

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°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-ft²-°F. After sterilization, the fermentor is cooled to 37°C with cooling water available at 17°C. You may assume that the initial medium is contaminated with 10⁵ spore/ml and 10⁷ 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 m³ 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 than 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 5×10^9 cell/ml and is operated at 37°C . If the sterilizer is operated at 62°C , what fraction of the protein in the cell will be denatured?

Some useful data:

Death constant for *E. coli* at 54°C $K = 0.25 \text{ min}^{-1}$

Denaturation constant for protein at 40°C $K_p = 5 \times 10^{-5} \text{ sec}^{-1}$

$R = \text{universal gas constant} = 1.99 \text{ cal/mol } ^\circ\text{K}$

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 ungasged 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 (N_a) 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 O₂/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 $\text{HP}/1000$ 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.

Yeast Concentration (gm/l)	Dilution Rate (Hr^{-1})	Power per Unit Volume ($\text{HP}/1000$ gal)	Superficial Gas Velocity (ft/Hr)
15	0.15	0.210	22.8
15	0.15	0.250	27.8
15	0.15	0.280	31.8
15	0.15	0.320	35.8
15	0.15	0.350	39.8
15	0.15	0.380	43.8
15	0.15	0.420	47.8
15	0.15	0.450	51.8
15	0.15	0.480	55.8
15	0.15	0.520	59.8
15	0.15	0.550	63.8
15	0.15	0.580	67.8
15	0.15	0.620	71.8
15	0.15	0.650	75.8
15	0.15	0.680	79.8
15	0.15	0.720	83.8
15	0.15	0.750	87.8
15	0.15	0.780	91.8
15	0.15	0.820	95.8
15	0.15	0.850	99.8
15	0.15	0.880	103.8
15	0.15	0.920	107.8
15	0.15	0.950	111.8
15	0.15	0.980	115.8
15	0.15	1.020	119.8
15	0.15	1.050	123.8
15	0.15	1.080	127.8
15	0.15	1.120	131.8
15	0.15	1.150	135.8
15	0.15	1.180	139.8
15	0.15	1.220	143.8
15	0.15	1.250	147.8
15	0.15	1.280	151.8
15	0.15	1.320	155.8
15	0.15	1.350	159.8
15	0.15	1.380	163.8
15	0.15	1.420	167.8
15	0.15	1.450	171.8
15	0.15	1.480	175.8
15	0.15	1.520	179.8
15	0.15	1.550	183.8
15	0.15	1.580	187.8
15	0.15	1.620	191.8
15	0.15	1.650	195.8
15	0.15	1.680	199.8
15	0.15	1.720	203.8
15	0.15	1.750	207.8
15	0.15	1.780	211.8
15	0.15	1.820	215.8
15	0.15	1.850	219.8
15	0.15	1.880	223.8
15	0.15	1.920	227.8
15	0.15	1.950	231.8
15	0.15	1.980	235.8
15	0.15	2.020	239.8
15	0.15	2.050	243.8
15	0.15	2.080	247.8
15	0.15	2.120	251.8
15	0.15	2.150	255.8
15	0.15	2.180	259.8
15	0.15	2.220	263.8
15	0.15	2.250	267.8
15	0.15	2.280	271.8
15	0.15	2.320	275.8
15	0.15	2.350	279.8
15	0.15	2.380	283.8
15	0.15	2.420	287.8
15	0.15	2.450	291.8
15	0.15	2.480	295.8
15	0.15	2.520	299.8
15	0.15	2.550	303.8
15	0.15	2.580	307.8
15	0.15	2.620	311.8
15	0.15	2.650	315.8
15	0.15	2.680	319.8
15	0.15	2.720	323.8
15	0.15	2.750	327.8
15	0.15	2.780	331.8
15	0.15	2.820	335.8
15	0.15	2.850	339.8
15	0.15	2.880	343.8
15	0.15	2.920	347.8
15	0.15	2.950	351.8
15	0.15	2.980	355.8
15	0.15	3.020	359.8
15	0.15	3.050	363.8
15	0.15	3.080	367.8
15	0.15	3.120	371.8
15	0.15	3.150	375.8
15	0.15	3.180	379.8
15	0.15	3.220	383.8
15	0.15	3.250	387.8
15	0.15	3.280	391.8
15	0.15	3.320	395.8
15	0.15	3.350	399.8
15	0.15	3.380	403.8
