Environmental Microbiology Learning Questions lecture 02.11.15

- **State the general equation of the free enthalpy and calculate the free enthalpy for the following reaction:**

  $C_6H_{12}O_6 + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O$

  $\Delta G_0\ (C_6H_{12}O_6) = -917.2$ kJ/mol
  $\Delta G_0\ (O_2) = 0$ kJ/mol
  $\Delta G_0\ (CO_2) = -394.4$ kJ/mol
  $\Delta G_0\ (H_2O) = -237.2$ kJ/mol

  Is the reaction endergonic or exergonic?

  Answer:

  ΔG_0 (rct) = Σ ΔG_0 (products) - ΔG_0 (educts)
  ΔG_0 (rct) = (6 * (-394,4 kJ/mol)) + (6 * (-237,2 kJ/mol)) - (- 917,2 kJ/mol) = - 2872.4 kJ/mol

  The reaction is exergonic.

- **How does the redox potential change if the concentration of one component of a redox couple is changed by one order of magnitude? (Standard conditions)**

  Answer:

  $E = E^0 + (RT/z_eF) \ln (\alpha_{ox}/\alpha_{red}) \sim E^0 + (0.06\ V/z_e) \log (c_{ox}/c_{red})$

  $= E^0 + (0.06\ V/z_e) \log (1/10^{-1})$

  For $z_e = 1 \rightarrow E^0 + 0{,}06\ V * 1$

  The redox potential changes by 0,06 V.

1. **Calculate the free energy for the incomplete oxidation of lactate to acetate at pH7.**

   2 lactate + SO_4^{2-} + $H^+ \rightarrow$ 2 acetate + 2 CO_2 + HS^- + 2 H_2O

   ΔG_0 (lactate) = - 517.8 kJ/mol
   $\Delta G_0\ (SO_4^{2-})$ = - 744.6 kJ/mol
   $\Delta G_0\ (H^+)$ = - 39.9 kJ/mol

ΔG_0 (acetate) = - 369.4 kJ/mol

ΔG_0 (CO_2) = - 394.4 kJ/mol

ΔG_0 (HS^-) = 12.5 kJ/mol

ΔG_0 (H_2O) = - 237.2 kJ/mol

Answer:

ΔG_0 (rct) = Σ ΔG_0 (products) - ΔG_0 (educts)

ΔG_0 (products)/(kJ/mol) = 2*(-369.4) + 2*(-394.4) + 12.5 + 2*(-237.2)

ΔG_0 (educts)/(kJ/mol) = 2*(-517.8) + (-744.6) + (-39.9)

→ ΔG_0 (rct) = -169.4 kJ/mol

Complete the following redox reaction and calculate ΔG. Does the reaction take place?

$C_6H_{12}O_6 + SO_4^{2-} \rightarrow CO_2 + H_2S$

$\Delta G_f°$ (H^+) = 0 kJ/mol

$\Delta G_f°$ ($C_6H_{12}O_6$) = -917.22 kJ/mol

$\Delta G_f°$ (SO_4^{2-}) = -744.63 kJ/mol

$\Delta G_f°$ (CO_2) = -394.36 kJ/mol

$\Delta G_f°$ (H_2S) = -33.56 kJ/mol

$\Delta G_f°$ (H_2O) = -237.18 kJ/mol

$\Delta G_f°$ (HS^-) = 12.05 kJ/mol

$\Delta G_f°$ (HCO^{3-}) = -586.85 kJ/mol

Solution:

$C_6H_{12}O_6 + 6H_2O \rightarrow 6\ CO_2 + 24\ e^- + 24\ H^+$

$SO_4^{2-} + 10\ H^+ + 8e^- \rightarrow H_2S + 4\ H_2O$ /*3

→ $C_6H_{12}O_6 + 3\ SO_4^{2-} + 6\ H^+ \rightarrow 3H_2S + 6\ CO_2 + 6\ H_2O$

ΔG = Σproducts – Σeducts

2. Why is it so important to control the pH in the anaerobic digester of a waste water treatment plant fermenter?
   If the pH is too low, the organisms will shift from acetate and butyrate production to alcohol production. Furthermore, the methanogenic communities will be inhibited and the whole fermenter will stop functioning.

