Environmental Microbiology – Seminar 31.10

1) What is the difference between aerobic respiration and fermentation?
Explain the mechanism and give examples.
In fermentation, energy is gained via substrate level phosphorylation. E.g, Phosphoenolpyruvate reacts with ADP and Pi to Pyruvate and ATP.

2) What happens if there is no O_2 in the body? In other words: no NAD^+ available and Glycolysis process stops.
Without oxygen humans die quickly. In muscles, however, oxygen is often used up and then the cells gain energy by substrate level phosphorylation in glycolysis and get rid of their electrons by lactic acid fermentation.

3) How can microbes be classified (energy source/carbon source/ e^- donor)?

Energy source: chemo-/phototroph
e^- donor: organo-/lithotroph
carbon source: hetero-/autotroph

4) Explain the processes in cellular respiration

-Glycolysis: Glucose → Pyruvate
-Krebs cycle: Pyruvate → CO_2
-Electron transfer system: conservation of energy (ADP→ATP)
➔32 ATP: $C_6H_{12}O_6+6O_2 \rightarrow 6H_2O+6CO_2$

5) Complete the following redox equation: $SO_4^{2-}+$ CH_2O ...→ …

$CH_2O+H_2O \rightarrow CO_2+4e^-+4H^+ \quad | \times 2$

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

$2CH_2O+\cancel{2H_2O}+SO_4^{2--}+\overset{2}{\cancel{10}}H^+ \rightarrow 2CO_2+\cancel{8H^+}+H_2S+\overset{2}{\cancel{4}}H_2O$

$2CH_2O+SO_4^{2-}+2H^+ \rightarrow 2CO_2+H_2S+2H_2O$

6) How can a microorganism use the energy released by a redox reaction?
In the oxidation reaction, energy can be conserved by substrate level phosphorylation. The electrons are transferred to an oxidized substrate. In the

reduction reaction, energy can only be conserved by electron transport phosphorylation (e.g. respiration).

7) List the categories of bacterial taxonomy from phyla to species with their endings according to bacterial code.

Phylum	not defined
Order	~ales
Family	~ceae
Genus	not defined
Species	not defined

8) In a batch experiment, the oxidation of acetate (M=59 $g \cdot mol^{-1}$; CH_3COO^-) by iron reducers is investigated. Write a complete redox equation and calculate the moles of CO_2 that result if half of the initial 1.18 g of the acetate has been degraded. (comment Meckenstock, nice question and a very good example why you should not use the elements but the electrons to balance the reaction)

a) $Fe(OH)_3 + CH_3COO^- \rightarrow Fe^{2+} + CO_2$

$CH_3COO^- + 2H_2O \rightarrow 2CO_2 + 7H^+ + 8e^-$

$Fe(OH)_3 + e^- + 3H^+ \rightarrow Fe^{2+} + 3 H_2O$! *8

$8Fe(OH)_3 + 8e^- + 24H^+ \rightarrow 8Fe^{2+} + 24 H_2O$

$CH_3COO^- + 8Fe(OH)_3 + 17H^+ \rightarrow 2CO_2 + 8Fe^{2+} + 22 H_2O$

b) 1.18 g Acetate/ 59 $g \cdot mol^{-1}$ = 20 mmol Acetate

→ 40 mmol CO_2 are produced by the expense of 320 mmol $Fe(OH)_3$

9) a) Write a redox reaction to show how microbes oxidize glucose. b) How much O_2 is needed for a complete reaction with 20g of glucose?

a) Oxidation: $C_6H_{12}O_6 \rightarrow CO_2$

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

Reduction: $O_2 \rightarrow H_2O$

$O_2 + 4e^- + 4H^+ \rightarrow 2H_2O$ | ×6

$6O_2 + \cancel{24e^- + 24H^+} \rightarrow 12H_2O$

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

b) Molecular weight of glucose:
carbon (12): 6 x 12 = 72
Hydrogen (1): 12 X 1 = 12
<u>Oxygen (16): 6 x 16 = 96</u>
Glucose $C_6H_{12}O_6$ = 72+12+96 = 180 g/mol

20 g glucose / 180 g mol^{-1} = 1/9 mol glc = 0.11 mol glc
0.11 mol glc x 6 = 0.66 mol O_2

