Problem 2

You are interested in analyzing the various pathway to generate succinate from glucose under anaerobic conditions. The conversion of glucose to succinate may be thought of as a two-step process: breakdown glucose into PEP (and pyruvate), and convert those intermediates into succinate.

There are four ways to breakdown glucose in *E. coli*:

1) via PTS and the EMP pathway
2) via glucokinase and the EMP pathway
3) via PTS and the PP pathway
4) via glucokinase and the PP pathway

There are also four ways to convert PEP into succinate:

1) via PEP carboxylase:
   PEP + CO_2 → oxaloacetate + Pi
   oxaloacetate + NADH → malate + NAD
   malate → fumarate + H_2O
   fumarate + NADH → succinate + NAD
2) via PEP carboxykinase:
   PEP + CO_2 + ADP → oxaloacetate + ATP
   oxaloacetate + NADH → malate + NAD
   malate → fumarate + H_2O
   fumarate + NADH → succinate + NAD
3) via pyruvate carboxylase
   PEP + ADP → pyruvate + ATP
   pyruvate + CO_2 +ATP → oxaloacetate + ADP + Pi
   oxaloacetate + NADH → malate + NAD
   malate → fumarate + H_2O
   fumarate + NADH → succinate + NAD
4) via the glyoxylate shunt
   2PEP + 2ADP → 2pyruvate + 2ATP
   2pyruvate + 2NAD + 2HS-CoA → 2acetyl-CoA + 2NADH + 2CO_2
   acetyl CoA + glyoxylate + H_2O → malate + HS-CoA
   isocitrate → succinate + glyoxylate
   malate + NAD → oxaloacetate + NADH
   oxaloacetate + acetyl-CoA + H_2O → citrate + HS-CoA
   citrate → isocitrate

Considering that the four glucose catabolic steps and the four succinate formation steps can be combined in any fashion, a total of 16 independent pathways may be used for the conversion of glucose to succinate.

Questions

A) Write the overall stoichiometric equations for the four glucose catabolic steps to PEP (and pyruvate). Note that for when the PTS is used, both pyruvate and PEP are products. When glucokinase is used, assume that only PEP is a product!
B) Write the overall stoichiometric equations for the four succinate formation steps from PEP.
C) Make all feasible combinations of these reactions to write the 16 possible complete stoichiometric equations. Note that in some cases (e.g., using the combination of the PTS, EMP pathway and PEP carboxylase), the pyruvate formed cannot be further metabolized, and therefore pyruvate will be a co-product. In other cases (e.g., PTS and glyoxylate shunt), pyruvate will be product instead of PEP, and you will have to modify the reaction you wrote for the conversion of PEP into succinate (in step B above).
D) Make a table of the 16 possible pathways, indicating for each
    a. The yield of succinate from glucose
    b. The yield of pyruvate from glucose
    c. The yield of CO_2 from glucose (some of them will be negative)
    d. The yield of ATP from glucose
    e. The yield of NADPH from glucose
    f. The yield of NADH from glucose
E) Any ideas about what would be a good way to make succinate?
F) Review Figure 1B on top of p. 1146 from Jantama et al., 2008 (link provided on course website). Is this an accurate depiction of redox balance in succinate production? Explain.

Note: The only carbon products can be CO_2, succinate, and in some cases, pyruvate.

Partial Answer to Problem 2

A) The catabolism of glucose via the PTS and EMP pathway is:

glucose + ADP + 2NAD + 2Pi → PEP + pyruvate + ATP + 2NADH + $2H_2O$

B) The formation of succinate via PEP carboxylase is:

PEP + CO_2 + 2NADH → succinate + 2NAD + H_2O + Pi

C) The complete reaction from glucose to succinate via the PTS/EMP pathway/PEP carboxylase is:

glucose + ADP + Pi + CO_2 → succinate + pyruvate + ATP + $3H_2O$

D) This reaction is redox balanced since the net NADPH and NADH consumption are zero. But, pyruvate can be considered to be a co-product.