Biotechnological processes: calculation exercises

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General

- Questions: jan.arends@ugent.be
- Case studies: contact Marlies Christiaens marlies.christiaens@ugent.be

➔ Put [Biotech] in the subject for any email concerning the course

- Course notes: VLK cursusdienst
- Subject first treated in theoretical course, so exercise sessions are complementary with theory
- New course notes: small errors possible!

General

- Goal: getting insight in biotechnological processes in wastewater treatment by **making calculations**
- Actively making exercises during courses, no passive listening
- Start with short theoretical background, then making exercises yourself !

When do we meet

Oct 29th	Stoichiometry	
Nov 5th	Removal of organic matter	13h00
	Sludge treatment	Denduyver
Nov 12th	Nutrient removal	13h00
	Sedimentation	Denduyver
Dec 2nd	Combined exercise // Overall design 8h30 HILO auditorium A	

Filters, aeration, fluidized-bed, oxidation ditch: additional information

Part 2: Stoichiometry

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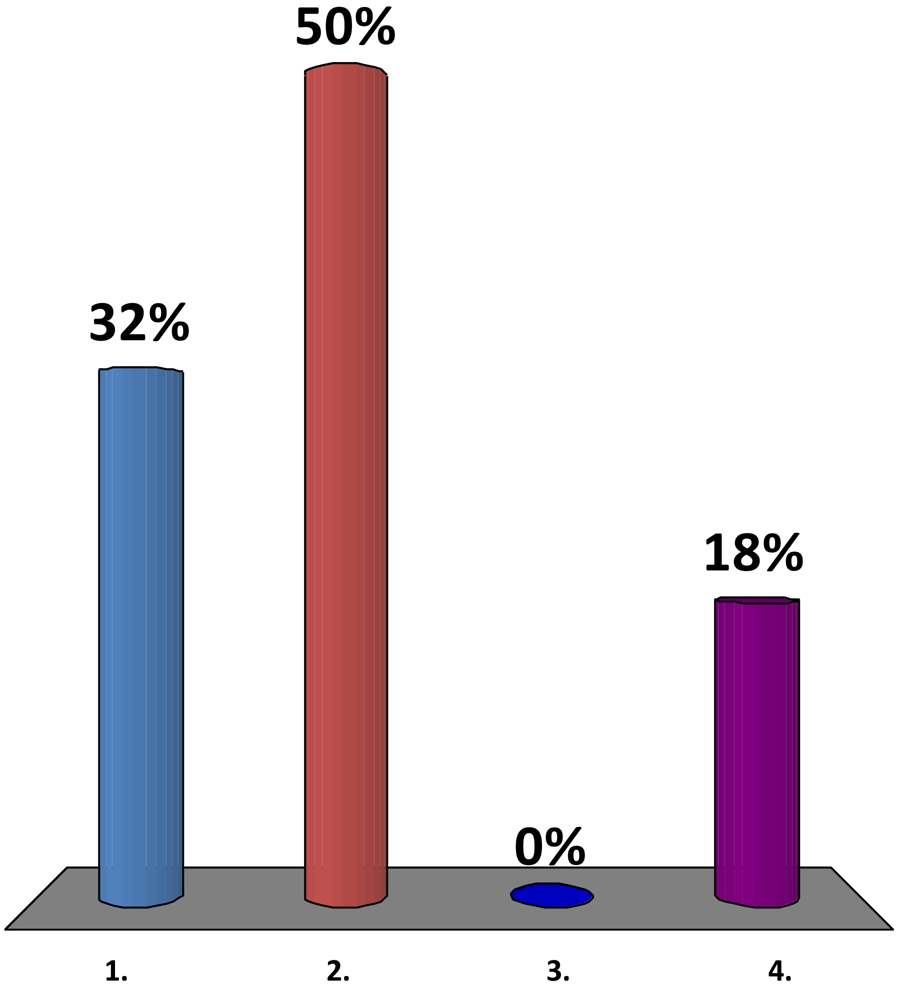
Chose your option

1. ManaMa Milieusanering
2. CES&T (IMENVI)
3. TIWM
4. Other



Stoichiometry: Balancing of reactions

Thermodynamics: calculation of Gibbs free energy change of a reaction

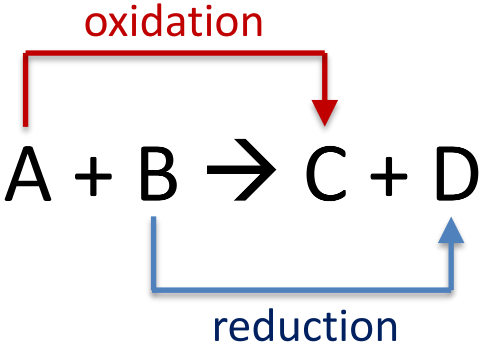
Wrap-up

At the end of the semester you can:

- Calculate the COD of simple molecules used in WWTP
- Determine the energy gain/input for biochemical reactions using the growth reference system (GRS)
- Comment on sludge production under various conditions in WWTP

Growth reference system (GRS)

- Biochemical processes: Redox reactions


oxidation

$$A + B \rightarrow C + D$$

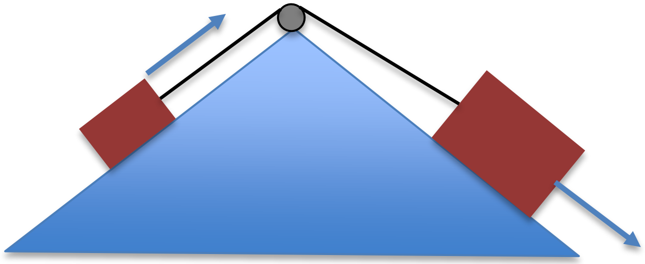
reduction

→ Thermodynamics: thermodynamically feasible

Driving force : $\Delta G^{\prime 0}$

$\Delta G^{\prime 0} < 0$ 👍

$\Delta G^{\prime 0} > 0$ 🚫



Different references to calculate $\Delta G^{\prime 0}$

Standard conditions (1 bar, 298.15 K, 1 mol/L, pH = 0)

BIOCHEMICAL Standard conditions (1 atm, 298.15 K, 1 mol/L, pH = 7)

Growth reference system (GRS)

- $\Delta G_r'^0 = \Sigma(\nu_x \cdot \gamma_x \cdot \Delta G_{e,x})$ $\nu_x > 0$ for products

