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Biochemical Engineering  
Problem Set for Continuous Culture

1. When growing aerobic organisms in a chemostat, compressed air is passed through the fermentor at typically one volume of air per volume of liquid per minute (VVM). Compressed air is dry on entering the fermentor but saturated with water on leaving. As a consequence, the liquid flow rates in and out are different.

a.) How will this affect the interpretation of analytical data obtained by measurement on the fermentor effluent? If the fermentor volume is 10 liter and the dilution rate is  $0.1 \text{ h}^{-1}$ , what will be the actual product concentration, corrected for evaporation, in the following system?

$$\begin{aligned}
 S_0 &= 50 \text{ g glucose/liter} \\
 K_s &= 10 \text{ mg glucose/liter} \\
 \mu &= 0.7 \text{ h}^{-1} \\
 Y_{x/s} &= 0.5 \text{ g cell/g-glucose} \\
 m &= 0.02 \text{ g glucose/g-cell-h} \\
 Y_{p/s} &= 0.5 \\
 q_p &= 0.2 \text{ g-product/g-cell-h} \\
 T &= 60 \text{ C}
 \end{aligned}$$

The vapor pressure of water at 60 C is 150 mm Hg.

2. Izuo *et al.* (J. Ferm. Tech. 58, p. 221, 1980) describes the production of dihydroxyacetone (DHA) from glycerol in a multistage continuous culture system with *Acetobacter suboxydans*. The medium contains: 15% glycerol, 3% corn steep liquor, 0.3%  $(\text{NH}_4)_2$ -fumarate, and 1%  $\text{CaCO}_3$ . The 20-liter fermentors contain 10 liter of medium and are operated at a dilution rate of  $0.1 \text{ h}^{-1}$ . At this dilution rate *A. suboxydans* is able to produce DHA at a specific rate of 1.4 mg DHA /mg cell-h in the presence of excess oxygen; under oxygen-limited conditions, little or no DHA is formed. The cell yield values on oxygen and on glycerol are approximately 1.0 g cell/g  $\text{O}_2$  and 0.4 g cell/g glycerol, respectively.

a.) Estimate the concentration of DHA and cell mass in the second stage fermentor. How does it differ from the first? Be sure to state all of your assumptions.

b.) If you were to operate with oxygen limitation to the first fermentor, what would be the concentration of DHA in the first and second stage? What is the oxygen demand (mmol  $\text{O}_2$ /liter-h) in the first and second stage?

3. Wall growth is a phenomenon, which plagues many investigators working with continuous culture. It can occur for a variety of reasons but in all cases the effect is that some portion of the total cell mass in the fermentor is attached to the fermentor wall and, as a consequence, the assumption that the vessel is well mixed no longer applies.

a.) Derive and solve the appropriate steady state equations for cell mass, substrate and product concentration describing a chemostat with growth and sketch the curves for residual substrate  $S$ , and effluent cell mass,  $X$ , as a function of dilution rate,  $D$ , comparing them to a "normal" system.

b.) Carefully list all assumptions in your derivation of these equations.

c.) Describe how you would quantitate the extent of wall growth and also suggest a rationale criterion to stop fermentation because of the extent of wall growth.

d.) In a 10 liter (liquid volume) fermentor with a height to diameter ratio of 2, how much cell mass on the wall to cause a deviation of growth rate from the dilution rate of 10%? How thick will the cell mass layer be?