INDIAN INSTITUTE OF TECHNOLOGY KHARAGPUR

End-Spring Semester Examination 2024-25

Date of Examination: 21-04-2025 Session: (FN/AN) FN Duration: 3 Hours

Full Marks: 50 Subject No.: CH40001 Subject: Biochemical Engineering

Department/Center/School: Department of Chemical Engineering

Specific charts, graph paper, log book etc., required: graph paper needed

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This question paper has 4 pages: Part A (4 questions) and Part B (8 questions)

Part - A

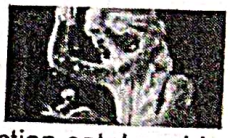
1. Let It Flow... or not?

In the land of Arendelle, Queen Elsa's icy powers accidentally mess up cellular metabolism. She's unknowingly inhibiting glycolysis in her royal cells. Her chief biochemist, Olaf the Snowman, suspects that Phosphofructokinase-1 (PFK-1) is being inhibited by high ATP levels—because Arendelle has had too many feasts and not enough sled rides.

PFK-1 catalyzes a key irreversible step in glycolysis and follows Michaelis-Menten kinetics with the following parameters:

K_m for fructose-6-phosphate = 0.3 mM

V_{max} = 150 µmol/min



a) Olaf measures the fructose-6-phosphate concentration at 0.1 mM. Calculate the rate of reaction catalyzed by PFK-1.

b) Olaf wants to activate glycolysis to generate heat for Arendelle's freezing citizens. What substrate concentration is needed for PFK-1 to reach 75% of its V_{max}?

c) Explain how high ATP levels act as allosteric inhibitors of PFK-1.

d) Based on your understanding of glycolysis, explain why this inhibition is important for metabolic balance, and how Anna's running up North Mountain might reverse it?

e) Meanwhile, Bruni, the magical gecko, scurries across icy ceilings without falling. What force allows Bruni to stick to vertical and inverted surfaces, and how is this similar to how enzymes bind substrates? **[5]**

2. The Glycolysis Gauntlet at Hogwarts

Professor Snape is furious. Someone in Potions class has accidentally added polyjuice potion to the glucose stock. The potion didn't transform students— but it altered the glycolysis spell, making it function with only one molecule of glyceraldehyde-3-phosphate (G3P) instead of the usual two.

Harry (the G3P) manages to move forward in the pathway, but Ron (the DHAP) never gets isomerized and is left behind. Snape assigns detention and a metabolic analysis.

a) Normally, each glucose molecule is split into 2 triose phosphates: G3P and DHAP. What enzyme converts DHAP to G3P, and why is this step important for ATP yield?

b) In standard glycolysis, how many net ATP molecules are generated per glucose, and what would happen to this number if only one G3P continues down the pathway?

c) List at least one step in glycolysis that produces ATP, and identify which enzyme is responsible for this.

d) If NAD^+ availability drops (due to magical stress or lack of oxygen in the dungeon), what happens to the step involving G3P, and how does this affect ATP generation?

e) Why is it more energy-efficient for the cell to allow both G3P and DHAP to proceed through glycolysis?

f) After glycolysis in the Hogwarts lab, Harry notices that when oxygen is present, cells produce much more energy than when it's not. What is the final product of glycolysis, and where does it enter for further reactions when oxygen is available?

g) When oxygen is present and the cell completes aerobic respiration, approximately how many ATP molecules are generated per glucose molecule in total? Briefly explain why this number is higher. **[7]**

3. **The Case of the Radioactive Whisper – A 1952 Lab Mystery**

The year is 1952. In a smoky lab filled with buzzing machines and vinyl records, two scientists—Hershey and Chase—are tracking a microscopic saboteur: the bacteriophage. The duo runs two experiments using radioactive isotopes to phage DNA and proteins:

In Experiment A, they use ^{35}S.

In Experiment B, they use ^{32}P.

