

## DOWNSTREAM PROCESSING

Exam #2

Open Book and Open Notes

### **Problem 1 (Membrane Filtration)**

Your goal is to concentrate a recombinant therapeutic protein, HR-I, that is eluted from an anion exchange column at 1.5 g-protein/L to a final concentration of 20 g/L. The concentration is done by tangential flow filtration across a 30,000 MW cut-off membrane in which the retentate is recirculated across the membrane from a reservoir. In laboratory experiments using a 100 cm<sup>2</sup> membrane the filtrate flux is 100 L/m<sup>2</sup>-hr at the initial concentration and cannot be increased by raising the pressure above 5 bar when the flow rate is 1 m/s. Also, the available literature data show that when the bulk protein concentration is continually increased under the same operating conditions, the flux becomes zero when the concentration of HR-I reaches 200 g/L.

In the pilot scale process being developed for eventual scale-up to manufacturing, you have available a 1 m<sup>2</sup> membrane system that can be operated at the same pressure and flow rate as the lab-scale apparatus. Both the lab and pilot scale systems use polysulfone membranes from the same lot of materials.

- a) Calculate the average of the beginning and ending fluxes during concentration of HR-I from 1.5 to 20 g/L.
- b) Use your answer from part a to estimate the time required to concentrate 100 L of column effluent to the desired end-point in the pilot scale process.

## Problem 2

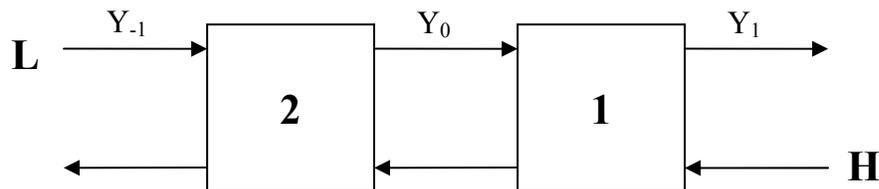
Bulk penicillin G is being recovered from a fermentation broth using rotary vacuum filtration, two centrifugal extraction stages, and crystallization.

The first of the two extraction steps is designed to extract penicillin, with a  $pK_a$  of 2.75, from the fermentation broth into an organic solvent at low pH; this provides significant purification. When the pH is raised prior to the second extraction stage, the distribution coefficient ( $K_d$ ) for partitioning of penicillin into the organic phase is reduced. This allows for removal of solvent prior to crystallization via extraction of the molecule back into the aqueous phase. The partition coefficient depends on pH in the following manner:

$$\begin{aligned}K_d(\text{pH } 2) &= 60 \\K_d(\text{pH } 5.6) &= 0.5\end{aligned}$$

A different centrifugal Podbielniak extractor is being used for each extraction step in this continuous process. Each machine has two theoretical extraction stages (this is the number of stages for use in design calculations).

- The throughput of the fermentation broth to the first extractor is 3,000 L per hr and the broth to solvent ratio is 10. Please calculate the recovery of penicillin from the first extraction stage. Note that the broth is acidified to pH 2 prior to extraction.
- In the second extraction stage, the pH is raised to 5.6 and penicillin is extracted out of the organic solvent and into an aqueous phase. Please derive an expression for the recovery of penicillin in the light phase. Refer to the following diagram in your derivation:



If the volume of the aqueous phase is 900 L per hr, what is the recovery of penicillin in the second extraction step and what is the overall recovery for both extraction steps?