  ν_x = stoichiometric coef. $\nu_x < 0$ for reagents

  γ_x = degree of reduction

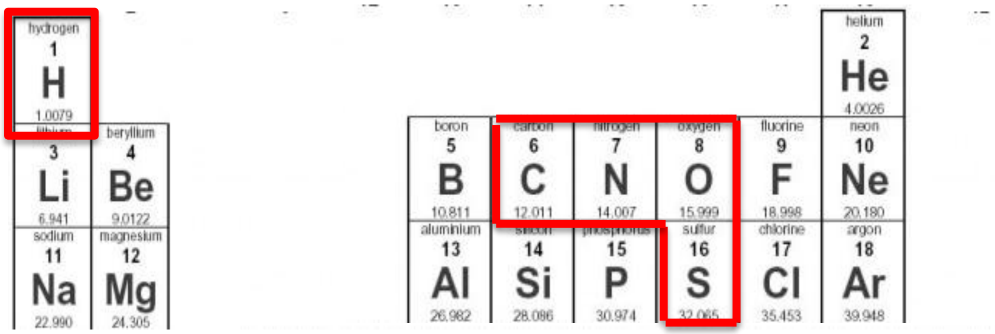
  $\Delta G_{e,x}$ = Gibbs free energy of formation (kJ/mol e^-) (Table 2)
- Gibbs energy of formation is 0 for stable end products in **microbial** systems
  - HCO_3^-, SO_4^{2-}, NO_3^-, H_2O, H^+
- At biochemical standard conditions
  - 1 atm, 298.15K, pH 7, 1 mol/L

  ➔ **Never mix these values with conventional thermodynamical data!!!!!!**

Heijnen, J.J. 2002. Bioenergetics of Microbial Growth. in: Encyclopedia of Bioprocess Technology

Degree of reduction (γ)

- See also table 1
  - C +4
  - H +1
  - O -2
  - S +6
  - P +5
  - N +5

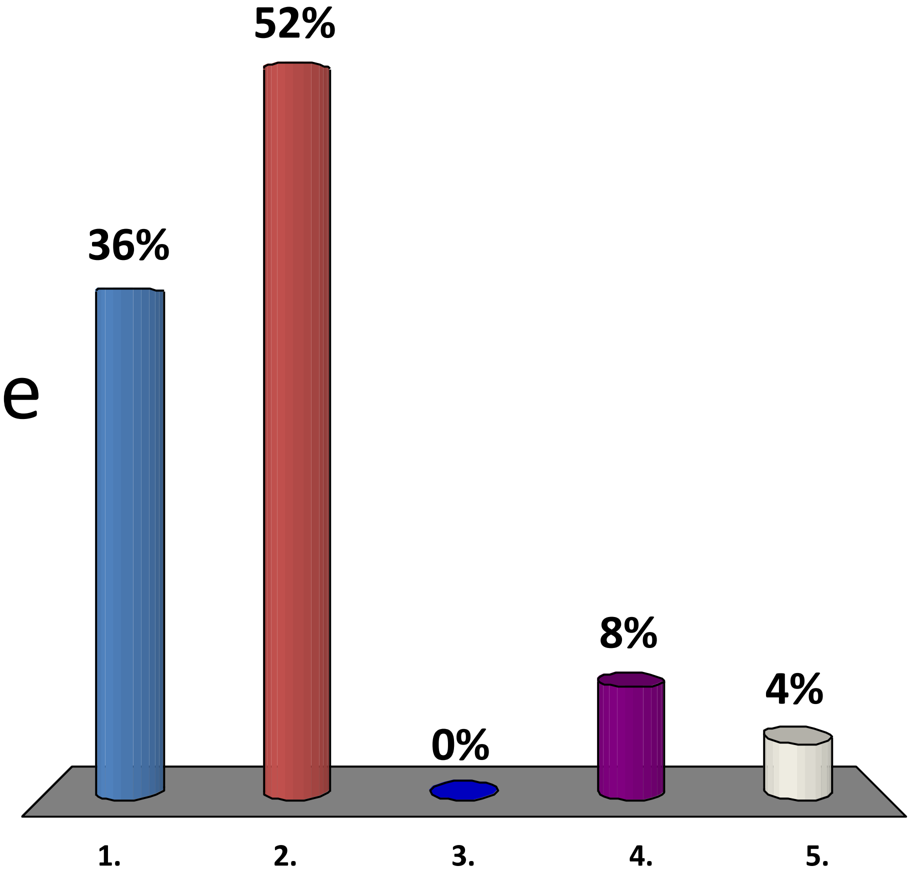


➔Degree of reduction = sum of reduction of all elements in a molecule

$SO_4^{2-} \rightarrow$
$NH_4^{+} \rightarrow$

What is the degree of reduction of: Sulfate and ammonium?

1. SO_4^{2-} =-2 & NH_4^+ = 1
2. [✓ SO_4^{2-} =0 & NH_4^+ = 8]
3. SO_4^{2-} =4 & NH_4^+ = 4
4. I don’t know?
5. My answer is not here



Degree of reduction (γ)

- See also table 1
  - C +4
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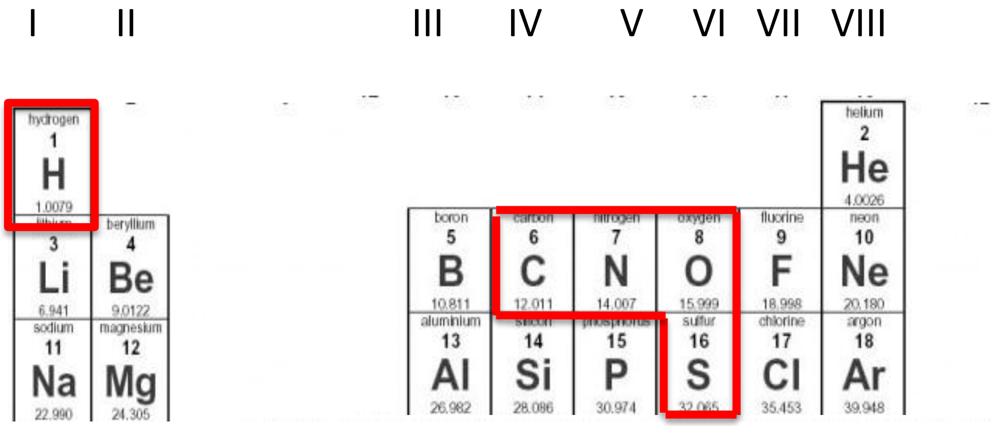
  - S +6
  - P +5
  - N +5



➔Degree of reduction = sum of reduction of all elements in a molecule

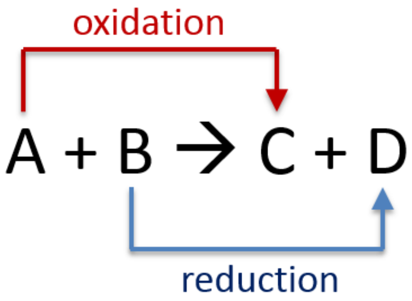
$SO_4^{2-} \rightarrow \gamma = 1 \times 6 + 4 \times (-2) + 2 \times 1$ (negative charge) $= 0$