3. In the thermodynamic of *Clostridium acetobutylicum*: How much NADH is recycled when metabolizing acetyl-CoA to acetone considering different end products. What is the determining factor?
   The determining factor is the pH. If too much acid is produced, the organism shifts to acetone and butanol production. No NADH is needed to produce Acetone, just one ATP is conserved in a transferase step from of acetoacetyl-CoA to acetoacetate. Thus, the organism always tries to produce acids because it can recycle lots of NADH from e.g. glycolysis.

4. Describe the main route of the fermentation of Glucose by *C. acetobutylicum*. What is the role of the hydrogen partial pressure and the acid production and where does the hydrogen and the acids come from?
   - First Step: Glucose → Pyruvate (in glycolysis)
   Pyruvate → Acetyl-CoA + CO_2 + Fd_{red}
   Ferredoxin → E° = -0.43 V → very negative → direct production of hydrogen
   H_2 partial pressure too high → more acid production → Acetyl-CoA → Butyryl-CoA → Butyrate (allows for ATP-production)
   Consequence: Covering of the pH; if pH gets too low Acetone and Butanol will be produced.

5. How much acetate can be oxidized with 2 mM of SO_4^{2-} ?
   CH_3COO^- + $2H_2O$ → 2 CO_2 + 7 H^+ + $8e^-$ (Ox.)
   SO_4^{2-} + 10 H^+ + $8e^-$ → H_2S + $4H_2O$ (Red.)
   CH_3COO^- + SO_4^{2-} + 3 H^+ → 2 CO_2 + H_2S +2 H_2O
   1 mole : 1mole, 1M of acetate is oxidized by 1 M of SO_4^{2-}.
   (2mM * 1M) / 1000 mM = 0.002 M of acetate will be oxidized by 2 mM of SO_4^{2-}

1. State how kinetics and thermodynamics are correlated

A: The thermodynamic determines if a reaction takes place or not. The kinetics determine the direction of the reaction. Thermodynamics influences also the kinetics. The more energy is released in the reaction, the faster it will proceed.

2. Why is ferodoxin so important for anaerobic microbes?

A: It's the electron carrier with the most negative redox potential in the cell. Hydrogen can be produced from ferredoxin and it is needed in some important metabolic reactions.

3. How much does the redoxpotential change if the redox couple changes by two orders of magnitude?

A: $E = E^0 + \left(\frac{0{,}06V}{n}\right) \log\left(\frac{c(ox)}{c(red)}\right)$

$$E = E^0 + \left(\frac{0{,}06V}{n}\right) \log\left(\frac{10^{-2}}{1}\right)$$

$$E = E^0 - 0{,}12V$$

4. What is the redox tower and for what do you need it? Give the values of 3 pairs.

A: O_2/H_2O -> 0,81 V

NO_3^-/N_2 -> 0,75 V

SO_4^{2-}/H_2S -> -0,218V

The redox tower gives the potential of a redox couple to accept or give away electrons -> Thus, we can deduce the ability of a compound to react with another compound.

5. What are the limitations of fermentation?

Fermenting organisms need to release electrons from the oxidative branch of substrate utilization (catabolism) on a redox potential that allows for the reaction. Thus, hydrogen partial pressures play an important role for many types of fermentation. E.g. for syntrophic interactions the hydrogen partial pressure is essential because some reactions that are endergonic under standard conditions can only take place if the hydrogen partial pressure is lowered by methanogens. Very important is also the pH as it changes fermentation patterns.

6. *Clostridium acetobutylicum* was used to produce 3 different products from starch. What are these products and name the process?

Fermentation to acetone, ethanol, butanol. But it also produces acetate and butyrate.