MANIPAL INSTITUTE OF TECHNOLOGY

MANIPAL

(A constituent unit of MAHE, Manipal)

VII SEMESTER B.TECH. END SEMESTER EXAMINATIONS

FEBRUARY 2021

SUBJECT: [BIO 4007] Metabolic Engineering [PE5]

Date of Exam: **01-02-2021** Time of Exam: **9AM-12 noon** Max. Marks: **50**

Instructions to Candidates:

- Answer ALL the questions & missing data may be suitably assumed

1A.	The genes involved in the informational processes such as replication, transcription, and translation are transferred between species much less often than the genes involved in metabolism - contemplate	**5**
1B.	From a chemical perspective, the glycolytic pathway can be divided in two stages. The first stage from glucose to glyceraldehyde 3-phosphate (G3P) prepares glucose so that it is spliced into G3P and an equivalent three-carbon fragment, which is then converted into G3P. The second stage - 'the payoff phase', from G3P to pyruvate, produce energy in the form of ATP and NADH. **i)** Write the balanced equation for the First stage of glycolysis (glucose →G3P) **ii)** Write the balanced equation for the second stage of glycolysis (G3P →pyruvate) **iii)** Write the balanced equation for the overall pathway (glucose → pyruvate)	**5**
2A.	Suppose you identify a gene that may play an essential role in the biosynthetic pathway that makes a specific compound. How would you predict the function or biochemical activity of the protein encoded by the identified gene? Choose the following and explain why your choice is correct? Also, add why the other choices are incorrect? **i)** Gene sequence comparison to all other genes to find if it is similar to known genes. **ii)** Express and purify the protein encoded by the gene and study its enzyme functions *in vitro*. **iii)** Express the gene in *E. coli* and determine whether *E. coli* can produce the compound. **iv)** Make mutations in the gene and determine how they affect synthesis of the compound.	**5**

2B.	Brief about the transcriptional network motifs such as FFL, double-input, multi-input, multi-output, and multi-Y motifs, and add a note on coherent and incoherent FFL.	**5**
3A.	Explain the Gene-Protein-Reaction (GPR) network with an example for succinate dehydrogenase (Sdh). Do the enzyme considered to be promiscuous? Explain why? Note: There are four gene loci for Sdh (b0721, b0722, b0723, and b0724, respectively)	**5**
3B.	Explain why the reactions of the citric acid cycle do not directly require the presence of oxygen	**5**
4A.	Explain about the regulation of metabolic pathways. Brief about positive and negative regulation both at protein and gene level. Also give some examples for sequential, concerted, and cumulative feedback inhibitions on a branched metabolic pathway.	**5**
4B.	In anaerobic conditions the glucose consumption is high at a steady rate. When oxygen is added, suddenly the glucose consumption drops and it is maintained at a lower rate. Explain why the glucose consumption is high in the absence of oxygen, whereas it is lower in the presence of oxygen?	**5**
5A.	Assume that the substrate (cycloalkane or glucose as a carbon source) for a particular microorganism. It was observed that the microorganism converts two-thirds (wt/wt) of the assimilated carbon into biomass. i) calculate the stoichiometric coefficients for the following metabolic reactions: Cyclohexane: C_6H_{12} + *a* O_2+ *b* NH_3 --> c ($C_{4.4}$ $H_{7.3}$ $N_{0.86}$ $O_{1.2}$) + *d* H_2O + e CO_2 Glucose: $C_6H_{12}O_6$ + *a* O2 + *b* NH_3 --> c ($C_{4.4}$ $H_{7.3}$ $N_{0.86}$ $O_{1.2}$) + *d* H_2O + e CO_2 ii) Calculate the yield coefficients $Y_{X/S}$ (g dw cell/g substrate), $Y_{X/O2}$ (g dw cell/g O_2) for both reactions. Comment on the differences	**10**