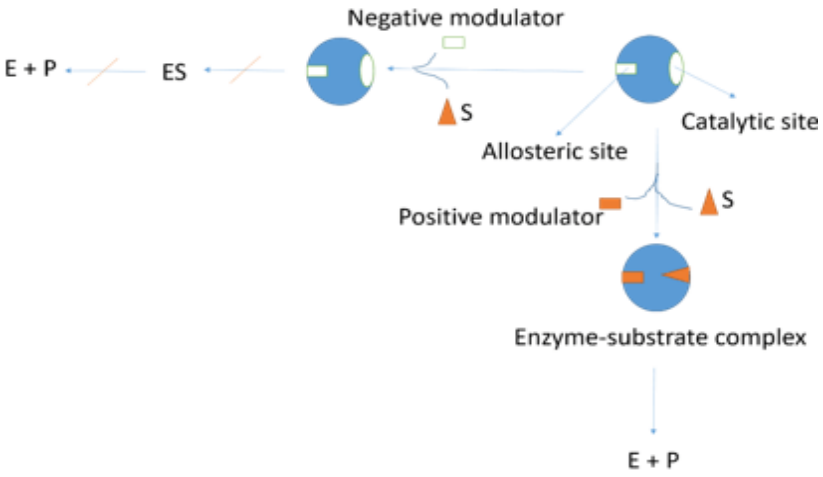
Name: **Enrolment No:**	UPES

UNIVERSITY OF PETROLEUM AND ENERGY STUDIES
End Semester Examination, December 2022

Course: Biochemistry and Metabolic Engineering **Semester: III**
Program: B.Tech Biotechnology **Duration: 03 hrs.**
Course Code: HSBT2001 **Max. Marks: 100**
Instructions:

	SECTION A **(Type the answers in test box)**	**(20Q x1.5M= 30 Marks)**	**CO**
	MCQs or Fill in the blanks		
Q1	Which of the following enzyme is present in the rate-limiting step in glycolysis? a) Enolase b) Phosphofructokinase c) Phosphohexose isomerase d) Glyceraldehyde-3-phosphate dehydrogenase	**1.5**	**CO1**
Q2	Which of the following is not a factor determining the activity of an enzyme? a) Association with regulatory protein b) Sequestration c) Allosteric regulation d) Nucleotides	**1.5**	**CO1**
Q3	Which of the following statements is true about brain metabolism in starvation? a) The brain can use glucogenic amino acids for energy b) The brain can only use glucose as fuel c) Up to a quarter of energy requirement of the brain can come from fatty acids d) Up to a half of energy requirement of the brain can come from ketone bodies	**1.5**	**CO1**
Q4	The First Product of Glycogenolysis is - a) Glucose-6-phosphate b) Glucose 1,6 diphosphate c) Glucose-1-phosphate d) Fructose-1-phosphate	**1.5**	**CO1**