$NH_4^{+} \rightarrow \gamma = 1 \times 4 + 1 \times 5 - 1$ (possitive charge) $= 8$

Reaction stoichiometry

Half reactions

Redox Reaction = oxidation half reaction + reduction half reaction



Oxidation: $A \rightarrow C + e^-$

Reduction: $B + e^- \rightarrow D$

- Half reactions
  1. Electron balance   Table (1 & 2)
  2. Carbon/nitrogen/sulfur/phosphorus balance
  3. Oxygen balance: H_2O
  4. Hydrogen balance: protons
  5. Charge balance as final check

→ e.g. Acetate to bicarbonate conversion

(oxidation half reaction)

Reaction stoichiometry

Half reactions

Conversion of <u>acetate</u> to <u>bicarbonate*</u> (oxidation half reaction)

* CO_2 at pH = 7 = HCO_3^-

1. e- balance (Table 1 & 2): $C_2H_3O_2^- \rightarrow 8\ \text{e-}$
2. Carbon balance (bicarbonate): $C_2H_3O_2^- \rightarrow 2\ HCO_3^- + 8\ \text{e-}$
3. Oxygen balance (water): $C_2H_3O_2^- + 4\ H_2O \rightarrow 2\ HCO_3^- + 8\ \text{e-}$
4. Hydrogen balance (protons): $\underbrace{C_2H_3O_2^- + 4\ H_2O}_{} \rightarrow \underbrace{2\ HCO_3^- + 8\ \text{e-} + 9\ H^+}_{}$
5. Charge balance (check): $-1 \rightarrow -1$

Reaction stoichiometry

Half reactions

Conversion of <u>oxygen</u> to <u>water</u> (reduction half reaction)

1. e^- balance: $O_2 + 4e^- \rightarrow$

2. Oxygen balance (water): $O_2 + 4\,e^- \rightarrow 2\,H_2O$

3. Hydrogen balance (protons): $O_2 + 4\,e^- + 4H^+ \rightarrow 2\,H_2O$

4. Charge balance (check): $0 \rightarrow 0$

***Remember: 1 O_2 gives 4 e^- then 8 gr O_2/ e^- Why?**

Complete reaction

$$C_2H_3O_2^- + 4\,H_2O \rightarrow 2\,HCO_3^- + 8\,e^- + 9\,H^+$$

$$2\,X\ \ (O_2 + 4\,e- + 4H^+ \rightarrow 2\,H_2O)$$

$$\mathbf{C_2H_3O_2^- + 2\,O_2 \rightarrow 2\,HCO_3^- + H^+}$$

Reaction stoichiometry

All at once

- Complete reactions

    1. Balance the 2 reagents based on the degree of reduction/number of electrons
    2. Carbon/nitrogen/sulfur/phosphorus balance
    3. Oxygen balance: H_2O
    4. Hydrogen balance: protons

➔Example: Oxidation of acetate

Reaction stoichiometry

Adjusting complete reaction directly

$\gamma_{C2H3O2-} = 8\ e^-$; $\gamma_{O2} = -\ 4\ e^-$ Acetate:O_2 = 1:2

1. Acetate and oxygen: $C_2H_3O_2^- + 2\ O_2 \rightarrow$

2. Carbon balance with bicarbonate: $C_2H_3O_2^- + 2\ O_2 \rightarrow 2\ HCO_3^-$

3. Oxygen balance with water: $C_2H_3O_2^- + 2\ O_2 \rightarrow 2\ HCO_3^-$

4. Hydrogen balance (protons): $C_2H_3O_2^- + 2\ O_2 \rightarrow 2\ HCO_3^- + H^+$

5. Charge balance (check): $\mathbf{C_2H_3O_2^- + 2\ O_2 \rightarrow 2\ HCO_3^- + H^+}$

Exercise 1: nitrification

- Nitrification: conversion of NH_4^+ to NO_3^-
  - – Oxidation reaction
    - Electrons: $NH_4^+ \rightarrow 8e^-$
    - Nitrogen: $NH_4^+ \rightarrow NO_3^- + 8\ e^-$
    - Oxygen: $NH_4^+ + 3\ H_2O \rightarrow NO_3^- + 8\ e^-$
    - Hydrogen: $NH_4^+ + 3\ H_2O \rightarrow NO_3^- + 10\ H^+ + 8\ e^-$

Exercise 1: nitrification

- Reduction reaction: reduction of O_2 to H_2O
  - Electrons: $O_2 + 4e^- \rightarrow H_2O$
  - Oxygen: $O_2 + 4e^- \rightarrow 2\ H_2O$
  - Hydrogen: $O_2 + 4e^- + 4H^+ \rightarrow 2\ H_2O$

➔Complete reaction = sum of oxidation and reduction

Or…

Exercise 1: nitrification

- Complete reaction:
  - Electrons: ammonium (8), oxygen (-4)

    $NH_4^+ + 2O_2$
  - Nitrogen: $NH_4^+ + 2O_2 \rightarrow NO_3^-$
  - Oxygen: $NH_4^+ + 2O_2 \rightarrow NO_3^- + H_2O$
  - Hydrogen: $NH_4^+ + 2O_2 \rightarrow NO_3^- + H_2O + 2H^+$

Calculation of the COD of an organic molecule

- See also theory course notes p. 12
- Steps:
  - – Calculate the degree of reduction of the molecule
  - – Multiply by 8 (8 g per electron of O_2)
  - – Divide by the molecular weight:
  - ➔ Result = g COD/ g substrate