After allowing infection and blending away the outer viral coats, they measure the radioactivity inside the bacterial cells.

a) If they find radioactivity inside the bacteria only in Experiment B, what does this tell us about which part of the virus entered the cell? What logical conclusion can they draw about the genetic material?

b) Suppose they had observed radioactivity inside the bacteria in both experiments. Would their conclusion change? Why or why not? **[2]**

4. **The Mystery of the Missing Metabolite – A Sherlock Holmes Whodunit**

Sherlock Holmes has been summoned to investigate a break-in at Cell HQ. One essential molecule has vanished, causing major disruption. Watson has narrowed down the suspects to four molecular entities: Carbohydrates, Lipids, Proteins, and Nucleic Acids. Holmes interrogates each macromolecule to determine their characteristics and functions.

Your task: Help Holmes deduce who does what and who's innocent. Also, since Holmes likes to brag about his powers of deduction, help him by mentioning the **detailed reasoning** of your deduction.

a) The first suspect is round and hydrophobic, often found hanging out in membranes and storing energy long-term. Who is it? Explain.

b) Another suspect is made of repeating sugar units and was seen providing quick energy and structural support in the cell wall. Who is this? Explain.

c) The third suspect insists they're innocent because they're too busy catalyzing reactions and building structures like hair and enzymes. Who is this? Explain.

d) One of the molecules left behind a strand of code at the crime scene. Who's this genetic informant? Explain.

e) Which of the four suspects is not a polymer in the traditional sense, and how is it structurally different?

f) Holmes finds that the stolen metabolite was involved in making cell membranes. Which macromolecule is now back under suspicion, and what specific type might it be? **[6]**

Part - B

Q.1. Explain the typical microbial cell growth cycle in detail. [4 Marks]

Q.2. In a continuous culture, using ideal chemostat show that dilution rate is equal to specific growth rate of cells. Also find the steady state substrate and cell mass concentration. [Please specify all the assumptions clearly] [4 Marks]

Q.3. Baker's yeast has a minimum doubling time of 2 h when grown on acetate in a chemostat that follows the Monod model. [6 Marks]

Given K_S = 1.5 g/L, $Y_{X/S}$ = 0.5 g cell / g acetate, S_0 = 80 g / L.

(a) Find out steady-state S and X when D = 0.8 D_{max}.

(b) Find the cell mass productivity at 0.5 D_{max}.

(c) Determine the value of $D_{washout}$.

Q. 4. A simple batch fermentation of an aerobic bacterium growing on methanol gave the results shown in the table. Calculate: [6 Marks]

a. Maximum growth rate (μ_{max})

b. Yield coefficient ($Y_{X/S}$)

c. Mass doubling time (t_d)

d. Saturation constant (K_s)

e. Specific growth rate at t = 10 h

f. Maximum expected cell mass concentration is expected when 150 g of substrate were used in the same size of inoculum.

Time (h)	X (g/l)	S(g/l)
0	0.5	100
2	1.0	95
5	2.1	85
10	4.8	58
15	7.7	30
20	9.6	12
25	10.4	5
30	10.7	2

Q.5. Volumetric flow rate in constant pressure filtration is given by

$$\frac{dV}{dt} = \frac{1}{0.5\,K_C V + 2\,Q_0}$$

Where V is the total volume of filtrate collected in time t, Kc and Q_0 are constants. [3 Marks]

a) What is the relation between V and t.
b) Draw a graph for the relation obtained in a.
c) For V = 1 lit at t = 41.3 sec and V = 2 lit at 108.3 sec, find the value of Kc
d) Find the value Q_0.
e) Find the time required to process 3.5 lit of volume.
f) Find the value of slope and intercept for graph obtained in b.

Q.6. Name different steps involved in downstream processing and mention different methods involved in each step. [2 marks]

Q.7. What do you mean by centrifugation and liquid - liquid extraction? What is the basic working principle in centrifugation and liquid-liquid extraction? Which method will you prefer for the separation of insoluble products and why? How the separation occurs in your preferred method. [3 Marks]

Q.8. A binary mixture containing 100 μm yeast cells having densities 2 gm/cm^3 and 4 gm /cm^3 is to be classified by the sedimentation technique using water. Estimate the range of velocities that can do a job and recommend a suitable value. [Hint: Assume the applicability of Stoke's law]. [2 Marks]

----------Best of luck----------