STUDENT NUMBER: _____
NAME (optional but I will figure out who you are eventually): _____

(This exam paper is a "HAND IN")

HAND IN
answers recorded
on question paper

QUEEN'S UNIVERSITY
FACULTY OF ENGINEERING AND APPLIED SCIENCE
DEPARTMENT OF CHEMICAL ENGINEERING

CHEE 380
BIOCHEMICAL ENGINEERING
FINAL EXAMINATION

DECEMBER 11, 2012

PROF. R.J. NEUFELD

INSTRUCTIONS: This examination is 3 hours in length. Please answer all questions in the answer booklets provided. You are allowed to use your course notes and calculator as aids. The answer will only be considered to be complete if it includes the units.

The exam consists of 5 pages, including this cover page. There are 6 questions, adding up to a total of 60 marks. Each question is worth the same number of marks (10 marks). **Up to 20% of the mark for a question may be deducted for answers that are messy, illegible, confused, unclear, or are missing units.**

Write your student number and name (optional) on the front of all answer booklets, and on the top of this exam paper. **This exam paper is to be handed in with your exam booklet.** Your final answers must be written on this exam paper when there is space provided, but the complete solution must appear neatly and clearly in the answer booklet.

PLEASE NOTE: Proctors are unable to respond to queries about the interpretation of exam questions. Do your best to answer exam questions as written.

1. Medium formulation

Saccharomyces cerevisiae (yeast) when grown aerobically on glucose produce biomass and CO_2 according to the following reaction stoichiometry. Consider yeast production in a batch reactor with working volume of 10^5 L and target cell concentration of 50 g/L. For your information, the atomic weights are C(12); H(1); O(16); N(14).

$$C_6H_{12}O_6 + 3O_2 + 0.48NH_3 \text{ -----> } 0.48C_6H_{10}NO_3 + 4.32H_2O + 3.12CO_2$$

a) Determine the total amount AND concentration of glucose required in the medium.
Answer: total amount: _____ concentration: _____

b) Calculate the yield coefficient $Y_{O2/X}$ on a mass basis. Answer: _____

c) Calculate the total amount of O_2 required on a mass basis? Answer: _____

d) If the volumetric rate of cell production during exponential phase is 0.7 g/L.h, find the volumetric rate of O_2 consumption. Answer: _____

e) Heat removal requirements on a volumetric basis (kcal/L.h). Answer:_____

2. Cell destruction kinetics

Regulatory agency guidelines require that organisms be killed prior to product recovery operations, if the organism is genetically modified. These guidelines apply for production volumes in excess of 10L. A microorganism that is commonly modified using DNA recombinant methods is *E. coli*. There is an idea that if the product of interest, such as an intracellular protein, is more stable than the microbe itself, it may be possible to heat kill the microbe, but recover a stable product.

Consider that you as new plant engineer are being asked by Fred Thermophilus, your boss, to devise a method to heat sterilize *E. coli* from a batch production in a 10 m^3 bioreactor, without damaging the intracellular recombinant protein. Because of the large volume of the vessel, you have decided to use continuous sterilization. Fran Flomass, your colleague responsible for downstream operations has suggested that you operate the piping between the bioreactor and the centrifuge as a continuous sterilization line by insulating the line. You realize that if steam is injected directly into the line with the medium, sterilization temperature can be achieved instantaneously. Flomass has also shown that at the flowrates you are considering with the pipe diameter available, the heated medium will flow such that that the axial dispersion coefficient essentially goes to zero, and the Peclet number is infinitely high.

Data provided in Figure 9.4 on next page provides the Arrhenius plot for *E. coli*. Since you are considering a sterilization temperature of 60°C, calculate the fraction of the protein product in the cell that will potentially be denatured in this sterilization protocol. You know from data provided from the lab that the bioreactor contains 5 x 10^9 cells/mL, and in your design, you choose to reduce viable cell numbers to 10^{-1} cells/batch. The denaturation constant for the recombinant protein at 60°C is 5 x 10^{-5} sec^{-1}.

Answer: _____

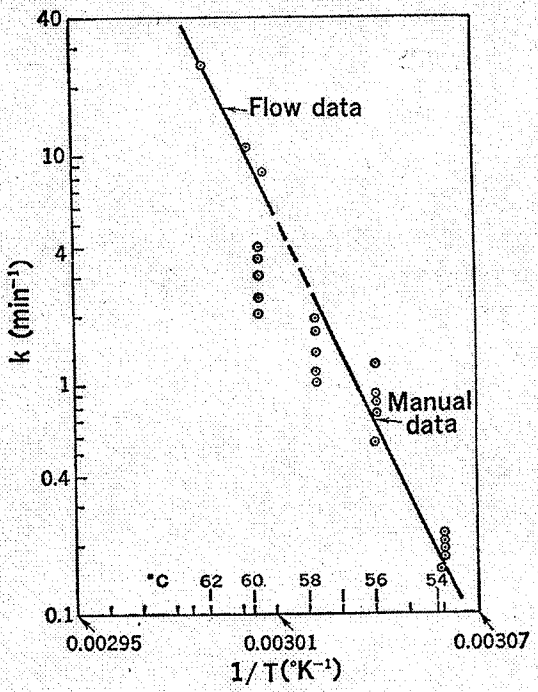


Fig. 9.4. Correlation of isothermal death rate data with temperature for *E. coli*, where k=reaction rate constant and T=absolute temperature. Data points at lower temperatures were measured with apparatus