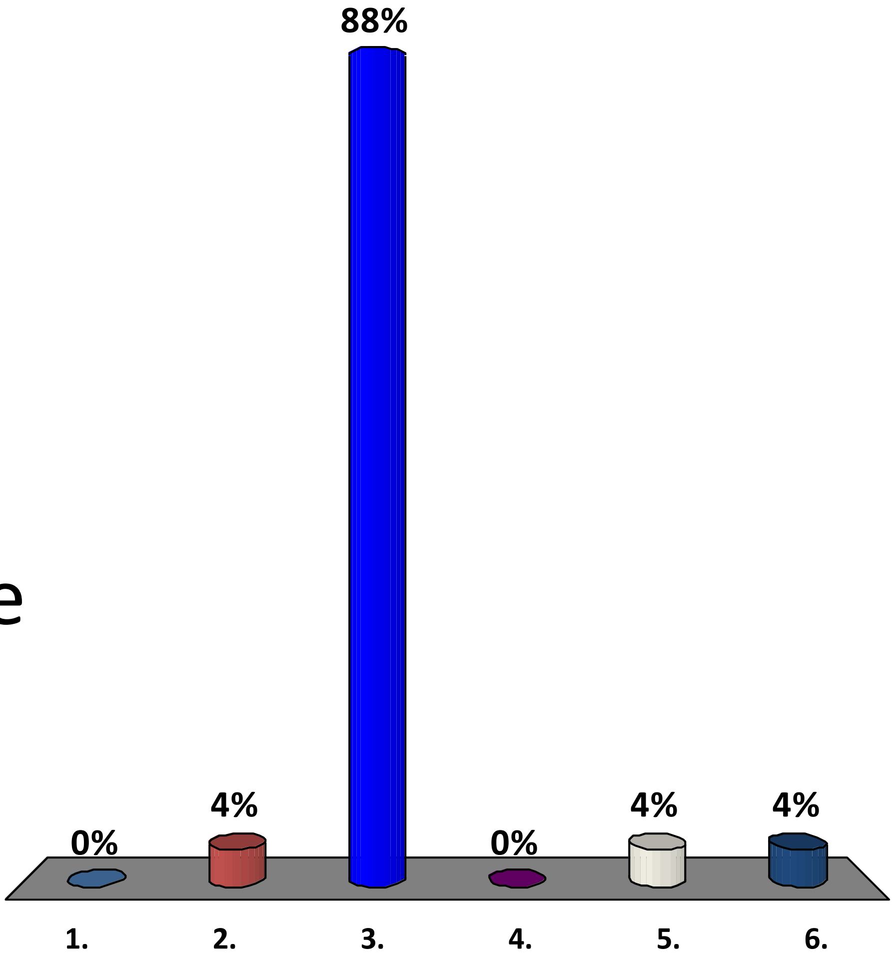
Example: metanol (CH_4O)

degree of reduction = 6 e^-

COD = 6 e^- X 8 grO_2/e^- = 48 gCOD/mol CH_4O = 1.5 gCOD/gCH_4O

Exercise 2: Calculate the COD of ethanol (g COD/g ethanol)

1. 1,39 g COD/g ethanol
2. 96 g COD/g ethanol
3. [✓ 2,09 g COD/g ethanol]
4. 2,29 g COD/g ethanol
5. I don’t know?
6. My answer is not here



Solution

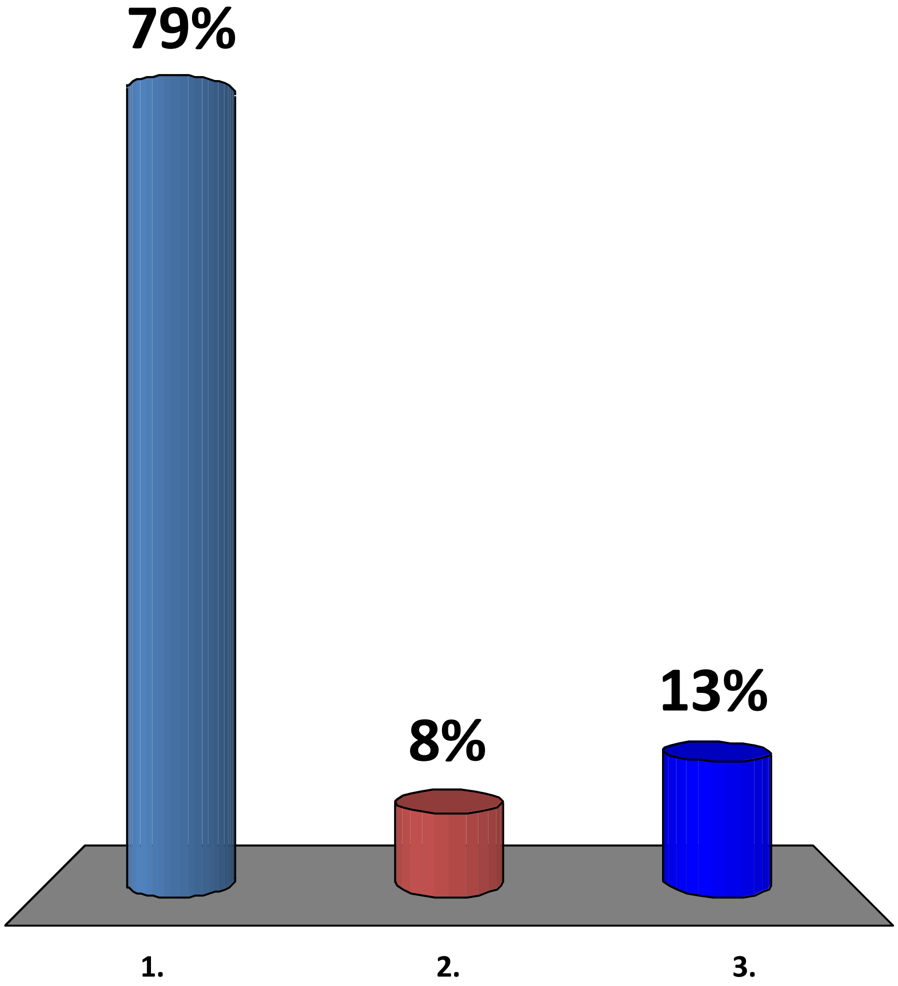
- Ethanol
  - Electrons: 2 X (+4) + 6 X (+1) + 1 X (-2)
    = 12 mol e^-/mol ethanol
  - COD = 12 X 8 = 96 g COD/mol ethanol
  - COD/weight = $\frac{96\ g\ COD/mol}{46\ g\ ethanol/mol}$

    = 2,09 g COD/ g ethanol
- Acetate: 1,06 g COD/g
- Glucose: 1,06 g COD/g

> - You need to know the formula of simple organic molecules:
> VFAs, glucose, fructose, lower alcohols, nitrate, nitrite, sulfide, sulfate, etc.

Do you understand the concept of COD calculations with the GRS?

1. Yes
2. No
3. I'm not sure



COD calculations (reminder)

- Steps:
  - Calculate the degree of reduction of the molecule

    γ = mol e^- / mol substrate
  - Multiply by 8 (8 g per electron of O_2)

    → g O_2 / mol substrate
  - Divide by the molecular weight:

  ➔ Result = g COD/ g substrate

EXAM!!!!!!

