10.442/542
Biochemical Engineering
Problem Set on Sterilization
Due April 13, 2000

1. A 1500-liter pilot plant fermentor containing 1000 liters of medium is to be sterilized with a holding temperature of 121^{o}C. The fermentor has a height to diameter ratio of 2 and the vertical portion is jacketed. Saturated steam is available to maintain steam in the jacket at 25 psig. The overall heat transfer coefficient when the fermentor is agitated is 75 BTU/hr-ft^{2}-^{o}F. After sterilization, the fermentor is cooled to 37^{o}C with cooling water available at 17^{o}C. You may assume that the initial medium is contaminated with 10^5 spore/ml and 10^7 vegetative cells/ml; you should design to a level of 10^{-3} cells/fermentor. The medium also contains 500 mg/liter of thiamin that is essential for your fermentation.

a. Calculate the degree of sterilization associated with each segment of a sterilization cycle designed to meet the specified criteria.
b. How much thiamin remains after sterilization?
c. If a 15 min hold time for sterilization is used instead of your design, how much thiamin will be present?
d. If you carried out the sterilization by direct steam injection instead of steam in the jacket, how long would heat-up and hold take, what would be the remaining thiamin, and what would be the volume change in the fermentor?

Please state all assumptions. There are useful data in *Media and Air Sterilization* by G. K. Raju and C. L. Cooney in Biotechnology Rehm and Reed 3, (1993) p157-184.

2. Continuous sterilization is used to prepare media for your 100 m^3 production fermentor (liquid volume). The media is sterilized at a rate to allow you to fill the reactor in two hours. However, it appears that there is inadequate sterilization of your fermentation medium and contamination occurs frequently. Random sampling (with 100 ml samples) of the sterilized media indicates that one out of every 500 samples becomes contaminated upon incubation of the samples.

The direct steam injection sterilizer operates at 140 C, it is 60 m long and 10 cm in diameter. Two engineers that report to you each have different suggestions. One engineer, Ted Thermophilus recommends that you simply increase the holding temperature so as to achieve greater thermal kill. The other engineer, Frank Flomass recommends a more complex solution in which you increase the flow rate through the sterilizer to reduce axial mixing and dispersion and then possibly make a more modest change in temperature then was suggested by Ted. The medium is a complex medium with yeast extract and casein hydrolyzate; thus it is susceptible to thermal degradation. Contamination is a serious problem and you have to take some action. Please quantitatively evaluate these two strategies, clearly state all of your assumptions, then select one of the proposed plans or *perhaps better yet suggest one of your own* in order to overcome the sterilization problem. As part of your recommendation, please specify the temperature, medium flow rate, and overall sterilization criteria that should be used in the plant.

3. The NIH guidelines for work with large volumes (>10 liter) of genetically engineered microorganisms requires that the organisms be killed or contained prior to further processing for product recovery. Fortunately, much of the recombinant DNA work is done in *E. coli* that is very sensitive to thermal death. A typical value for the activation energy for thermal death of *E. coli* is 75 kcal/mol. Many of the products of interest from genetically engineered *E. coli* are proteins; the activation energy for thermal denaturation of protein is typically 25 kcal/mol. The greater sensitivity of *E. coli* over proteins to thermal destruction suggests that heat treatment of *E. coli* at the end of the fermentation may provide a means of killing cells without causing substantial damage to the protein product. You may use a continuous sterilizer with steam injection to heat kill the cells to 10^{-1} viable organisms/fermentor as they are pumped from the fermentor to a collection vessel. The 10 m^3 fermentor contains *E. coli* at 5 x 10^9 cell/ml and is operated at 37^{o}C. If the sterilizer is operated at 62^{o}C, what fraction of the protein in the cell will be denatured?

Some useful data:

Death constant for *E. coli* at 54^{o}C K= 0.25 min^{-1}

Denaturation constant for protein at 40^{o}C $K_p = 5x10^{-5}$ sec^{-1}

R = universal gas constant = 1.99 cal/mol ^{o}K

BIOCHEMICAL ENGINEERING (10.442/10.542)

PROBLEM SET NUMBER 5

1. Some experimental data have been obtained for the pilot plant operations of a yeast fermentation. The data were obtained from a 30,000 liter (total volume) fermentor operated at 20,000 liters of broth. A six bladed turbine impeller at impeller speeds of 50, 70, and 85 RPM and with aeration rates of 200 and 320 cu. meter/hr. were employed. The fermentor is fully baffled.

