FACULTY OF SCIENCE

DEPARTMENT OF BIOTECHNOLOGY AND FOOD TECHNOLOGY

B-TECH DEGREE IN BIOTECHNOLOGY

MODULE BIC1YB4

MICROBIAL BIOCHEMISTRY 4

CAMPUS DFC

NOVEMBER EXAM 2014

DATE: 01/11/14	**SESSION 12:30-15:30**
ASSESSOR(S)	**MR F.Y FONKUI**
EXTERNAL MODERATOR	**DR V. MAVUMENGWANA**
DURATION 180 MINUTES	**MARKS: 107**
TOTAL PAGES:	**4 PAGES**

INSTRUCTIONS:

- **ALL QUESTIONS ARE COMPULSORY.**
- **EACH CANDIDATE SHOULD HAVE ONE ANSWER SCRIPT AND A GRAPH PAPER.**
- **CALCULATORS ARE ALLOWED IN THE EXAM HALL AND CELL PHONE SHOULD NOT FOR ANY REASON BE USED AS CALCULATOR.**

Question 1

Enzymes such as RNA polymerase present in *E. coli* are known to be involved in certain metabolic activity essential for life.

a) What is the name of the reaction catalyzed by this enzyme? (1)
b) This polymerase belongs to which class or family of organized enzyme system among the three different groups known? (Be specific because no mark will be allocated for answer like complex system). (2)
c) Which are the most frequent signals that trigger the termination of the reaction catalyzed by this particular enzyme? (2)

Pyruvate dehydrogenase (PDH) complex is one among several other multienzymes complexes or multienzyme polypeptides found both in eukaryote and prokaryotes sharing some common features and disparities.

d) Name two other multienzyme complexes found in these organisms. (2)
e) What are the similarities and differences shared by pyruvate dehydrogenase and the other multienzyme system mentioned in question 1.d? (8)

[15]

Question 2

Carbohydrates like glucose are known as energy sources for many living organisms. Yet literature reveals that bacteria such as *Escherichia coli* are unable to directly make use of free glucose because it cannot cross their cellular membranes. With the aid of equations describe the mechanism through which this molecule is transported into *E. coli* cells. **[10]**

Question 3

An enzyme exhibiting Michaelis Menton kinetics has a velocity of 0.2 V_{max} at a substrate concentration of 1.0 mM. What substrate concentration is required to triple the velocity? Show all calculation to have full mark. **[5]**

Question 4

After defining the EMP and ED pathways, clearly differentiate between these two pathways making use of the right terminologies. **[15]**

Question 5

Ten micrograms of a pure enzyme with a molar mass (MW = 102,000) catalyzes a reaction at a rate of 0.250 µmoles/min under optimum conditions. Calculate:

a) The specific activity of the enzyme in terms of units/mg protein and units/mol (2)
b) The turn over number (1)
c) How long is one catalytic cycle. (2)

[5]

Question 6

Discuss three experimental approaches biotechnologists normally use to determine the mechanism of enzyme action.

NB. Please note that no mark will be allocated for enumerating them. **[12]**

Question 7

Draw a velocity vs substrate concentration {v = f([S])} and Lineweaver-Burk plot for an enzyme catalysed reaction exhibiting Michaelis Menton kinetics; on each plot, indicate how the plots would be affected by the presence of an uncompetitive inhibitor. Clearly indicate how each plot can be used to determine kinetics data for the enzyme. **[10]**

Question 8

An enzymatic assay was carried under two different sets of conditions out using a pure substrate S. The results are tabulated below.

[S]/10^{-5} M	**V_o**	
	Condition A	**Condition B**
1.5	**0.21**	**0.08**
2.0	**0.25**	**0.1**
3.0	**0.28**	**0.12**
4.0	**0.33**	**0.13**
8.0	**0.44**	**0.16**
16.0	**0.4**	**0.18**

1. Using Lineweaver-Burke plot, determine V_{max} and K_m for both sets of conditions (10)
2. Assuming that **condition B** refers to data obtained in the presence of a competitive inhibitor. What effects will be expected as Michaelis Menton data are concerned? (2)
3. Determine the type of inhibition. (2)
4. Determine K_I following the condition defined in question 6.3 above if inhibitor's concentration is 5µM. (5)
5. If the total enzyme concentration was 1 nM, what would Kcat be for condition A? (3)
6. Calculate Kcat/K_m for the enzyme and draw conclusions on the efficiency of the enzyme? (3)
7. The same enzyme was inhibited by an irreversible inhibitor. Sketch on the same graph the expected curve for this inhibition. (3)

[23]

Question 9

Discuss the regulation of glycolysis focussing mainly on the activity of 6-PFK. **[12]**