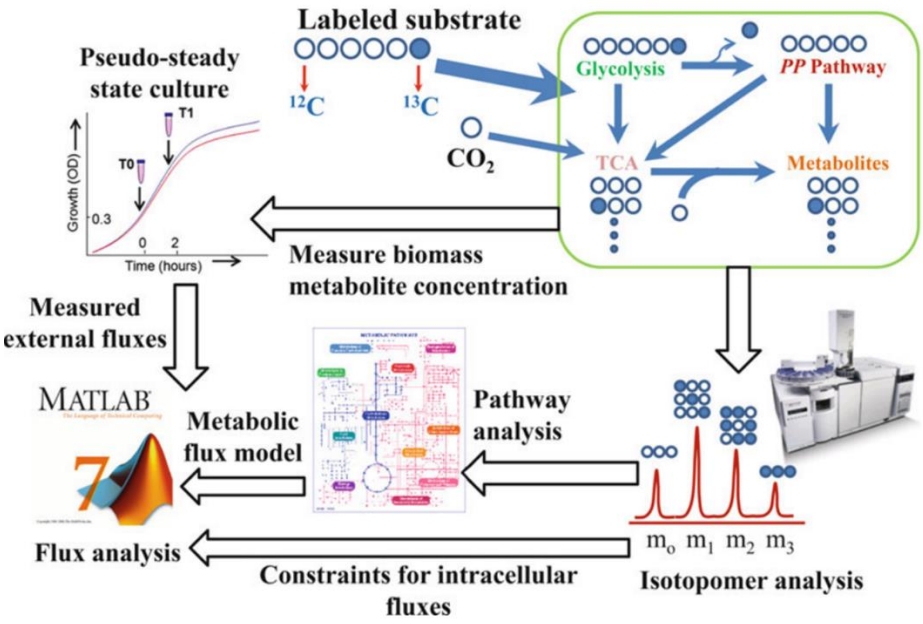
Q5	Which of the following hormone is not used in the hydrolysis of triacylglycerol into the fatty acids in adipose tissues? a) Epinephrine b) Norepinephrine c) Glucagon d) Insulin	**1.5**	**CO2**
Q6	Which of the following is not true for allosteric enzyme? a) Greek word 'allo' means other and 'steros' means site b) Enzymes having another site c) Regulatory metabolites are called effector or modulator or modifier d) Each of two or more enzymes with identical function but different structure	**1.5**	**CO2**
Q7	Which of the following is TRUE about feedback inhibition? a) Feedback inhibition has no physiological importance. b) Multiple products are required for feedback inhibition. c) Feedback inhibition of a pathway can only be accomplished by the products of that pathway. d) Feedback inhibition involves products binding to the active site to prevent enzyme activity.	**1.5**	**CO2**
Q8	What is represented in the following diagram?  a) Induced fit model b) Mechanism of allosteric enzymes by modulators c) Biosensor d) Fluidized bed reactor	**1.5**	**CO2**
Q9	The inducer of lac operon is a) βgalactosidase b) Galactose c) Lactose	**1.5**	**CO3**

	d) Glucose		
Q10	Which of the following is not true of the secondary metabolites a) They have direct role in photosynthesis, respiration and reproduction b) They act as toxin or pigment or lectins c) Distribution is limited and restricted to special taxonomic group d) Organic compound of diverse nature	**1.5**	**CO3**
Q11	Which of the following is NOT the class of secondary metabolite. a) Amino acids b) Terpenes c) Phenolics d) Alkaloids	**1.5**	**CO3**
Q12	Flux control coefficient measures the _____ a) Steady state change b) pH change c) Temperature change d) Hormonal change	**1.5**	**CO3**
Q13	Some regulated enzymes rebalance the level of metabolites in response to the change in flux, this activity is _____ a) Regulation b) Control c) Metabolism d) Anabolism	**1.5**	**CO4**
Q14	Define Flux Balance Analysis.	**1.5**	**CO4**
Q15	What is the principle of metabolomics?	**1.5**	**CO4**
Q16	Which of the following detection methods is not commonly used to detect isotopically labelled drug metabolites? a) Infra red spectroscopy b) Nuclear magnetic resonance spectroscopy c) Scintillation counting (detection of radioactivity) d) Mass spectrometry	**1.5**	**CO4**
Q17	Which of the following statements best describes an intermediate trapping experiment? a) A labelled sample of a known biosynthetic precursor is fed to a culture. An enzyme inhibitor is added to block one of the stages in the biosynthetic pathway, such that the levels of labelled biosynthetic intermediate increases in the cell and can be extracted. b) A labelled sample of a known biosynthetic precursor is fed to a culture. Th culture is grown for a short period of time such that there will be more	**1.5**	**CO5**