Gibbs energy per electron

$$\Delta G_r{}'^{0} = \Sigma(\nu_x \cdot \gamma_x \cdot \Delta G_{e,x})$$

$\nu_x > 0$ for products
$\nu_x < 0$ for reagents

ν_x = stoichiometric coef.
γ_x = degree of reduction
$\Delta G_{e,x}$ = Gibbs free energy of formation (kJ/mol e^-)

- ΔG_r : Gibbs free energy of reaction
  - Calculated with conventional values, after production with the reference substances (bicarbonate, nitrate, …)
- ΔG_e : Gibbs free energy of formation per electron
  - Divide ΔG_r by the degree of reduction (γ)

Simplifying thermodynamical reactions

- Reaction potentials can be calculated accordingly (e.g. for microbial fuel cells)

$$\Delta E^{0\prime} = -\frac{\Delta G_r^{\;0\prime}}{nF}$$

OR

$$\Delta E^{0\prime} = -\frac{\Delta Ge^{0\prime}}{nF}$$

$E^{0\prime} > 0$ 👍 $\Delta G^{\prime 0} < 0$

$E^{0\prime} < 0$ 🚫 $\Delta G^{\prime 0} > 0$

2nd semester: Microbial Technologies for Re-use

Concentration effects

- Conditions can differ from 1 atm, 298.15 K, 1 mol/L and pH 7
- If not: correct:
  - Temperature
  - Concentration
  - Partial pressure
  - pH

$$\Delta E_r = \Delta E_r^{0\prime} - \frac{RT}{nF}\ln(\Pi) + \frac{RT}{nF}\ln\left(\left[\frac{10^{-pH}}{10^{-7}}\right]^{\nu_H}\right)$$

➔ Part 1.4 + exercise 3 (@home)

Excersise 3

The reaction HCOOH → H_2 + CO_2 is not thermodynamically favourable. Prove this by calculating the ΔG_r. However, it is possible to microbially produce hydrogen from formate if hydrogen is consumed by another species, i.e. the methanogens: $4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$.

Calculate the new ΔG_r assuming that the methanogens keep the concentration of H_2 $[H_2]$ at 10^{-5} atm. The reaction occurs at pH 7 and temperature 25°C.

- Solution: 2 ways to calculate

1°

$HCOOH \rightarrow H_2 + CO_2$

$HCOOH + H_2O \rightarrow H_2 + CO_2 + H_2O$

$HCOOH + H_2O \rightarrow HCO_3^- + H^+ + H_2$

γ for H_2O, HCO_3^- and H^+ are 0

$\Delta G_r°' = v_{H2} * \gamma_{H2} * \Delta G_{H2} – v_{HCOOH} * \gamma_{HCOOH} * \Delta G_{HCOOH}$

$\Delta G_r°' = 80 – 78 = 1.368$ kJ/mol → does not allow growth

If concentration $[H_2]$ is kept low (at 10^{-5} atm):

(at pH 7)

→ allows growth

1°

If concentration $[H_2]$ is kept low (at 10^{-5} atm):

(at pH 7)

$$\Delta G_r = \Delta G_r^{0'} + RT\ln(\Pi) - RT\ln\left(\left[\frac{10^{-pH}}{10^{-7}}\right]^{\nu_H}\right) = \Delta G_r^{0'} + RT\ln(\Pi)$$

$$\Pi = \frac{a_C{}^{\nu_C}\, a_D{}^{\nu_D}}{a_A{}^{\nu_A}\, a_B{}^{\nu_B}} \approx \frac{[C]^{\nu_C}[D]^{\nu_D}}{[A]^{\nu_A}[B]^{\nu_B}} = \frac{[HCO_3^-][H_2]}{[HCOOH]} = \frac{1*(10^{-5})}{1} = 10^{-5}$$

$$\Delta G_r = 2kJ/mol + RT\ln(\Pi) = 1.368kJ/mol + 8.314\frac{m^2\,kg}{1000s^2.K.mol}(25+273)K * \ln(10^{-5})$$

$$\approx -27\,kJ/mol$$

→ allows growth

2°

$2H^+ + 2e^- \rightarrow H_2$ $\Delta G_r°' = ?$

$HCOOH + H_2O \rightarrow HCO_3^- + 2H^+ + 2e^- + H+$

$\Delta G_r°' = -78$ kJ/mol

$HCOOH + H_2O \rightarrow HCO_3^- + H_2 + H^+$

$$\Delta G_r = \Delta G_r^{0'} + RT\ln(\frac{1}{H_2})$$

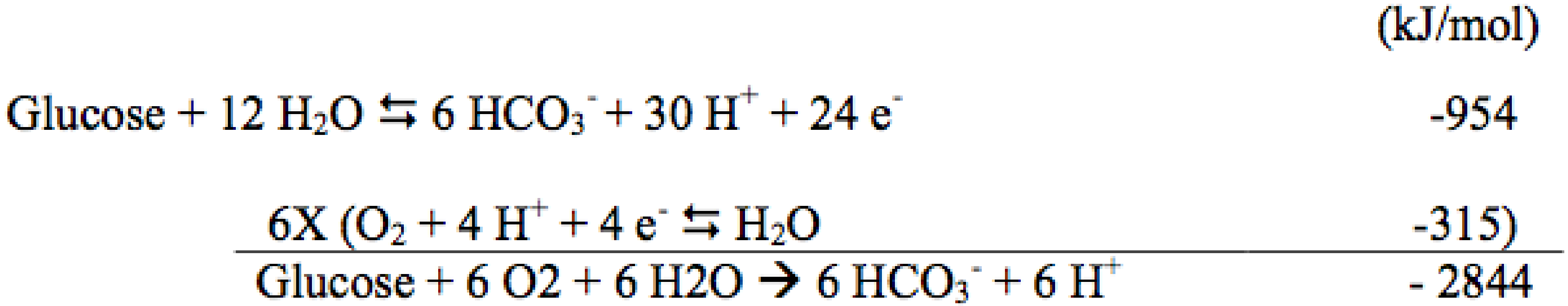
$$\Delta G_r = \Delta G_r^{0'} + RT * 2.303 * (\log[H_2] - 2\log[1]) = \Delta G_r^{0'} + RT * 2.303 * (-5) of \Delta G_r^{0'} + RT * \ln(10^{-5})$$

