UNIVERSITY OF TORONTO
FACULTY OF APPLIED SCIENCE AND ENGINEERING

FINAL EXAMINATION, DECEMBER 14, 2001
Fourth Year
CHE 466F - BIOPROCESS ENGINEERING

EXAMINER - E.A. EDWARDS

NAME:________________________________________

All programmable and non-programmable calculators permitted.

Marks indicated on each question (Total = 100)

**As always, in multiple part questions, if you think you need an answer from a previous part of the question that you couldn't solve, assume a value to illustrate how you would solve the question.**

Additional Information:
Ideal gas law: $PV = nRT$ $R = 0.08206$ L atm/mol K
Solubility of pure oxygen at 1 atm in water@ 25°C = 1.26 mmol/L = 40.3 mg/L
Air is 21% oxygen and 79% nitrogen
Molar mass: H - 1 g/mol; C - 12 g/mol; O - 16 g/mol; N - 14 g/mol

**Bioreactor Design Parameters and Equations**

$$\theta = \frac{V}{Q^o}$$

$$\mu = \mu_{max}\frac{S}{K_s + S} - k_d$$

$$\mu_{max} = Yk$$

$$\theta_c = \frac{VX}{X^e Q^e + X^w Q^w}$$

$$\frac{1}{\theta_c} = \frac{YkS}{K_s + S} - k_d$$

$$X\ (\text{biomass}) = \frac{\theta_c}{\theta}\left[\frac{Y(S^o - S^e)}{1 + k_d\theta_c}\right]$$

$$W = \frac{X\,V}{\theta_c^d} \quad \text{(biomass production rate)}$$

Substrate Removal Rate $= Q^o(S^o - S^e)$

Required $O_2 = Q^o(S^o - S^e) - 1.42W$ (if substrate as COD)

$$S = \frac{K_s(1 + k_d\theta_c)}{\theta_c(Yk - k_d) - 1}$$

$$E = 100\left(\frac{S^o - S^e}{S^o}\right)$$

**The Genetic Code**

| First Position (5' end) | Second Position U | C | A | G | Third Position (3' end) |
|---|---|---|---|---|---|
| U | Phe | Ser | Tyr | Cys | U |
| | Phe | Ser | Tyr | Cys | C |
| | Leu | Ser | Term | Term | A |
| | Leu | Ser | Term | Trp | G |
| C | Leu | Pro | His | Arg | U |
| | Leu | Pro | His | Arg | C |
| | Leu | Pro | Gln | Arg | A |
| | Leu | Pro | Gln | Arg | G |
| A | Ile | Thr | Asn | Ser | U |
| | Ile | Thr | Asn | Ser | C |
| | Ile | Thr | Lys | Arg | A |
| | Met* | Thr | Lys | Arg | G |
| G | Val | Ala | Asp | Gly | U |
| | Val | Ala | Asp | Gly | C |
| | Val | Ala | Glu | Gly | A |
| | Val† | Ala | Glu | Gly | G |

Abbreviations: Ala - alanine, Arg - arginine, Asn - asparagine, Asp - aspartate, Cys - cysteine, Gln - glutamine, Glu - glutamate, Gly - glycine, His - histidine, Ile - isoleucine, Leu - leucine, Lys - lysine, Met - methionine, Phe - phenylalanine, Pro - proline, Ser - serine, Thr - threonine, Trp - tryptophan, Tyr - tyrosine, Val - valine, Term - chain termination.

**Question 1: (24 marks total)**
Briefly define or explain the following. Each part is worth **3 marks**.

a. Electrophoresis;
b. The overall reaction for the Krebs cycle;
c. The basis for separation in gel permeation chromatography;
d. Prof. Yip's seminar topic;
e. Chemolithotrophy;
f. Chemiosmosis;
g. Antisense strand;
h. Extremophile

**Question 2: (5 marks)**
Show the location of the control and information sequences on a gene that guide the process of gene expression in a cell. Briefly describe the purpose of each region of the gene.

**Question 3: (5 marks)**
What are the major forms of sulphur in the sulphur cycle?

**Question 4: (3 marks)**
Dolly was the first mammal (sheep) cloned from an adult cell. This was a remarkable achievement. What about this achievement was so surprising to developmental biologists?

**Question 5: (5 marks)**
How is enzyme activity regulated in a cell? List the two fundamentally different methods a cell uses for regulating enzyme activity.

