DOWNSTREAM PROCESSING

Problem Set #1

## Problem 1

Dunnill and Lilly ("Protein Extraction from Microbial Cells", Single Cell Proteins II, S. Tannenbaum and D.I.C. Wang (eds.), MIT Press, Cambridge, Mass, 1975) observed that protein recovery from passage of baker's-yeast cell suspensions through a large pressure drop confined to a small volume (homogenizer) conformed to the following equation:

$$\log\left(\frac{R_m}{R_m - R}\right) = KN$$

where  K     =     first-order rate constant

=     $\kappa P^{\alpha}$ where $\kappa$ and $\alpha$ depend on the microbe and P is the operating pressure

N     =     number of passes through homogenizer

$R_m$     =     maximal achievable protein release

R     =     protein release after N passes

They also found that the relationship between K and pressure for baker's yeast is represented by the data shown in Figure 1.
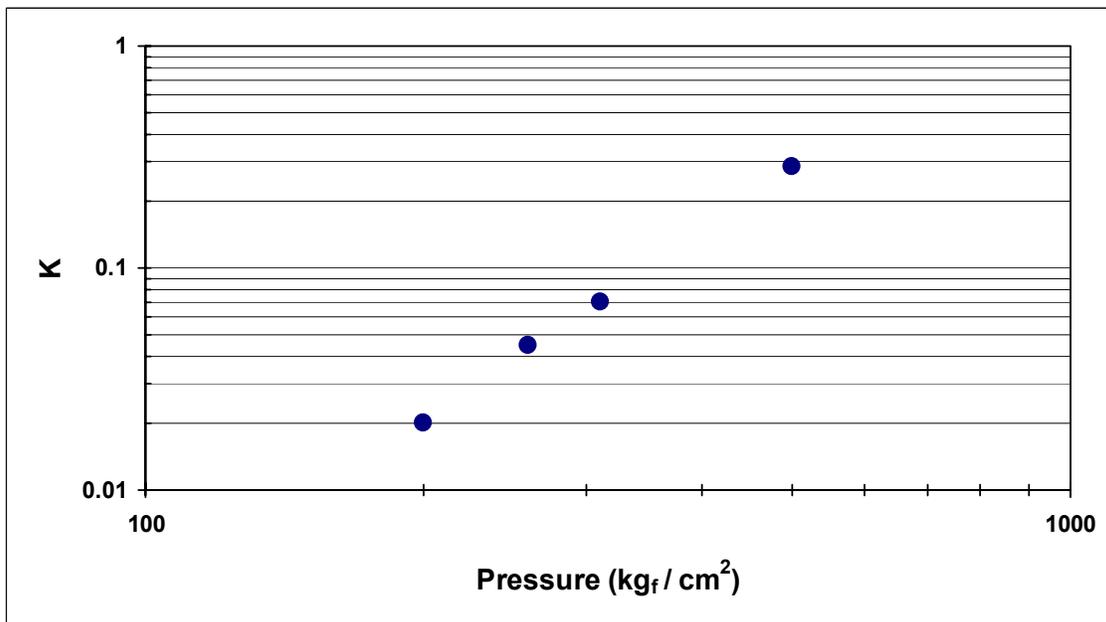


**Figure 1: Relationship Between K and Pressure for Baker's Yeast (N = 1)**

a) Show that a first-order rate law gives the expression observed, treating N as a continuous variable.

b) Dunnill and Lilly showed that power input per pass varies with operating pressure, increasing linearly by 7.63 x $10^{-3}$ kW-cm$^2$/kg$_f$. Show graphically that a maximum exists in the curve of Q (percent protein released per kilowatt power input) vs. pressure. What is the optimal operating pressure with regard to power efficiency for N = 1. Is it more efficient to operate with two passes (i.e. N = 2)?

## Problem 2

Your objective is to recover the enzyme glucose 6-phosphate dehydrogenase (G6PDH) from *Leuconostoc mesenteriodes* by high pressure homogenization in one or more Gaulin type machines in a 6 hour period. Your production fermentor has a capacity of 100 m$^3$ and you are able to grow the cells to 7 g/L. It is necessary to concentrate the cells by centrifugation to 80 g/L for cell disruption. *L. mesenteriodes* is a very difficult organism to break and requires up to 5 passes at 8000 psi to achieve 90% recovery of the intracellular protein. You are considering the purchase of machines to accomplish the task. The available sizes and costs are shown in Table 1.

Table 1:  Gaulin-Type Homogenizer Capacities and Costs

| Capacity (m$^3$/hr) | Cost ($) |
|---|---|
| 0.45 | 22,000 |
| 1.14 | 32,000 |
| 2.27 | 45,000 |
| 4.54 | 69,000 |

a) What is the optimal allocation of machines for 1, 2, 3, 4, and 5 passes if the objective is to minimize capital cost?

b) For each number of passes in part a, what operating pressure is necessary to achieve 90% disruption? How does operating pressure affect your final decision as to how many passes to use for cell disruption? For this organism, the disruption rate constant is dependent on pressure to the power 1.8 (i.e. K $\propto$ P$^{1.8}$).

## Problem 3

A recombinant therapeutic protein is being produced as an intracellular yeast product. In lab-scale studies pertaining to the recovery of this protein, a horizontally operated high-speed agitator bead mill with 7 spiral disks is being investigated for cell disruption on a continuous basis.

Experiments show that when 100 L of fermentation broth containing yeast at a density of 30 grams dry cell weight per liter are processed in the mill, the total amount of intracellular protein released as a function of time is as shown in Table 2.

**Table 2: Intracellular Protein Release as a Function of Time**

| Time (min) | Protein (grams) |
|---|---|
| 1 | 400 |
| 2 | 990 |
| 3 | 1,200 |
| 4 | 1,100 |
| 5 | 1,410 |
| 6 | 1,425 |
| 7 | 1,475 |
| 8 | 1,473 |
| 9 | 1,483 |
| 10 | 1,490 |

If cell disruption is to occur on a continuous basis in which fermentation broth is fed to the 100 L bead mill at a flow rate F, what is the maximum dilution rate ($D = F/V$) that can be used in the process while still achieving 95% release of intracellular protein? You may assume that yeast are 50% protein by dry cell weight.