10) Name a balanced redox-equation relevant in a chemo-lithotroph metabolism. Explain why your chosen reaction is relevant: (Comment Meckenstock, equation is correct but impossible due to the thermodynamics → have a look to the redox potentials)

Ox: $Fe^{2+} \rightarrow Fe^{3+}+e^-$ | ×8
Red: $SO_4^{2-}+8e^-+9H^+ \rightarrow HS^-+4H_2O$
Redox: $8Fe^{2+}+SO_4^{2-}+9H^+ \rightarrow 8Fe^{3+}+HS^-+4H_2O$
$8Fe(OH)_2+SO_4^{2-}+9H^+ \rightarrow 8Fe(OH)_3+HS^-+ \ldots$

Ox: $Fe(OH)_2+H_2O \rightarrow Fe(OH)_3+e^-+H^+$ | ×8
Ox: $8Fe(OH)_2+\cancel{8}H_2O \rightarrow 8Fe(OH)_3+\cancel{8e^-+8H^+}$
Red: $SO_4^{2-}+\cancel{8e^-}+\cancel{9}H^+ \rightarrow HS^-+\cancel{4H_2O}$
Redox: $8Fe(OH)_2+SO_4^{2-}+4H_2O \rightarrow 8Fe(OH)_3+HS^-$

11) Name the product of the oxidation and reduction branch in the general conservation of energy with glucose and oxygen as substrates. In which of the branches is the energy conserved?

Ox $C_6H_{12}O_6$ → product CO_2 (ATP from substrate level phosphorylation through high energy ~P bonds

Red O_2 → product H_2O (ATP by respiration of the electrons from $NADH+H^+/FADH_2$)

12) Describe the process of carbon dioxide to methane including the redox equation?

ox: $4\ H_2 \rightarrow 8e^- + 8\ H^+$

red: $CO_2 + 8e^- + 8\ H^+ \rightarrow CH_4 + 2\ H_2O$

Sum: $CO_2 + 4\ H_2 \rightarrow CH_4 + 2\ H_2O$

13) Draft the general principle of energy conservation in microorganisms

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substrate red               substrate ox
        |                             |
        |------------->[H]------------>|
        |                             |
product ox                  product red
oxidizing branch            reducing branch
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14) What is the energy source, carbon source and e^- donor of a photolithoautotrophic microorganism?

photo:	light	(energy)
auto:	CO_2	(carbon)
litho:	inorganic	(e^-donor)

15) PhD student Bernd Böhmermann finally made some progress on his field of research. He isolated a microorganism that seems to be able to reduce sulfate by oxidizing formaldehyde to formic acid.

a.) Write down the related redox equation!

b.) What kind of “respiration” is this (e.g. photolithoautotroph)?

a)

Ox) $H_2CO + H_2O \rightarrow HCOOH + 2e^- + 2\ H^+$ / x 4

Red) $SO_4^{2-} + 8\ e^- + 10\ H^+ \rightarrow H_2S + 4\ H_2O$

Ox x 4) $4H_2CO + 4H_2O \rightarrow 4HCOOH + 8e^- + 8\ H^+$

Sum) $4H_2CO + SO_4^{2-} + 2\ H^+ \rightarrow 4HCOOH + H_2S + 4\ H_2O$

b) Chemo-organo-heterotroph

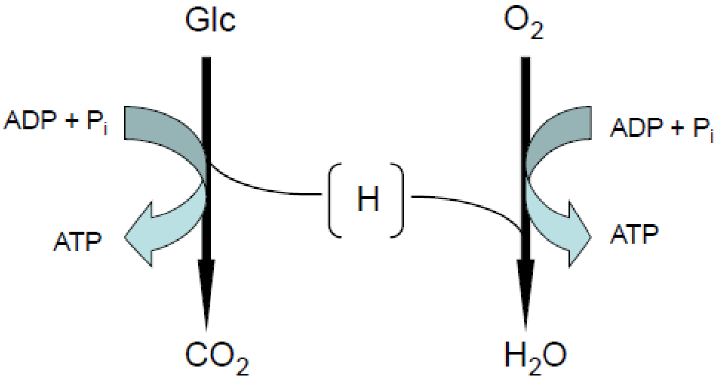
16) Give an example of ATP-conservation in a fermentation and respiration process.