3. Growth kinetics of mammalian cell culture/reactor power requirements

Cellular immunotherapy involves the treatment of a patient with large doses of their own T cells (T lymphocytes are a type of white blood cell produced in the Thymus). The large numbers of freshly grown T cells are then injected into the patient to combat certain types of cancers or infections. It is necessary to culture the T cells in a bioreactor to achieve the numbers required.

a) Under optimal conditions, it is possible to get an 18 fold expansion in cell numbers in 4 days. If you assume that growth is first order in T cell concentration, determine the first order rate constant for T cell growth. Answer: _____

b) If you assume that 10^7 cells are originally isolated from the patient, how long would it take to produce 10^{10} cells to return to the patient? Answer: _____

c) To culture the T cells described above, it was decided to retrofit (modify) an existing bioreactor operating with 2 Rushton flat-blade turbine impellers. Your objective is to reduce shear forces on the cells, and to promote more of a vertical mixing pattern by replacing the turbine impellers with 1 marine propeller type mixer. What would be the relative value of ungassed power per unit volume for the retrofitted vessel, expressed as a percentage of the ungassed power available with the existing bioreactor? Be sure to state your assumptions in answering the question. Answer: _____
Assumptions: _____

4. Oxygen transfer rate

a) The oxygen consumption rate in a bioreactor was measured during active growth by shutting off the aeration and measuring the rate of decline in dissolved oxygen levels. The following results were obtained.

Time (sec)	O_2 (% of saturation)	O_2 (mmol O_2/L)
0	75	0.165
10	58	0.128
30	12	0.026

a) Using this data, calculate the volumetric oxygen transfer rate to the bioreactor, prior to shutting off the aeration, and express your result in mmol/L.h.

Answer: _____

Growth kinetics (related to problem above)

b) In the hours preceding the above test, cell density was measured, and results are shown below. Calculate the yield of cells on oxygen (g_{cells}/g_{O2}) for this culture. Use the oxygen consumption rate data presented above.

Time before test (h)	Cell mass (g/L)
-4	0.1
-2	0.5
0	2.1

Answer: _____

5. Heat transfer: Bioreactor cooling

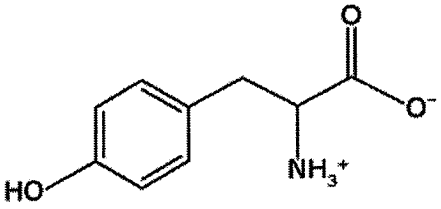
The major limitations in scale-up to very large bioreactors is thought to be the removal of heat, and not so much in the mass transfer of oxygen. The reason is that the heat transfer area increases in proportion to the radius squared, while the volume (related to the total heat generated by the microorganism in the medium) increases with the radius cubed.

I will describe a bioreactor to you, and will ask you to calculate and tell me the largest feasible reactor vessel possible for this process. Answer in L or m^3: _____

Bioreactor specifications:
- cooling equipment: cooling jacket around wall of vessel (no coils)
- height/diameter (H_L/D_T) ratio of 2/1
- material of construction: stainless steel
- cooling water available at 15°C
- discharged water regulated at no higher than 30°C
- bioreactor operating temperature 35°C
- overall heat transfer coefficient during cooling: 350 kcal/m^2h°C
- heat generation by culture: 30 kcal/L.h
- power input from agitation: 1.5 kcal/L.h

6. Downstream processing

L-tyrosine is an amino acid produced as an extracellular product through biological processing, and used therapeutically to reduce fatigue and stress (tempting during exam time, but don't even think about it without consulting with your doctor). Assume that you are responsible for developing the conceptual design for the downstream operations to purify L-tyrosine to high level of purity necessary for therapeutic use. Think carefully about what unit operations may be appropriate to purify this molecule, given its particular size, structure (illustrated below) and properties. In the answer booklet, on one page, neatly draw a process flow sheet starting with the bioreactor through to final product. Beware, since this question is a trap. Throw in too many unit operations in crazy order, and you will convince me that you really don't understand the key stages in downstream operations. On the other hand, providing a smaller number of unit operations is gutsy, but if assembled in a logical order can be very convincing. Label each of the units and in a few words (6 will do), explain the purpose of each unit in the separation scheme. Also, clearly identify the various key grouping of steps in your downstream operation so I know what you are doing.



All the best in your exams. Have a restful and safe holiday break. Hope you had as much fun as I did with chee 380. See you in 2013. **R.J. Neufeld (ho ho ho)**