$$= 51.476 kJ/mol$$

→51.476 – 78 = <u>-27 kJ/mol</u>

Stoichiometry in WWTP

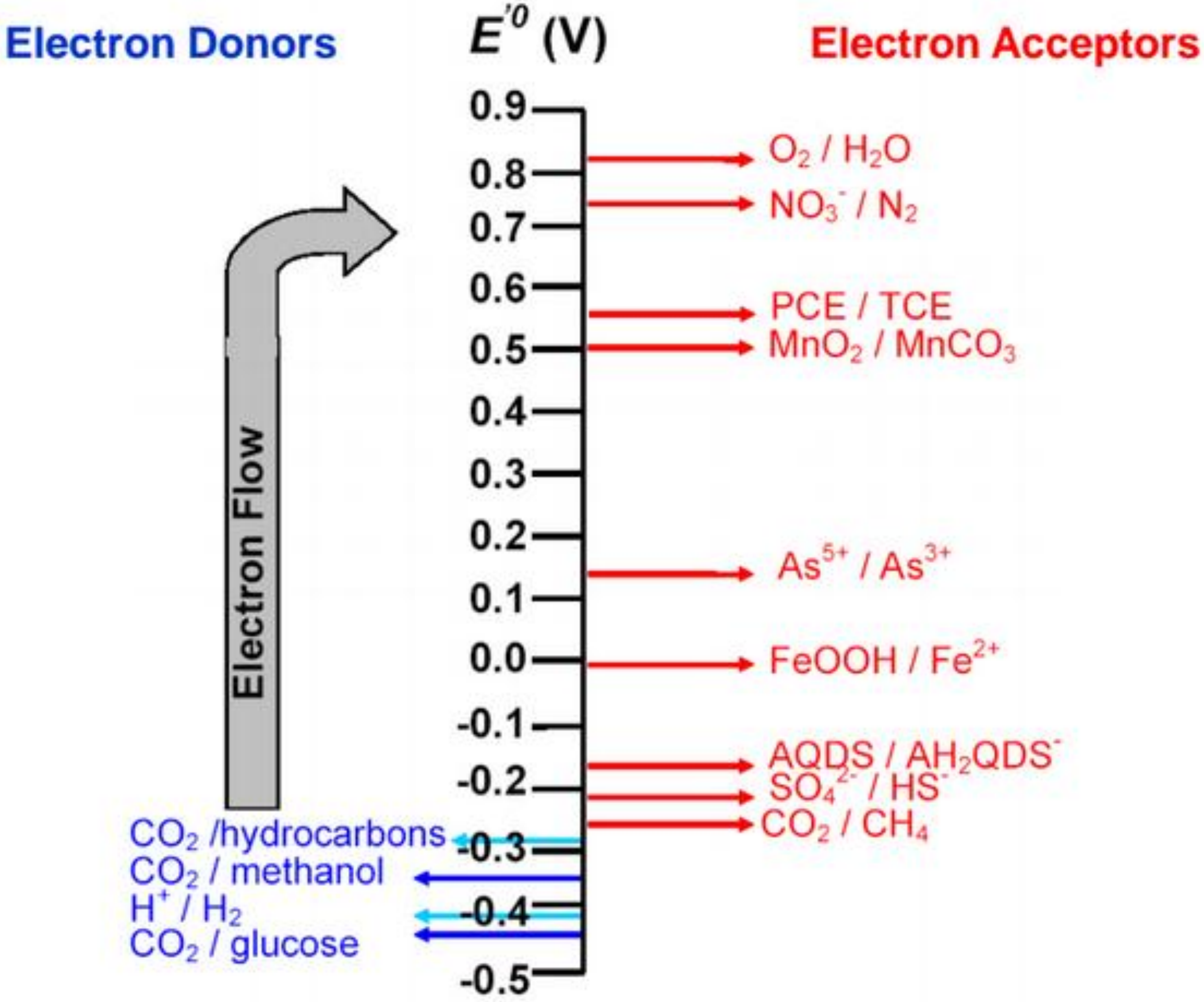
- Organic matter removal
  - Domestic waste: $C_{10}H_{19}O_3N$,
    glucose as simplification ($C_6H_{12}O_6$)
  - Aerobic: O_2 as electron acceptor



	(kJ/mol)
Glucose + $12\ H_2O \leftrightarrows 6\ HCO_3^- + 30\ H^+ + 24\ e^-$	-954
$6X\ (O_2 + 4\ H^+ + 4\ e^- \leftrightarrows H_2O$	-315)
Glucose + 6 O2 + 6 H2O $\rightarrow 6\ HCO_3^- + 6\ H^+$	- 2844

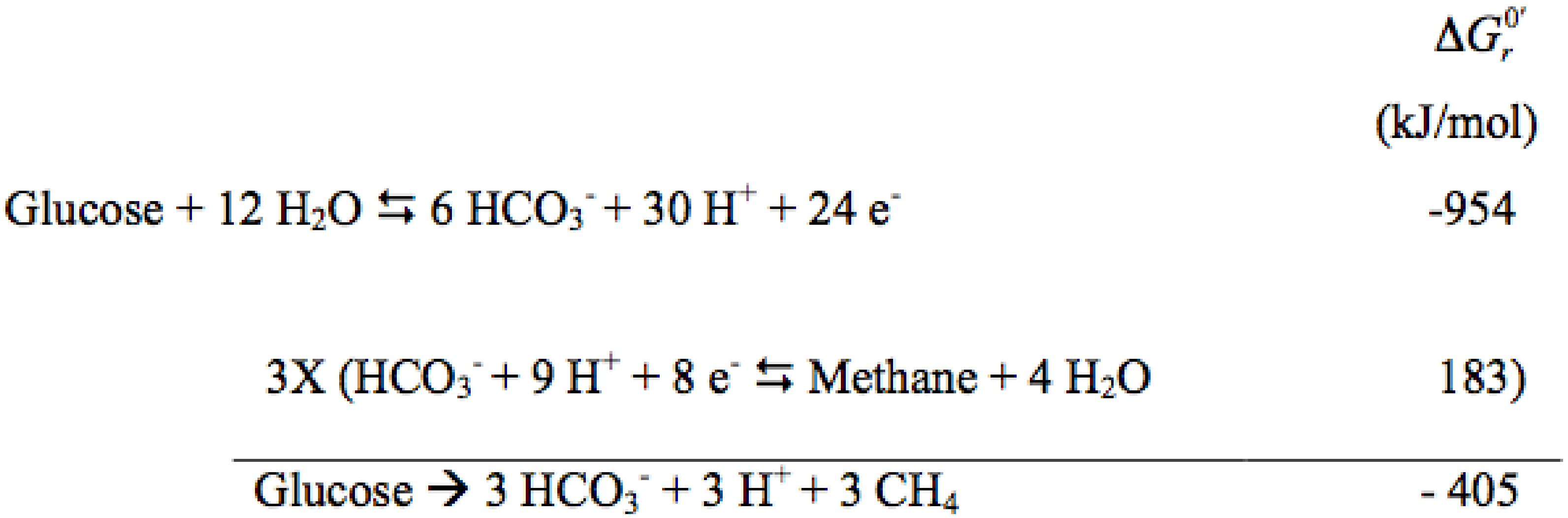
Stoichiometry in WWTP

In the absence of O_2 some bacteria can use different e- acceptors



Stoichiometry in WWTP

- Anoxic/Anaerobic: No oxygen as electron acceptor; thermodynamically less favourable
- HCO_3^- as electron acceptor (e.g. anerobic digestion)