**Question 6: (3 marks)**
Explain the difference between substrate-level phosphorylation and oxidative phosphorylation

**Question 7: (18 marks total)**
In many enzyme-catalyzed reactions, another metabolite is used in order to activate or inhibit the enzyme. In the Krebs Cycle pathway, the enzyme isocitrate dehydrogenase catalyzing the conversion of isocitrate to α-ketoglutarate is inhibited by adenosine triphosphate (ATP).

$$S \xrightarrow{E} P + \text{other products}$$

Use the following nomenclature for this question:

E: Enzyme isocitrate dehydrogenase
S: Substrate (isocitrate)
P: the main product (α-ketoglutarate)
I: The inhibitor (ATP)
A: The activator (ADP; see parts c and d)

Use lower case (s, e, p, i, a) to denote concentrations.

a. Propose a mechanism for this reaction, assuming that the ATP inhibition takes place non-competitively. Show the mechanism only **(3 marks)**

b. If the reaction described in part (a) has a Michaelis Menten constant of 0.001 M when no ATP is present and the concentration of isocitrate in the cell is 0.1 M, what can the cell do to increase the rate of the reaction at a given concentration of ATP? **(3 marks)**

c. In actual fact, the situation is more complex than that outlined in part (a). In addition to the inhibition by ATP, the reaction is activated by ADP. The reaction rate is increased when ADP binds to the enzyme. In other words, the reaction can be catalyzed just by enzyme only, or at a higher rate by Enzyme-ADP complex (EA). As in part (a), the binding of ATP inactivates the enzyme non-competitively. The site of activation and inactivation are the same. Propose a mechanism for this process. **(5 marks)**

d. Set up the equations that you would need to solve for the reaction velocity (rate of product formation) as a function of the concentrations s, $e_o$, i, a, and any rate or equilibrium constants. Make all the assumptions normally made for Michaelis Menten Kinetics. **<u>Do not solve the equations</u>**. **(5 marks)**

e. The other products are carbon dioxide and 2H. Where do the 2H go? **(2 marks)**

**Question 8: (10 marks total)**
An engineer has postulated that a particular sequence of amino acids in an enzyme (Glycine, Alanine, Valine) are key to the tertiary structure of the active site. In order to test this hypothesis, the engineer decides to genetically modify the gene that produces the protein so that these 3 amino acids will be changed. The idea is to substitute 3 new amino acids that would cause the shape of the active site to change dramatically. The kinetic activity of this modified enzyme would be tested to see if it is decreased in comparison with the unmodified enzyme.

a. List 3 amino acids that the engineer might use to replace the three in the active site. Briefly justify your choice. **4 marks**

b. What could be the base sequence for the three amino acids in the template strand of the DNA in the gene for the "normal" enzyme and the modified enzyme. You only need to show one example for each gene. What type of mutation(s) are required for any one of the codons (pick one) of the amino acids? **6 marks**

**Question 9: (27 marks total)**
Consider an activated sludge process. The influent wastewater soluble substrate concentration is 200 mg COD/l. The wastewater flow is 10,000 $m^3/d$. The design $\theta_c$ is 3.8 days. The following growth parameters apply:

| Parameter | Activated Sludge |
|---|---|
| Y | 0.45 g cells/g COD |
| k | 12 g COD/g cells.d |
| Ks | 200 mg COD/l |
| $k_d$ | 0.15 per day |

a. Calculate the effluent substrate concentration. **(1mark)**

**Part 1:** The waste is to be treated in a complete mix reactor with <u>no</u> recycle:

b. Calculate the volume of the reactor. **(2 marks)**

c. Calculate the concentration of microorganisms in the reactor. **(2 marks)**

**Part 2:** The Engineers decide that a reactor with no recycle is too big and expensive. They have at their disposal a tank with a volume V= 3000 $m^3$, and propose to set up a system with recycle of settled sludge.

d. Calculate the hydraulic residence time in this new tank. **(2 marks)**

e. What would the biomass ($X_b$) concentration be in this tank (assuming recycle)? **(2 marks)**

f. Calculate the mass of sludge wasted per day. **(2 marks)**

g. What tests should the engineers perform to make sure that this value of $X_b$ could be maintained in the reactor? **(2 marks)**

h. If the mass transfer coefficient for aeration ($k_l a'$) is 0.1 $s^{-1}$, will the system be oxygen-limited? **(3 marks)**

**Part 3:** Actually, biomass is not the only kind of suspended solids in the bioreactor. The incoming wastewater also contains suspended solids that contribute to the overall suspended solids in the process. (see diagram below)

i. Consider the case when the <u>influent</u> to the activated sludge process now contains 60 mg/l of inert inorganic suspended solids. Derive an equation for the steady state concentration of inorganic suspended solids in the reactor ($X_{in}$) as a function of $\theta_c$, $\theta$, and the influent concentration ($X_{in}^{o}$). Assume that these suspended solids settle at the same rate as biomass. In other words: $(\theta_c)_{X_{in}} = (\theta_c)_{X_b} = \theta_c$ **(3 marks)**

j. The wastewater also contains 100 mg/l degradable suspended solids, and that the first order degradation rate for these solids is 0.2 $d^{-1}$. Derive an equation for the steady state concentration of degradable suspended solids ($X_d$), assuming first order decay. Again, assume that these suspended solids settle at the same rate as biomass. In other words: $(\theta_c)_{X_d} = (\theta_c)_{X_b} = \theta_c$ **(3 marks)**

k. Calculate the total suspended solids ($X_t$) in this system with recycle including the contributions of these influent suspended solids. **(2 marks)**

l. Calculate the total mass of sludge wasted per day. Compare this to your answer in Part f. and comment on the impact of influent suspended solids on sludge production. **(3 marks)**

**THE END**