Question n1

Escherichia coli, a widely used organism in laboratories, it is a facultative anaerobic bacterium.

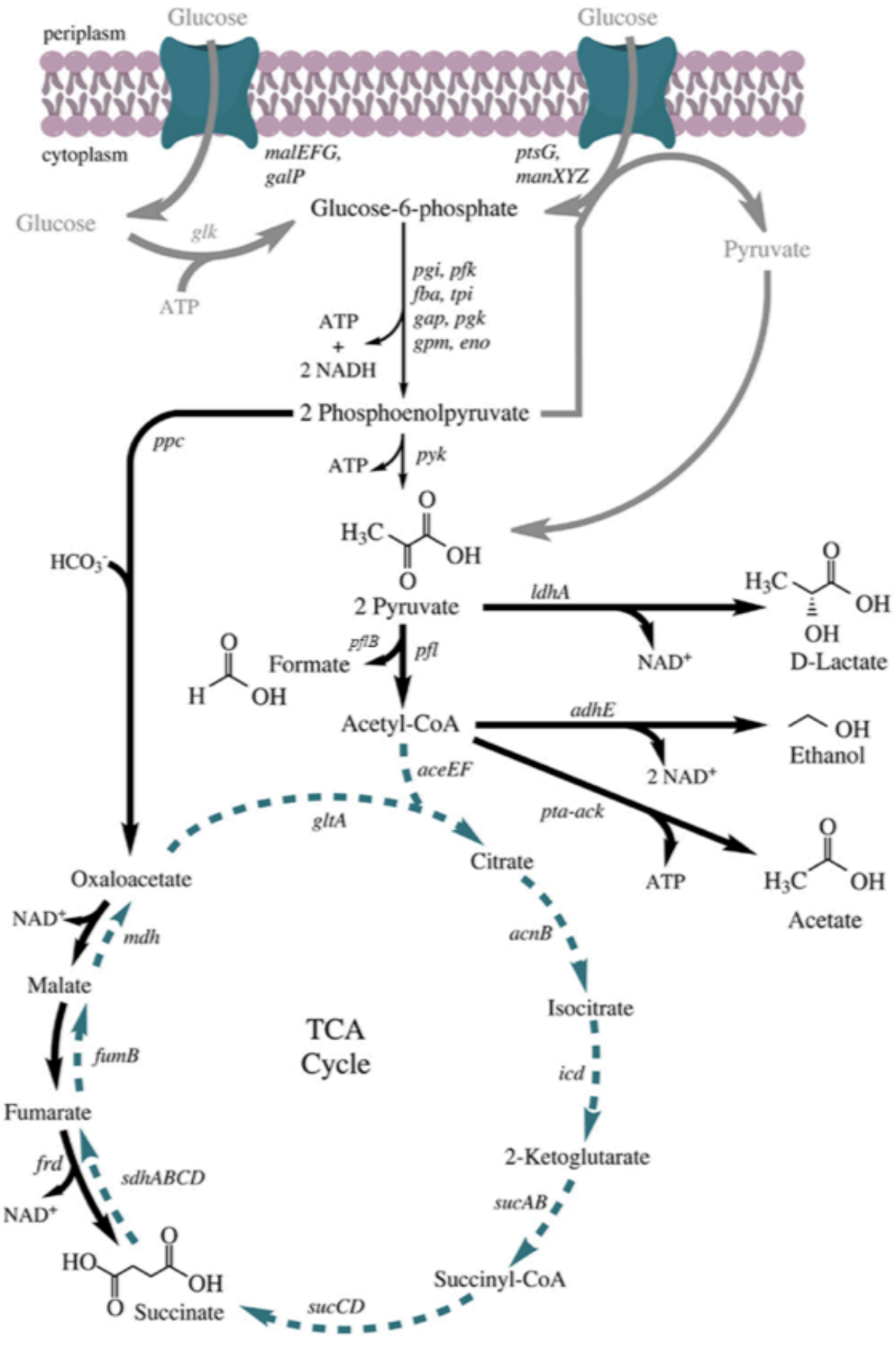
1) Define what a facultative anaerobic organism is.

2) E. coli fermentation is considered to be mixed, meaning that it is able to produce both lactate and ethanol. Could you describe the two types of fermentations?

3) E. coli is capable of producing ethanol through an endogenous process in which one mole of glucose is metabolized into two moles of formate, two moles of acetate, and one mole of ethanol. Considering the vast knowledge we have on its genome and metabolism (see below), E. coli is the primary choice for the production of biofuels. If you wanted to enhance the production of ethanol using genetic engineering, which gene(s) would you inhibit?

