

\[
D = 0.8 \mu_m \quad Y_{x/s} = 0.5 \frac{\text{gX}}{\text{g S}}
\]

\[
\mu_m = 1.0 \text{ h}^{-1} \quad S_0 = 1000 \text{ mg/l}
\]

\[
K_s = 10 \text{ mg/l} \quad \beta = 0.5 \text{ h}^{-1} \text{ mg P/g X}
\]

\[
\alpha = 0.4 \text{ mg P/g X}
\]

6.20. Consider a chemostat. You wish to know the number of cells in the reactor and the fraction of the cells that are viable (i.e., alive as determined by ability to divide).

a. Write an equation for viable cell number (\( n_v \)). Assume the following:

\[
\mu_{net,rep} = \frac{\mu_{m,rep} S}{K_{r,rep} + S} - k_d
\]
Here $\mu_{\text{net,rep}}$ = net specific replication rate, $\mu_{\text{m,rep}}$ = maximum specific replication rate, and $k'd$ = death rate. $K_{\text{rep}}$ is the saturation parameter.

b. Derive an expression for the value of $S$ at steady state.

c. Write the number balance in the chemostat on dead cells ($n_d$).

d. Derive an expression for the fraction of the total population which are dead cells.

6.21. *E. coli* is cultivated in continuous culture under aerobic conditions with a glucose limitation. When the system is operated at $D = 0.2 \, \text{h}^{-1}$, determine the effluent glucose and biomass concentrations by using the following equations ($S_0 = 5 \, \text{g/l}$):

a. Monod equation: $\mu_m = 0.25 \, \text{h}^{-1}, K_s = 100 \, \text{mg/l}$

b. Tessier equation: $\mu_m = 0.25 \, \text{h}^{-1}, K = 0.005 \, (\text{mg/l})^{-1}$

c. Moser equation: $\mu_m = 0.25 \, \text{h}^{-1}, K_s = 100 \, \text{mg/l}, n = 1.5$

d. Contois equation: $\mu_m = 0.25 \, \text{h}^{-1}, K_{sx} = 0.04, Y_{X/S}^M = 0.4 \, \text{g} \, X/\text{g} \, S$

$S_0 = 5 \, \text{g/l}$

Compare and comment on the results.

6.22. Consider steady-state operation of a chemostat. Assume that growth is substrate inhibited and that endogenous metabolism can be ignored such that we get the following:

$$\mu_{\text{net}} = \frac{\mu_m S}{K_s + S + S^2/K_1}$$

a. Derive an expression for the residual substrate concentration (i.e., $S$) as a function of dilution rate and the kinetic parameters ($\mu_m, K_S, K_I$).

b. What are the implications for operation of a chemostat when the organism is subjected to substrate inhibition?

6.23. Formation of lactic acid from glucose is realized in a continuous culture by *Streptococcus lactis*. The following information was obtained from experimental studies:

$S_0 = 5 \, \text{g/l}, \mu_m = 0.2 \, \text{h}^{-1}, K_s = 200 \, \text{mg/l}, k_d = 0.002 \, \text{h}^{-1}, Y_{X/S}^M = 0.4 \, \text{g} \, X/\text{g} \, S,$

$Y_{P/S} = 0.2 \, \text{g} \, P/\text{g} \, S, q_p = 0.1 \, \text{g} \, P/\text{g} \, X \cdot \text{h}.$

a. Plot the variations of $S, X, P, DX$, and $DP$ with dilution rate.

b. Determine (graphically) the optimum dilution rate maximizing the productivities of biomass (DX) and the product (DP).