Department of Biochemical Engineering and Biotechnology

L 4-21

BE [illegible]: Metabolic Regulation and Engineering

Major Test

April 29, 2008

Max Marks: 50

Note : Attempt all questions.

Part-A

Q.1. How is yield (or metabolic yield) defined? Why is it important to know the yield of a fermentation product (especially of those which are high-volume low-value products)? How can it be increased? **(1+2+3=6)**

Q.2 Differentiate between productivity (volumetric or fermentor productivity) and specific productivity. Mention two different ways to improve the productivity of ethanol fermentation process, which uses cellulose or glucose as raw material. **(2+4=6)**

Q.3 Almost all natural xylose-utilizing microbes exhibit diauxic growth behavior when grown on a medium containing both glucose and xylose sugars. Why does it happen? Which genetic methods can be employed to overcome this phenomenon? **(3+3=6)**

Q.4 (a)*Saccharomyces cerevisiae*, a powerful ethanologen, can ferment xylulose to ethanol but not its isomer, xylose. Two enzymes, xylose reductase (XR) and xylitol dehydrogenase (XDH), are required to impart this capability to the yeast. XR can carry out xylose to xyliol conversion in which NADPH or NADH serves as cofactor. For XDH-catalyzed reaction, only NAD+ serves as its cofactor.

To construct a recombinant *S cerevisae* strain, which can ferment both glucose and xylose efficiently to ethanol, the properties of XR were studied in various microbes. The XR enzymes from three different yeasts had the following properties:

(i) XR from *Pichia stipitis*: Km for NADPH = 2 mM, and for NADH = 10 mM
(ii) XR from *Pachysolen tannophilis*: Km for NADPH = 30 mM and for NADH = 1 mM
(iii) XR from *Candida shehatae*: Km for NADPH = 40 mM and for NADH = 0.1 mM

Which one of the three XR genes would you prefer to use for construction of recombinant *S. cerevisae* strain and why? Assume that all the three XR have the same Km for xylose, and that the intracellular concentrations of NADPH and NADH are 1 mM and 0.1 mM respectively in S. cerevisiae. **(6)**

(b) Mention the salient features of a metabolic engineering approach which has been used for construction of a recombinant strain for efficient conversion of both glucose and xylose to ethanol. **(5)**

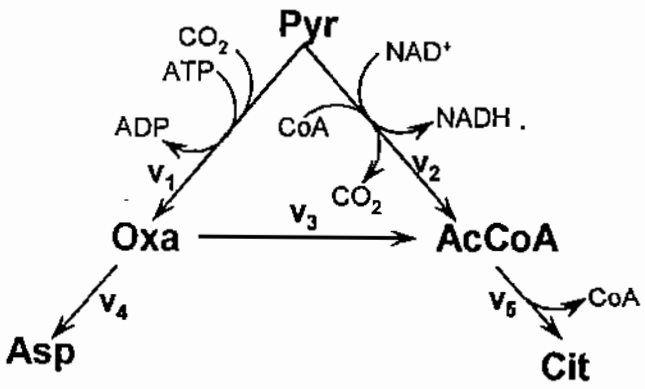
Q.5. Consider glycerol fermentation by *Klebsiella pneumoniae* such that it produces 1,3 –PD and acetate only (refer to the relevant portion of Figure-1 in the attached annexure-1).

(i) What will be the theoretical yield of 1,3 – PD per mole of glycerol? **(2)**

(ii) Figure 2 mentions the "genetically limited" and "metabolically limited" steps of the pathway. How will you differentiate these steps? And how will you overcome these limitations? **(4)**

PART – B

1. The sequence of reactions leading to the synthesis of Aspartic acid and Citric acid is from pyruvate is shown below in Fig 1.
   a) Identify the intermediates and justify why or why not a compound appearing in the pathway may be considered as an intermediate. Is the system exactly, over or under determined under this consideration?
   b) If only Oxa, AcCoA and NADH can be measured, determine Asp and Cit in terms these measurements.



(10 marks)

2. Describe gene regulation by attenuation with the help of a specific example.

(5 marks)