	of the labelled biosynthetic intermediate present than labelled natural product. c) A labelled sample of a known biosynthetic precursor is fed to a culture. An unlabelled sample of a proposed biosynthetic intermediate is then added. Some time later, the proposed intermediate is extracted from the culture to see if it is labelled. d) A labelled sample of a known biosynthetic precursor is fed to a culture. An alkylating agent is added some time later that reacts with any labelled biosynthetic intermediate that might have been formed, and prevents it from being converted to the fnal product. The alkylated intermediate builds up in the cell and can be extracted		
Q18	Radioisotopes are frequently used in the study of cells. Assume a culture of *E. coli* is grown in a culture medium containing radioactive phosphorous. At the end of 48 hours, it is expected to find the radioactive label located in a) Enzymes b) RNA c) Phospholipids d) All of these	**1.5**	**CO5**
Q19	In the presence of lactose, how long does it take for the lac operon to be expressed? (a) when lactose equals glucose concentration (b) when glucose is more than lactose concentration (c) as long as lactose is more than glucose concentration (d) as long as lactose is more than galactose concentration	**1.5**	**CO5**
Q20	This organism is used in the preparation of alcohol a) *Saccharomyces* b) *Acetobacter* c) *Lactobacillus* d) *Penicillium*	**1.5**	**CO5**
	SECTION B ***(Scan and upload)***	**(4Qx5M=20 Marks)**	**CO**
Q1	Discuss allosteric and feedback inhibition in biochemical pathways with the help of a neat diagram	**5**	**CO1**
Q2	What are the various factors affecting distribution of fluxes across metabolic pathways	**5**	**CO2**
Q3	What are extracellular and intracellular fluxes. Discuss the methodology for determination of metabolic fluxes	**5**	**CO3**
Q4	List any three applications of metabolic engineering in pharmaceuticals with the help of examples	**5**	**CO4**

	SECTION C (Scan and upload)	(2Qx15M=30 Marks)	CO
Q1	Noscapine has been commonly used in the past and is being explored for its potential use as a chemotherapy drug. Like many other opioids, noscapine can only be sourced from opium poppy plants to meet the current demands. Although present in opium, noscapine is a nonnarcotic drug that has been used worldwide for over 50 years as a safe, nonnarcotic antitussive. Noscapine has also been demonstrated to exhibit anticancer activity. Noscapine and its analogs (collectively referred to as noscapinoids) serve as an emerging class of microtubule-modulating anticancer agents that exhibit fewer side effects than traditional chemotherapy drugs. Industrial cultivation of microorganisms, such as the baker's yeast *Saccharomyces cerevisiae*, occurs over days, whereas poppies are annuals. The fundamental knowledge base about yeast has led to the development of a number of tools for pathway construction in this organism, including methods for controlling functional expression of heterologous genes and ensuring the genetic stability of the introduced genes. Also, because microbes are grown in closed fermentation vessels, the production process is not susceptible to external environmental factors and could provide greater consistency in product composition and impurity profiles across batches. Finally, by engineering a microorganism that does not make the broader profile of narcotic alkaloids present naturally in the opium poppy, one can remove the unnecessary hurdles associated with a tightly controlled supply chain. Li et al. metabolically engineered *Saccharomyces cerevisiae* to alter its metabolic pathways to produce noscapine. They did so by introducing desired foreign genes, incorporating them into the genome and forcing the yeast to produce those enzymes to make noscapine. Engineered strain contains 25 heterologous plant, bacteria, and mammalian genes and 6 mutant or overexpressed yeast genes. The noscapine biosynthetic pathway incorporates seven endomembrane-localized plant enzymes, highlighting yeast's ability to functionally express and properly localize large numbers of heterologous enzymes into the endoplasm reticulum. The initial yeast strain was engineered to express 29 enzymes from plant, bacteria, mammals, and yeast and produced ∼120–230 ng/L noscapine. Through engineering rate-limiting pathway enzymes, optimizing enzyme expression levels, introducing modifications to the endogenous yeast metabolism to enhance NADPH supply, and optimizing fermentation conditions, we improved noscapine titers by over 18,000-fold to ∼2.2 mg/L. Noscapine titers were improved by 18,000-fold (to low mg/L levels) via a combination of enzyme engineering, pathway and strain engineering, and fermentation optimization. Further optimization of the pathway and process will enable scale up to a commercially relevant, cost-effective fermentation process, offering a supply chain for noscapine and related molecules that does not rely on opium poppy farming. They demonstrated	**15 (3 marks each)**	**CO3**