4) In order to study the efficiency of the conversion of glucose into ethanol, you decide to label the glucose with 14C. If you could label the glucose in just one position, which carbon would you choose to obtain labelled ethanol as a product?

5) Why wouldn't you get any labelled ethanol if you chose position 3?


periplasm
cytoplasm
Glucose
Glucose
malEFG,
galP
ptsG,
manXYZ
Glucose
glk
ATP
Glucose-6-phosphate
Pyruvate
pgi, pfk
fba, tpi
gap, pgk
gpm, eno
ATP
+
2 NADH
2 Phosphoenolpyruvate
ppc
ATP
pyk
HCO3-
2 Pyruvate
ldhA
NAD+
D-Lactate
pflB
pfl
Formate
Acetyl-CoA
adhE
2 NAD+
Ethanol
aceEF
pta-ack
ATP
Acetate
gltA
Citrate
Oxaloacetate
NAD+
mdh
acnB
Malate
Isocitrate
TCA
Cycle
fumB
icd
Fumarate
2-Ketoglutarate
frd
sdhABCD
NAD+
sucAB
Succinyl-CoA
Succinate
sucCD

Question n2

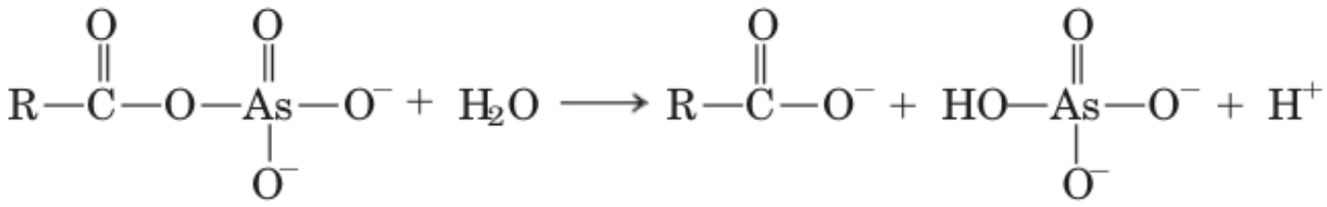
Fermentation to Produce Soy Sauce Soy sauce is prepared by fermenting a salted mixture of soybeans and wheat with several microorganisms, including yeast, over a period of 8 to 12 months. The resulting sauce (after solids are removed) is rich in lactate and ethanol. How are these two compounds produced? To prevent the soy sauce from having a strong vinegar taste (vinegar is dilute acetic acid), oxygen must be kept out of the fermentation tank. Why?

Question n3

Equivalence of Triose Phosphates ^{14}C-Labeled glyceraldehyde 3-phosphate was added to a yeast extract. After a short time, fructose 1,6-bisphosphate labeled with ^{14}C at C-3 and C-4 was isolated. What was the location of the ^{14}C label in the starting glyceraldehyde 3-phosphate? Where did the second ^{14}C label in fructose 1,6-bisphosphate come from? Explain.

Question n4

Arsenate Poisoning Arsenate is structurally and chemically similar to inorganic phosphate (P_i), and many enzymes that require phosphate will also use arsenate. Organic compounds of arsenate are less stable than analogous phosphate compounds, however. For example, acyl *arsenates* decompose rapidly by hydrolysis:



On the other hand, acyl *phosphates,* such as 1,3-bisphosphoglycerate, are more stable and undergo further enzyme-catalyzed transformation in cells.

(a) Predict the effect on the net reaction catalyzed by glyceraldehyde 3-phosphate dehydrogenase if phosphate were replaced by arsenate.

(b) What would be the consequence to an organism if arsenate were substituted for phosphate? Arsenate is very toxic to most organisms. Explain why.

Question n5

Role of the Vitamin Niacin Adults engaged in strenuous physical activity require an intake of about 160 g of carbohydrate daily but only about 20 mg of niacin for optimal nutrition. Given the role of niacin in glycolysis, how do you explain the observation?

Question n6

Free-Energy Change for Triose Phosphate Oxidation The oxidation of glyceraldehyde 3-phosphate to 1,3-bisphosphoglycerate, catalyzed by glyceraldehyde 3-phosphate dehydrogenase, proceeds with an unfavorable equilibrium constant ($K'_{eq} = 0.08$; $\Delta G'^{\circ} = 6.3$ kJ/mol), yet the flow through this point in the glycolytic pathway proceeds smoothly. How does the cell overcome the unfavorable equilibrium?