   A. Calculate the ungassed power requirement at the different impeller speeds. Express your answer in total horsepower required and in HP/1000 gallon.

   B. Calculate the gassed power requirement using the aeration number (Na) correlation as well as the correlation of Michael and Miller.

   Note: the answers to this problem will be required for calculations in the next problem (no. 2).

2. Some mass transfer data for the production of food yeast on molasses in a 20,000 liter fermentor have been obtained by Hospodka (1964). The results along with the fermentor design are tabulated in the Table below and in the attached figure.

Table 1: Mass Transfer Measurements in 20,000 Liter Fermentor

Impeller Speed (RPM)	Oxygen Adsorption Rate (mM/L-Hr.)	Measured Power (Kilowatt-Hr/Kg yeast)	Air Flow Rate (m^3/Hr.)
50	16.7	0.475	200
70	19.4	0.615	200
85	23.7	0.572	200
50	21.8	0.528	320
70	26.3	0.589	320
85	27.8	0.615	320
85	39.0	0.610	600

The dissolved oxygen was essentially zero when the oxygen adsorption rates were measured. Saturated dissolved oxygen at 0.21 atm partial pressure was found to be 7.2 mg O_2/liter.

A. Using this data obtain the best correlation of the mass transfer coefficient, $k_L A$ in Hr^{-1}, to the power per unit volume in HP/1000 gallon and the superficial gas velocity in ft/Hr.

B. How does the experimentally measured power compare with the calculated power?

C. It is assumed that this data can be used for scale-up calculations. It has been proposed that a 50,000 gallon fermentor (liquid volume) be employed for the continuous cultivation of yeast on hydrocarbon. The steady-state yeast concentration shall be maintained at 15 gm/liter at a dilution rate of 0.15 Hr^{-1}. Specify the fermentor dimensions, agitator size, operating speed, gas flow rate, cost of agitation (KWH/Kg yeast) and any other information which may be pertinent. Assume a yield constant of 0.35 grams of yeast per gram of oxygen in your calculations for the hydrocarbon fermentation.