- Respiration: $C_6H_{12}O_6 + O_6 \rightarrow 6CO_2 + 6\ H_2O$
  (Oxidation of glucose, aerobic)

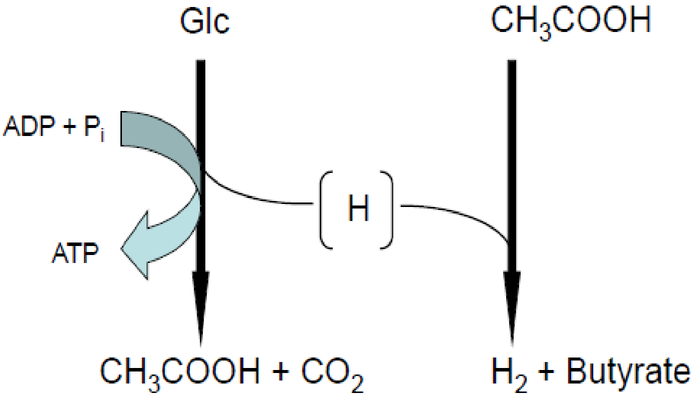
- Fermentation: anaerobic fermentation of glucose to lactate (Pyruvate is an intermediate compound → e^- acceptor)

17) Illustrate the different ways of energy conservation:

- Envi. Microbiology 1. Lecture, slide 12 (aerobic)



- Envi. Microbiology 1. Lecture, slide 13 (anaerobic)



18) How much benzene can you oxidize with an average nitrate concentration of 120 µM NO_3^-?

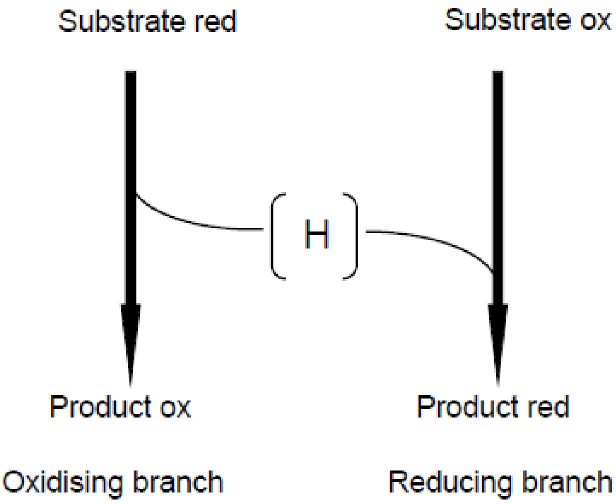
1) $C_6H_6 + 12\ H_2O \rightarrow 6\ CO_2 + 30\ e^- + 30\ H^+$
2) $2\ NO_3^- + 12\ H^+ + 10\ e^- \rightarrow N_2 + 6\ H_2O$ | x 3
3) $6\ NO_3^- + 36\ H^+ + 30\ e^- \rightarrow 3\ N_2 + 18\ H_2O$
    - $C_6H_6 + 6\ NO_3^- + 6\ H^+ \rightarrow 6\ CO_2 + 3\ N_2 + 6\ H_2O$

$[NO_3^-]$ = 120 µM

$$\frac{[C6H6]}{[NO3-]} = \frac{1}{6} \ <=> \ [C6H6] = \frac{1}{6}\ x\ [NO3-] \ = \frac{1}{6}\ x\ 120\ \mu M = 20\ \mu M$$

19) Draw the general principle of energy conservation? What is the driving force of energy conservation?

- Principle: [Lecture 1, slide 11]



The difference in redox potential between the reduced substrate electron donor) and the oxidized substrate (electron acceptor) is the driving force.

20) <u>Give three examples for anaerobe respiration with total formula.</u>

- Denitrification: $5\,[H] + NO_3^- + H^+ \rightarrow \frac{1}{2}\,N_2 + 3\,H_2O$
- Sulfate-Reduction: $8\,[H] + SO_4^{2-} + 2\,H^+ \rightarrow H_2S + 4\,H_2O$
- Nitrate-Ammonification: $8\,[H] + NO_3^- + 2\,H^+ \rightarrow NH_4^+ + 3\,H_2O$