15	0.15	3.420	407.8
15	0.15	3.450	411.8
15	0.15	3.480	415.8
15	0.15	3.520	419.8
15	0.15	3.550	423.8
15	0.15	3.580	427.8
15	0.15	3.620	431.8
15	0.15	3.650	435.8
15	0.15	3.680	439.8
15	0.15	3.720	443.8
15	0.15	3.750	447.8
15	0.15	3.780	451.8
15	0.15	3.820	455.8
15	0.15	3.850	459.8
15	0.15	3.880	463.8
15	0.15	3.920	467.8
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15	0.15	3.980	475.8
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15	0.15	4.180	499.8
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15	0.15	4.250	507.8
15	0.15	4.280	511.8
15	0.15	4.320	515.8
15	0.15	4.350	519.8
15	0.15	4.380	523.8
15	0.15	4.420	527.8
15	0.15	4.450	531.8
15	0.15	4.480	535.8
15	0.15	4.520	539.8
15	0.15	4.550	543.8
15	0.15	4.580	547.8
15	0.15	4.620	551.8
15	0.15	4.650	555.8
15	0.15	4.680	559.8
15	0.15	4.720	563.8
15	0.15	4.750	567.8
15	0.15	4.780	571.8
15	0.15	4.820	575.8
15	0.15	4.850	579.8
15	0.15	4.880	583.8
15	0.15	4.920	587.8
15	0.15	4.950	591.8
15	0.15	4.980	595.8
15	0.15	5.020	599.8
15	0.15	5.050	603.8
15	0.15	5.080	607.8
15	0.15	5.120	611.8
15	0.15	5.150	615.8
15	0.15	5.180	619.8
15	0.15	5.220	623.8
15	0.15	5.250	627.8
15	0.15	5.280	631.8
15	0.15	5.320	635.8
15	0.15	5.350	639.8
15	0.15	5.380	643.8
15	0.15	5.420	647.8
15	0.15	5.450	651.8
15	0.15	5.480	655.8
15	0.15	5.520	659.8
15	0.15	5.550	663.8
15	0.15	5.580	667.8
15	0.15	5.620	671.8
15	0.15	5.650	675.8
15	0.15	5.680	679.8
15	0.15	5.720	683.8
15	0.15	5.750	687.8
15	0.15	5.780	691.8
15	0.15	5.820	695.8
15	0.15	5.850	699.8
15	0.15	5.880	703.8
15	0.15	5.920	707.8
15	0.15	5.950	711.8
15	0.15	5.980	715.8
15	0.15	6.020	719.8
15	0.15	6.050	723.8
15	0.15	6.080	727.8
15	0.15	6.120	731.8
15	0.15	6.150	735.8
15	0.15	6.180	739.8
15	0.15	6.220	743.8
15	0.15	6.250	747.8
15	0.15	6.280	751.8
15	0.15	6.320	755.8
15	0.15	6.350	759.8
15	0.15	6.380	763.8
15	0.15	6.420	767.8
15	0.15	6.450	771.8
15	0.15	6.480	775.8
15	0.15	6.520	779.8
15	0.15	6.550	783.8
15	0.15	6.580	787.8
15	0.15	6.620	791.8
15	0.15	6.650	795.8
15	0.15	6.680	799.8
15	0.15	6.720	803.8
15	0.15	6.750	807.8
15	0.15	6.780	811.8
15	0.15	6.820	815.8
15	0.15	6.850	819.8
15	0.15	6.880	823.8
15	0.15	6.920	827.8
15	0.15	6.950	831.8
15	0.15	6.980	835.8
15	0.15	7.020	839.8
15	0.15	7.050	843.8
15	0.15	7.080	847.8
15	0.15	7.120	851.8
15	0.15	7.150	855.8
15	0.15	7.180	859.8
15	0.15	7.220	863.8
15	0.15	7.250	867.8
15	0.15	7.280	871.8
15	0.15	7.320	875.8
15	0.15	7.350	879.8
15	0.15	7.380	883.8
15	0.15	7.420	887.8
15	0.15	7.450	891.8
15	0.15	7.480	895.8
15	0.15	7.520	899.8
15	0.15	7.550	903.8
15	0.15	7.580	907.8
15	0.15	7.620	911.8
15	0.15	7.650	915.8
15	0.15	7.680	919.8
15	0.15	7.720	923.8
15	0.15	7.750	927.8
15	0.15	7.780	931.8
15	0.15	7.820	935.8
15	0.15	7.850	939.8
15	0.15	7.880	943.8
15	0.15	7.920	947.8
15	0.15	7.950	951.8
15	0.15	7.980	955.8
15	0.15	8.020	959.8
15	0.15	8.050	963.8
15	0.15	8.080	967.8
15	0.15	8.120	971.8
15	0.15	8.150	975.8
15	0.15	8.180	979.8
15	0.15	8.220	983.8
15	0.15	8.250	987.8
15	0.15	8.280	991.8
15	0.15	8.320	995.8
15	0.15	8.350	999.8
15	0.15	8.380	1003.8
15	0.15	8.420	1007.8
15	0.15	8.450	1011.8
15	0.15	8.480	1015.8
15	0.15	8.520	1019.8
15	0.15	8.550	1023.8
15	0.15	8.580	1027.8
15	0.15	8.620	1031.8
15	0.15	8.6	