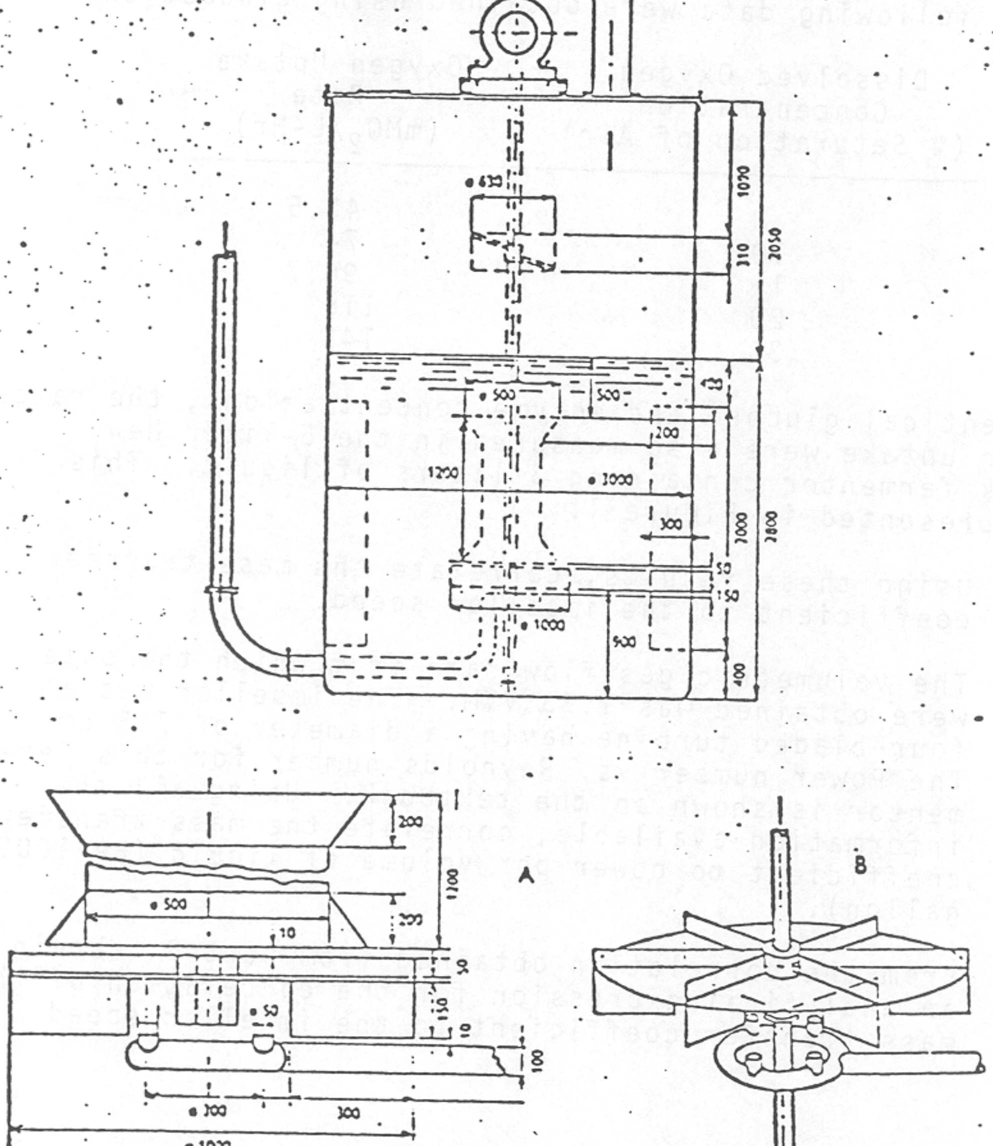

Fig. 9. Turbine aeration equipment, A, B – details of the impeller.

NOTE: ALL DIMENSIONS SHOWN ARE IN MILLIMETERS

3. The use of glucose oxidase to oxidize glucose to gluconic acid has been reported by Hsieh, Silver and Mateles (1968) as a good method of estimating the mass transfer coefficient in laboratory fermentors. Using a polarographic oxygen probe the following data were obtained using glucose oxidase:

Dissolved Oxygen Concentration (% Saturation of Air)	Oxygen Uptake Rate (mMO_2/L-Hr)
5	43.5
10	74
15	96.7
20	116
30	145

Using identical glucose and enzyme concentrations, the rates of oxygen uptake were also measured in the 5-liter New Brunswick fermentor containing 3 liters of liquid. This data is presented in Figure 1.

A. Using these results, correlate the mass transfer coefficient to the impeller speed.

B. The volumetric gas flow rate from which the data were obtained was 1.33 VVM. The impeller was a four-bladed turbine having a diameter of 7.5 cm. The Power number vs. Reynolds number for this fermentor is shown in the textbook. Using all the information available, correlate the mass transfer coefficient to power per volume of liquid (HP/1000 gallon).

C. From the correlation obtained from part B, obtain an analytical expression for the correlation of the mass transfer coefficient to the impeller speed.