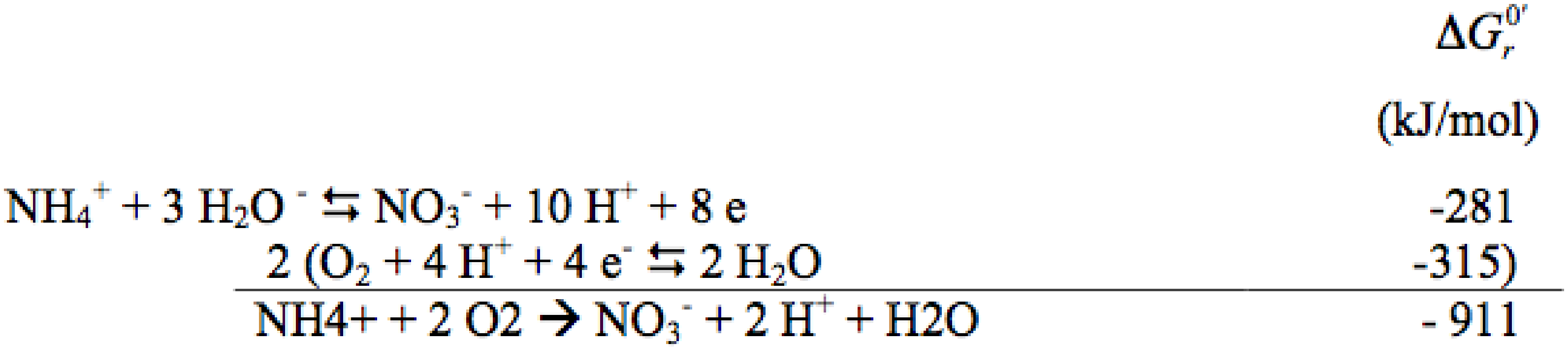


	$\Delta G_r^{0'}$ (kJ/mol)
$\text{Glucose} + 12\ H_2O \leftrightarrows 6\ HCO_3^- + 30\ H^+ + 24\ e^-$	-954
$3X\ (HCO_3^- + 9\ H^+ + 8\ e^- \leftrightarrows \text{Methane} + 4\ H_2O$	183)
$\text{Glucose} \rightarrow 3\ HCO_3^- + 3\ H^+ + 3\ CH_4$	- 405

- Other electron acceptors: see removal of nutrients

Stoichiometry in WWTP

- Nitrogen removal
  - Nitrification: autotrophic bacteria



	$\Delta G_r^{0'}$ (kJ/mol)
$NH_4^+ + 3\,H_2O\ ^- \leftrightarrows NO_3^- + 10\,H^+ + 8\,e$	-281
$2\,(O_2 + 4\,H^+ + 4\,e^- \leftrightarrows 2\,H_2O$	-315)
$NH4+ + 2\,O2 \rightarrow NO_3^- + 2\,H^+ + H2O$	- 911

How much O_2 needed per gram of N?

Stoichiometry in WWTP

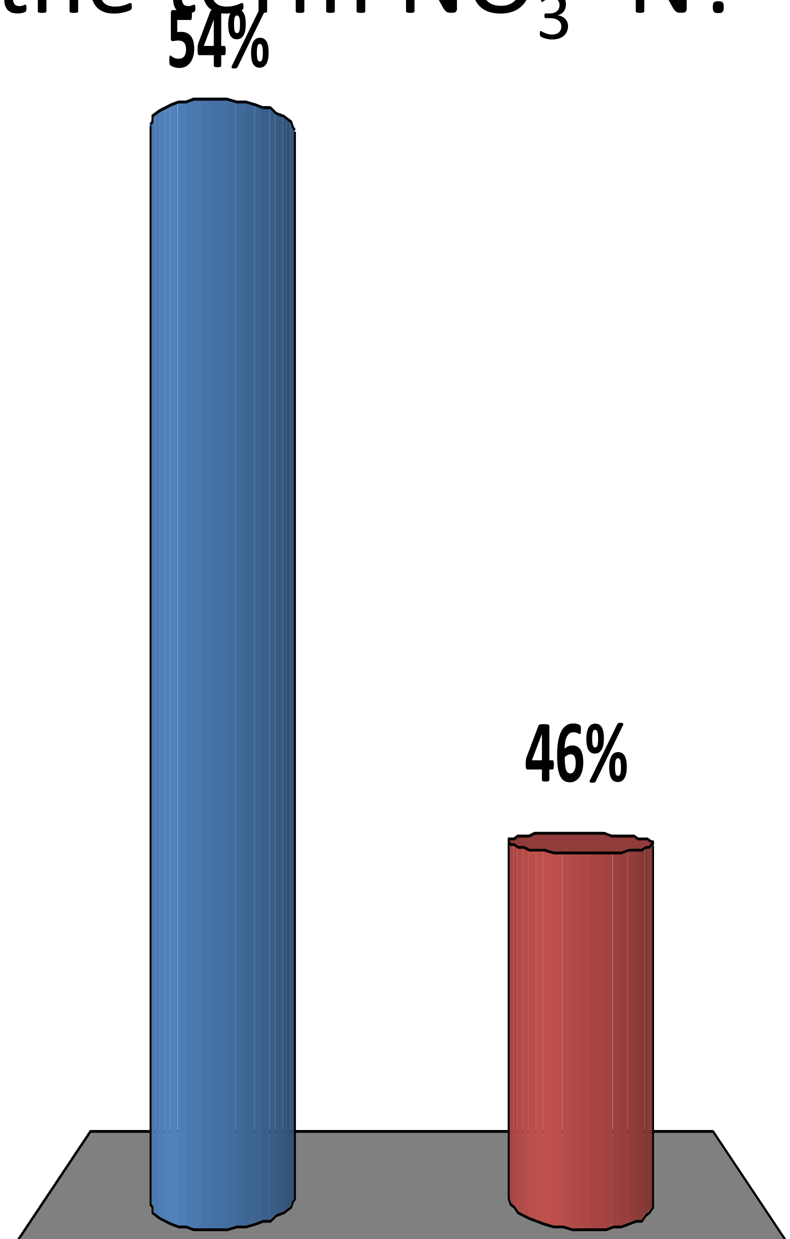
- Nitrogen removal
  - Denitrification: heterotrophic bacteria: need a carbon source!

	$\Delta G_r^{0'}$ (kJ/mol)
$5\ (\text{Glucose} + 12\ H_2O \leftrightarrows 6\ HCO_3^- + 30\ H^+ + 24\ e^-$	$-954)$
$24(NO_3^- + 6\ H^+ + 5\ e^- \leftrightarrows 0.5\ N_2 + 3\ H_2O$	$-361)$
$5\ \text{Glucose} + 24\ NO_3^- \rightarrow 12\ N_2 + 6\ H^+ + 12\ H_2O$	$-\ 13434$

How much glucose needed per gram of N?