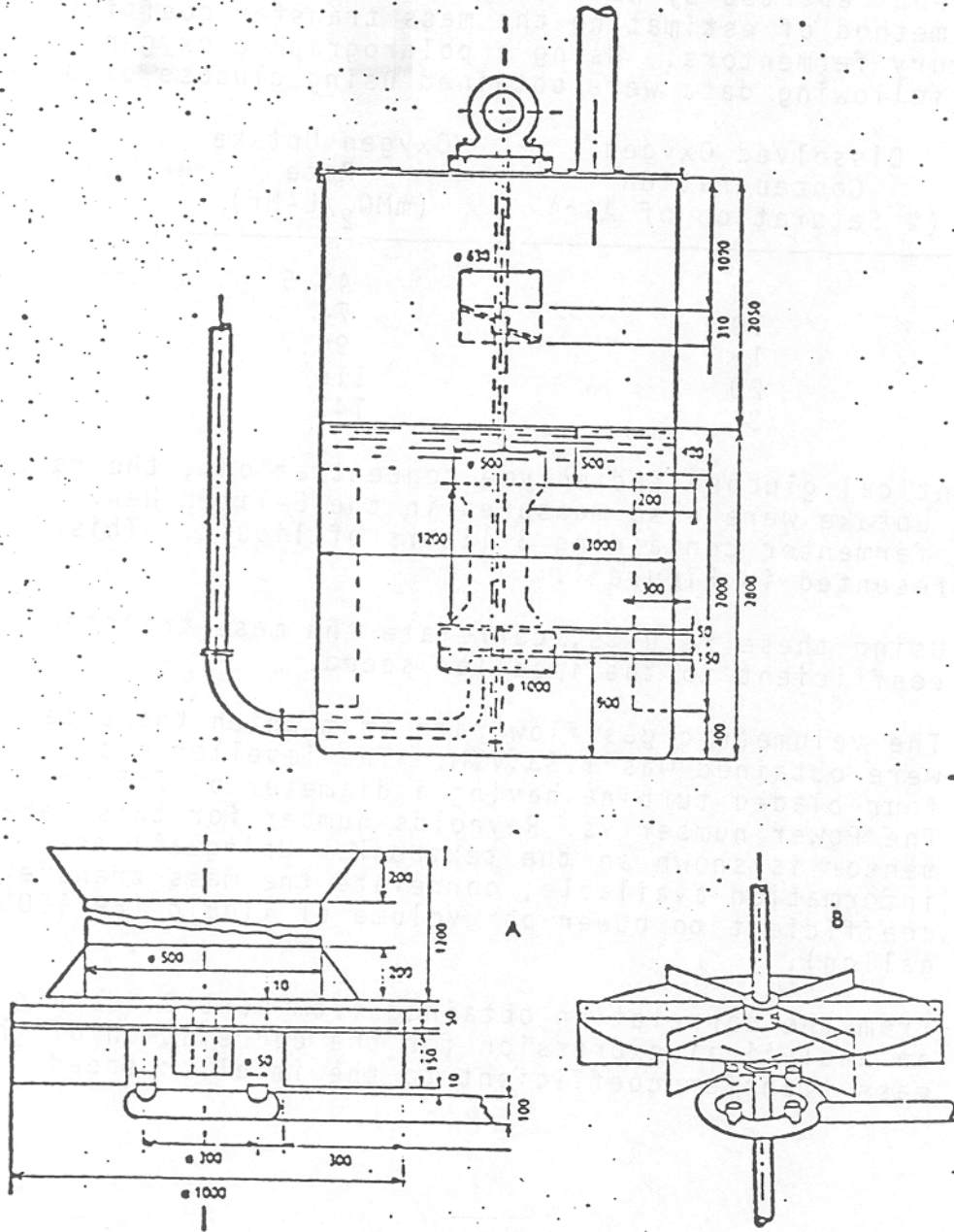


Fig. 9. Turbine aeration equipment, A, B - details of the impeller.