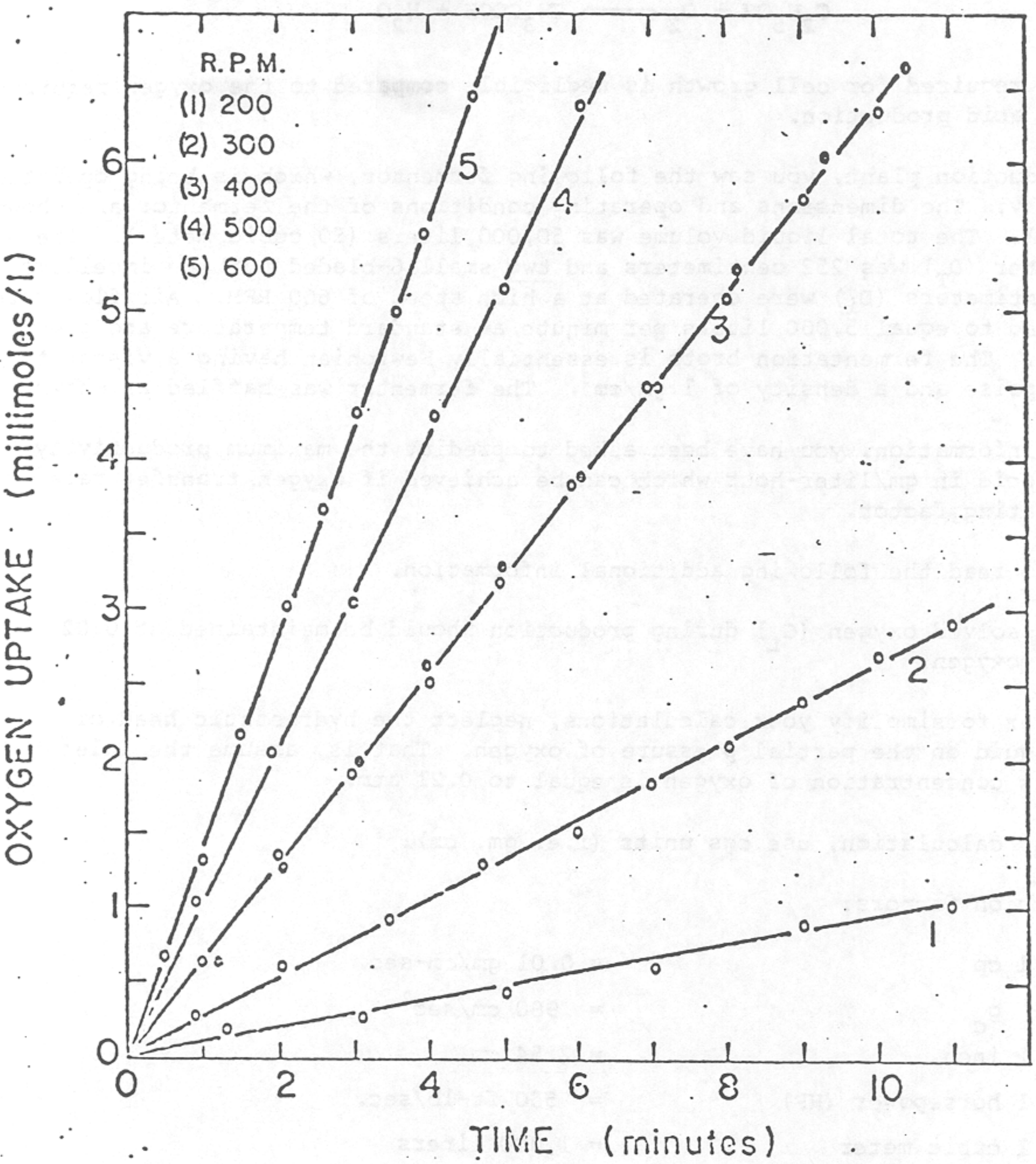

Figure 1: Oxygen Uptake Using Glucose Oxidase

4. You have been asked to consult on the production of vinegar (acetic acid) by the aerobic oxidation of ethanol using Acetobacter suboxydans. The stoichiometry of the reaction is:

$$C_2H_5OH + O_2 \longrightarrow CH_3COOH + H_2O$$

The oxygen required for cell growth is negligible compared to the oxygen required for acetic acid production.

In the production plant, you saw the following fermentor, which is being operated continuously. The dimensions and operating conditions of the fermentor are shown in Figure 1. The total liquid volume was 50,000 liters (50 cubic meter). The tank diameter (D_T) was 252 centimeters and two small 6-bladed turbine impellers each 30 centimeters (D_i) were operated at a high speed of 600 RPM. Air flow rate was measured to equal 5,000 liters per minute at standard temperature and pressure (STP). The fermentation broth is essentially Newtonian having a viscosity of 1 centipoise and a density of 1 gm/cm^3. The fermentor was baffled as shown.

From this information, you have been asked to predict the maximum productivity of acetic acid in gm/liter-hour which can be achieved if oxygen transfer rate is the limiting factor.

Please also read the following additional information.

1. The dissolved oxygen (C_L) during production should be maintained at 0.02 atm of oxygen.

2. In order to simplify your calculations, neglect the hydrostatic head of the liquid on the partial pressure of oxygen. That is, assume the inlet gaseous concentration of oxygen is equal to 0.21 atm.

3. In your calculation, use cgs units (i.e. gm, cm):

   Conversion factors:

   a. 1 cp = 0.01 gm/cm-sec.

   b. g_c = 980 cm/sec^2

   c. 1 inch = 2.54 cm

   d. 1 horsepower (HP) = 550 ft-lb/sec.

   e. 1 cubic meter = 1,000 liters

   f. 1,000 cubic centimeter (cm^3) = 1 liter

   g. 22.4 liters (STP) = 1 gm-mole

   h. To convert from gm-cm/sec. into horsepower (HP), divide by 7.6 X 10^6

FIGURE 1: SCHEMATIC DIAGRAM OF FERMENTER