DOWNSTREAM PROCESSING

Exam #1

<u>Open Book and Open Notes</u>

Problem 1 (Cell Disruption)

The data given from a particle size analysis (NICOMP model 270) of yeast cells before and after one pass through a Gaulin-type high-pressure homongenizer at 8,000 psi indicate a mean particle size before homogenization of 5.2 micron and a mean particle size after homogenization of 3.5 micron. After one pass through the homogenizer, only 28% of the cellular protein is released.

a) How many passes are required to achieve 90% release of cellular protein?

b) Concerned about degradation of your desired protein product, you decide to attempt to achieve a high level of recovery by increasing pressure rather than number of passes. Your equipment will allow you to operate at a maximum pressure of 10,000 psi, so this is the pressure at which you conduct further experiments. At 10,000 psi and a single pass through the homogenizer, you are disappointed to find that you can release only 40% of the cellular protein. What is the pressure dependence of the disruption rate constant?

Problem 2 (Centrifugation)

The first step in a process to recover an intracellular protein from yeast is to harvest the cells from the fermentation with a disk-stack centrifuge. The machine that you have available has a Σ of 12,000 m^2. The cell concentration in the fermentor is 30 kg/m^3 and the desired feed to the homogenizer is at least 140 kg/m^3.

a) Estimate the feed rate to the centrifuge that will enable you to recover at least 95% of the cells.

b) What is the heavy-phase flow rate from the centrifuge?

c) Due to increased costs associated with the process, it has become necessary to increase batch productivity, which means performing centrifugation in less time with the same level of biomass recovery. One of your colleagues takes the initiative to solve this problem by purchasing a holding tank large enough to dilute the fermentation broth to a concentration of 10 g/L prior to centrifugation. Why do you think he decided to attack the problem in this manner? Do you think that his plan will work or has he simply wasted a lot of money? Please provide a quantitative reason for your opinion.

(Hint: Data found in the reading material will be necessary to solve this problem.)

Problem 3 (Chromatography)

In a high-resolution separation process, two proteins are eluted from an ion exchange column with the profiles shown in Figure 1. The interstitial void volume for the column is 1.0 ml. The molecular weight of the antibody is 150 kD and that of the impurity is 50 kD. The column is operated at 0.5 ml/min and has 130 theoretical plates.
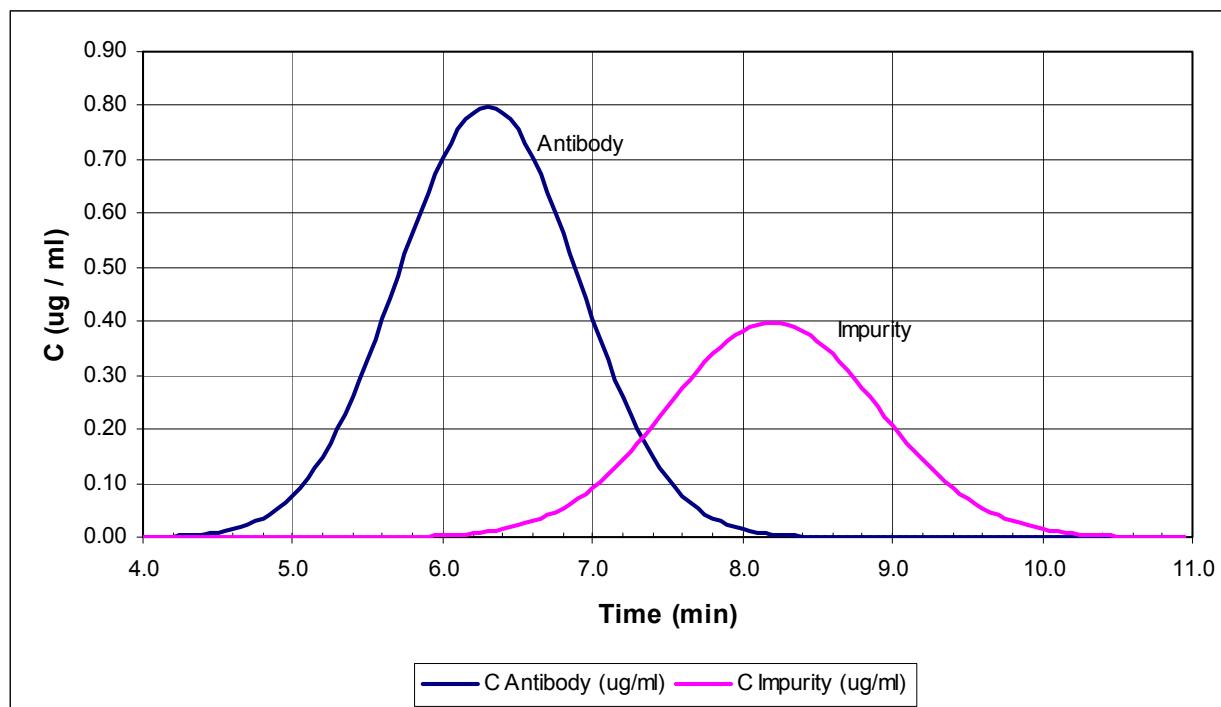

Figure 1: Antibody and Impurity Chromatographic Profiles

a) What is the selectivity achieved in this separation? What is the resolution?

b) Because your annual incentive bonus is dependent on achieving good economic performance in this separation, you have set a goal of baseline resolution of the antibody and impurity. This will allow you to collect as much of the product as possible. What is the **minimum** increase in column length necessary to meet this separation goal? Please carefully consider resolution in your answer.

c) By extending the column length, by what factor will the peak spread (σ) increase?