	that microbial fermentation can be used to produce halogenated alkaloid derivatives, which can ultimately serve as potential drug leads, through feeding amino acid derivatives to strains. Li et al. maneuvered rate-limiting pathway enzymes, gene expression optimization, manipulated existing yeast genome and fermenting yeast cells in optimized media under ideal conditions to source the maximum yield of noscapine. Thus, the biosynthesis of halogenated noscapinoids could be achieved through two routes: (i) engineering of tailoring enzymes, such as halogenases, to accept noscapinoids as substrates and (ii) engineering limiting enzymes in the noscapine pathway, such as berberine bridge enzyme, to exhibit increased activity on halogenated substrates. Based on the above case study, answer the following: a) What are the advantages of using *Saccharomyces cerevisiae* as a host for metabolite overproduction. b) Which engineering strategy is discussed above in meeting the increasing demand of noscapine and how is it helpful? c) What do you understand by term "engineered strain". Comment on the capability of improved/engineered yeast strain in comparison with initial yeast strain. d) What is the rate limiting step in a pathway and how optimization can help us in achieving a higher production of noscapine. e) What do you conclude from this study. What are the other engineering approaches that can be used for an improved production of metabolite.		
Q2	The goal of metabolic engineering is to design and build engineered biological systems that can produce chemicals, materials, food, and drugs at high yield using the appropriate microorganisms. However, the lack of fundamental understanding of cellular responses during industrial bioprocesses often prevents metabolic engineers from achieving satisfactory goals in biochemical production. In the past decade, ^{13}C-MFA has been widely used to provide insightful information on metabolism of various microorganisms, thus helping metabolic engineers to successfully improve biochemical production. Stable isotope labeling is a powerful technique with promising applications. Depending upon this powerful technology, labeled tracers can sensitively and accurately track changes according to the location and quantity of peptides, amino acids, or carbohydrates containing isotope-labeled in vivo or in vitro. It enables direct analysis of nutrient distribution, metabolism, conversion into metabolites, and the fate of the resulting metabolites. In contrast to radioactive labeling, there are no dangers or safety concerns, making this technique particularly well suited for metabolism studies in humans. As a result, isotope labeling technology has	**15 (3 marks each)**	**CO4**

	received progressively more recognition in the fields of medicine and biochemistry.  Look at the diagram carefully and answer the following: a) Which approach is depicted in the diagram to estimating metabolic fluxes for biochemical production. Explain this approach and its significance in metabolic engineering. b) With the help of the given diagram, list five key procedural steps in estimating the metabolic fluxes c) What are isotopomers? Why is isotopic labelling important? d) Explain isotopomer analysis and two primary analytical methods used for measuring the labelled metabolites. e) List any two promising applications of stable isotope labeling.		
	SECTION- D **(Scan and upload)**	**(2Qx10M=20 Marks)**	**CO**
	Long Answer type Question		
Q1	a) What are primary and secondary metabolites. Give examples. What is their significance in metabolism b) How are the metabolic pathways regulated. Discuss in brief c) With the help of a neat and labelled diagram, discuss the regulation of lipid metabolism	**10 (3+3+4 marks each)**	**CO3**
Q2	a) What is Flux Balance Analysis (MFA). What are the steps involved in developing genome-scale models	**10 (4+4+2 marks each)**	**CO5**

	b) Discuss various methods in the analysis of optimality in natural and perturbed metabolic networks c) Discuss ^{13}C-Isotope Labeling and its significance in estimating metabolic fluxes		