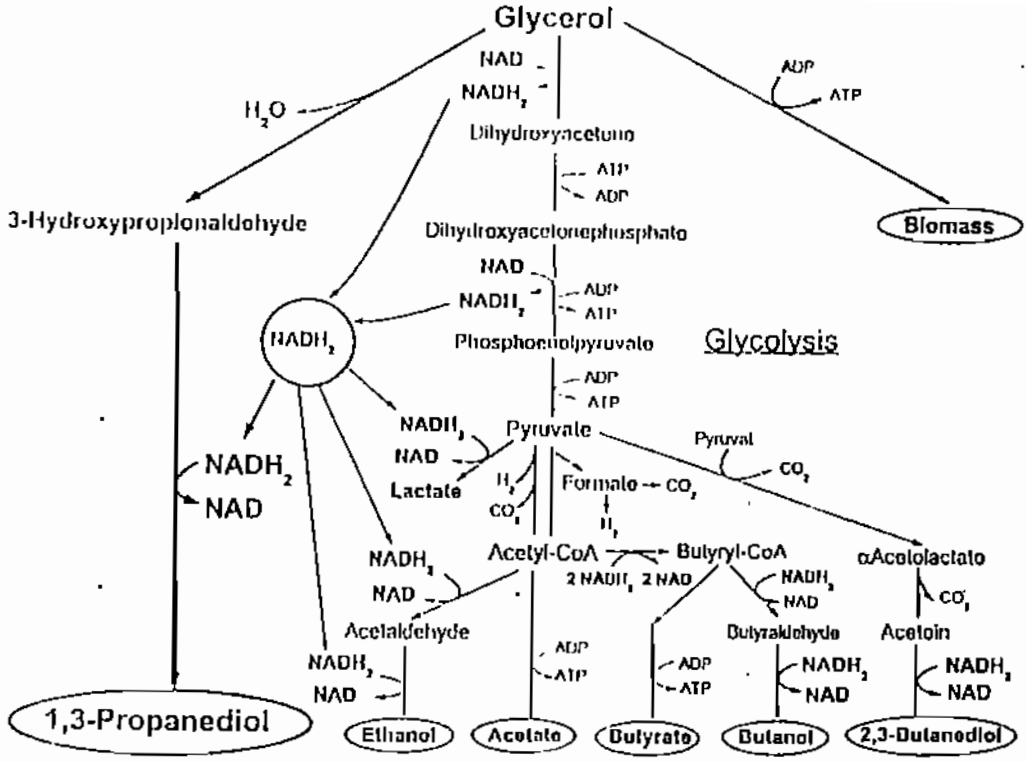


Fig. 1. Metabolic pathways of glycerol metabolism

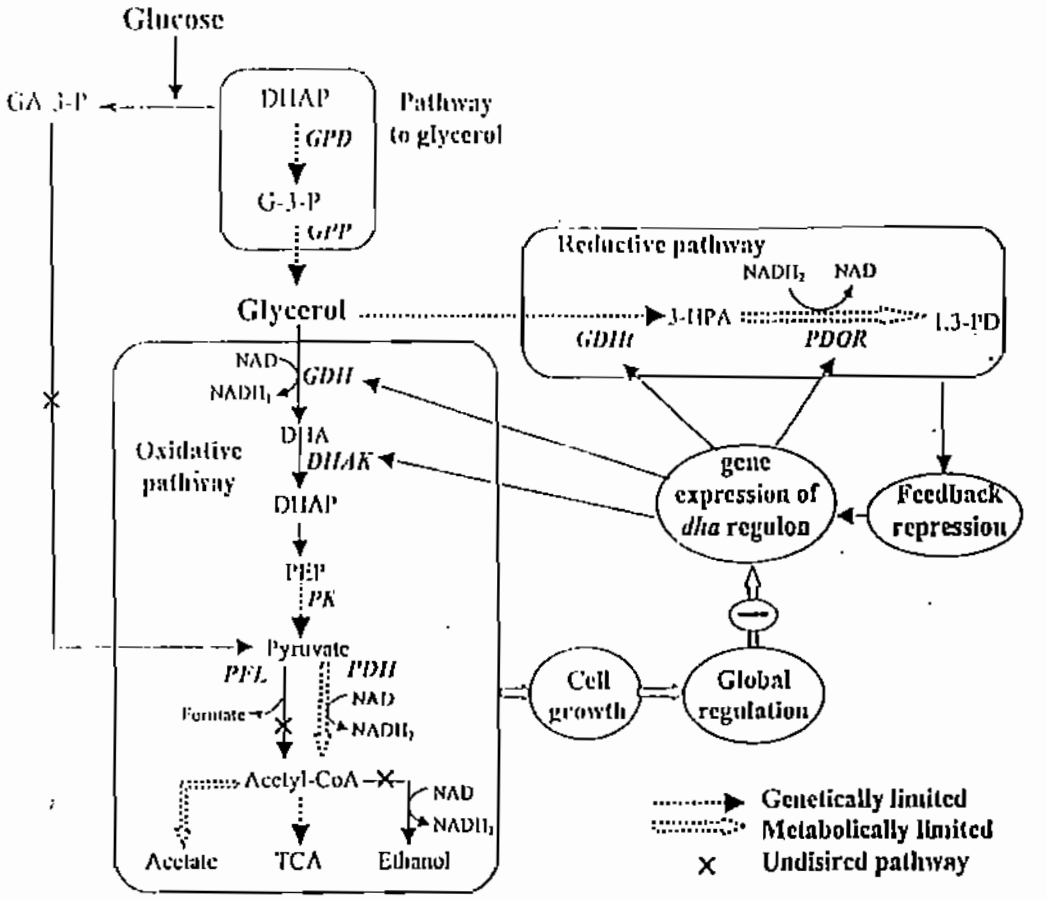


Fig. 2. Multiple targets for metabolic engineering of 1,3-PD production