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 ($\text{mMO}_2/\text{L-Hr}$)
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.

- Using these results, correlate the mass transfer coefficient to the impeller speed.
- 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).
- From the correlation obtained from part B, obtain an analytical expression for the correlation of the mass transfer coefficient to the impeller speed.

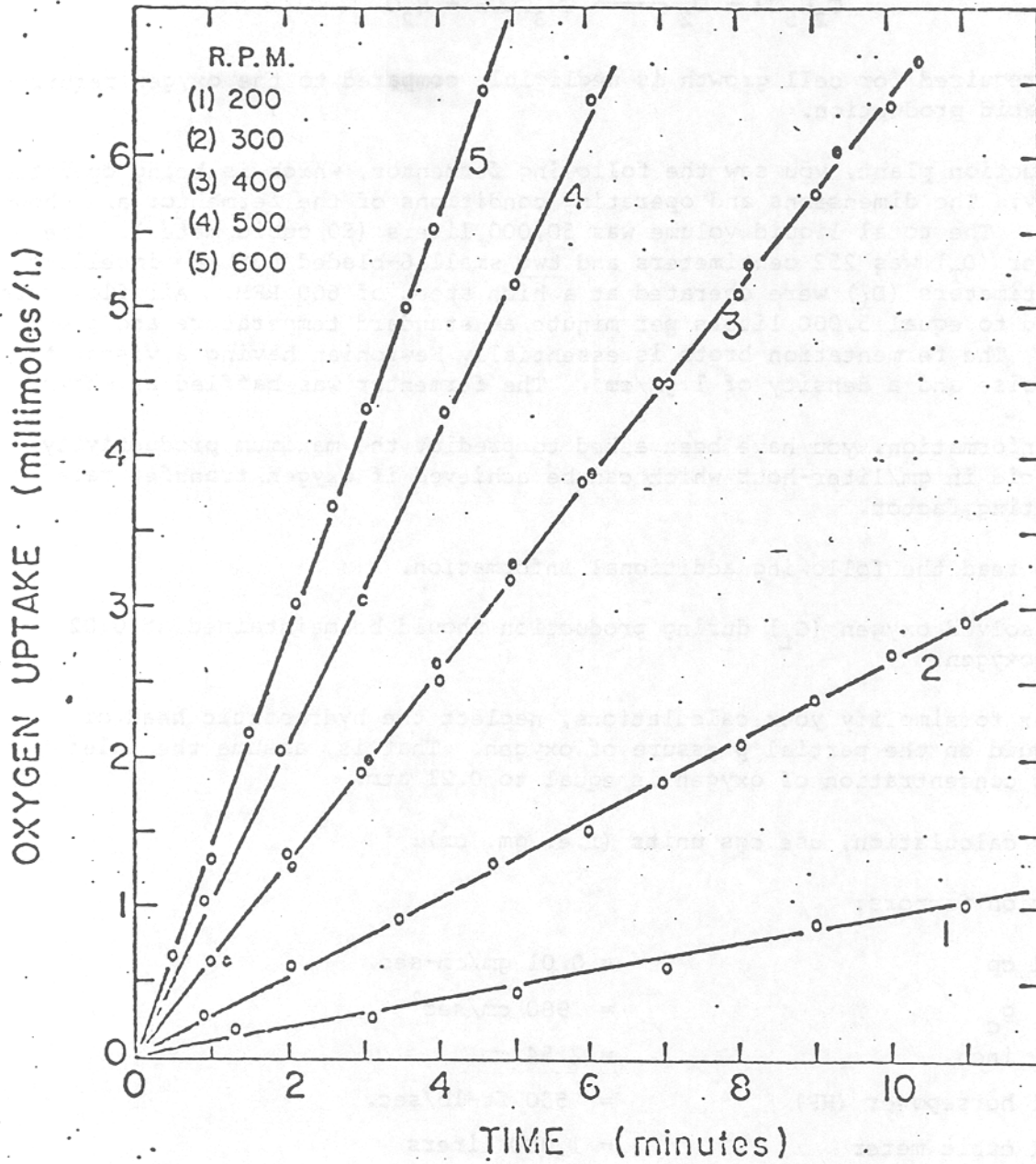


Figure 1: Oxygen Uptake Using Glucose Oxidase

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:



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/cm³. 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 7.6×10^6 |

FIGURE 1: SCHEMATIC DIAGRAM OF FERMENTER