Do you understand the term NO_3^--N?

1. Yes
2. No

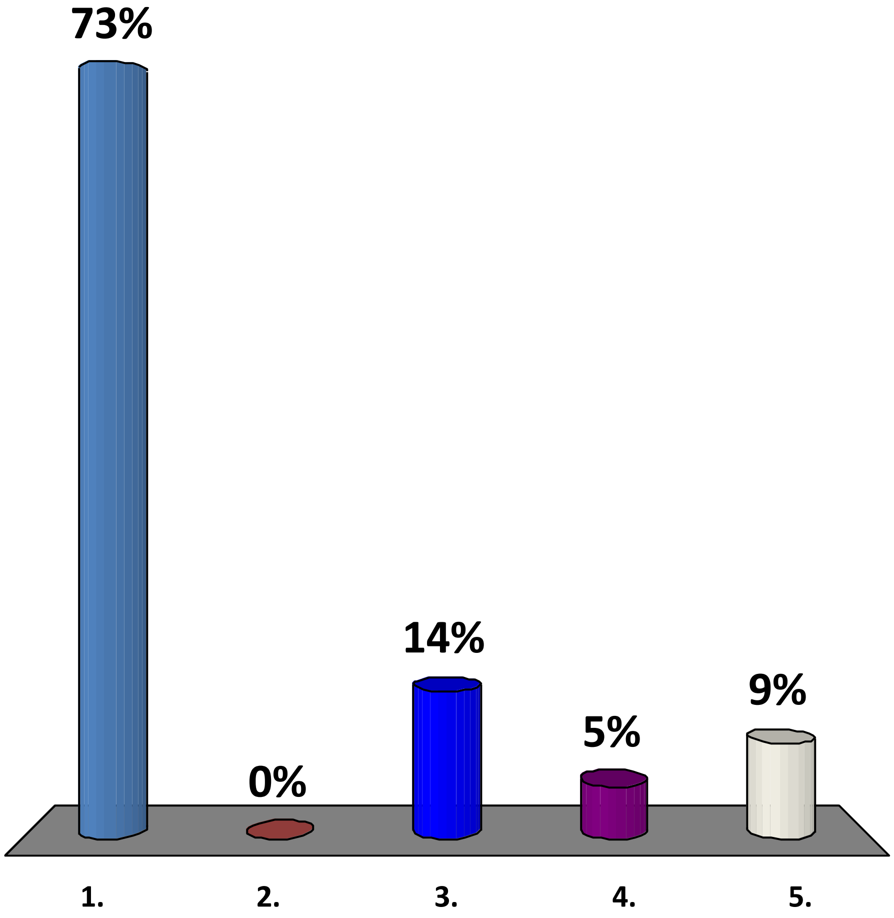


NH_4^+-N, NO_2^--N, NO_3^--N

- Nitrogen
- Origin of nitrogen source is added
- e.g. NH_4^+-N, NO_3^--N
- Only take molecular mass of N into account, no other elements
- Also for other compounds: SO_4^{2-}-S , PO_4^{3-} -P

How much glucose is needed per g N removed (through denitrification)

1. [✓ 2,68 g]
2. 0,60 g
3. 0,21 g
4. 12,86 g
5. ?

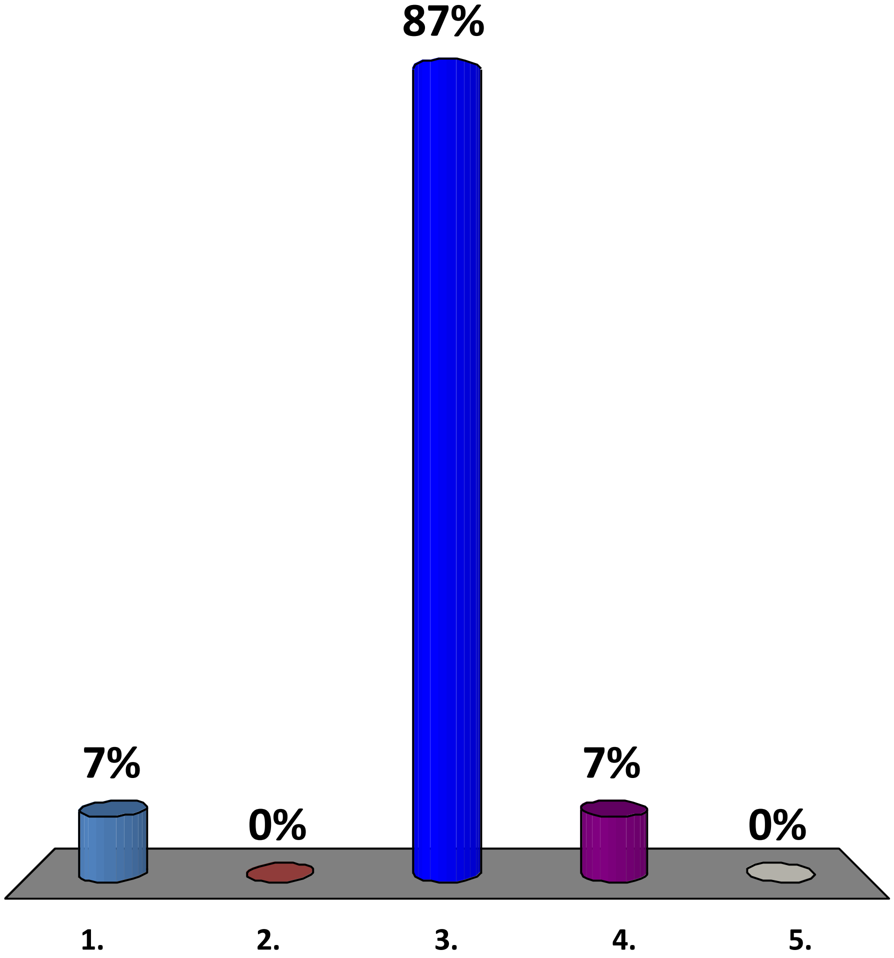


Exercise 4a

5 mol glucose per 24 mol N = 900 g glucose per 336 g N = 2,68 g glucose per g N

Calculate the price of methanol per kg NO_3^--N removed

1. 0,80 €/kg N
2. 0,15 €/kg N
3. [✓ 0,67 €/kg N]
4. 1,00 €/kg N
5. ?



Exercise 4b

$5\,(\text{Methanol} + 2\,H_2O \leftrightarrows HCO_3^- + 7\,H^+ + 6\,e^- \quad)$

$\underline{6\,(NO_3^- + 6\,H^+ + 5\,e^- \leftrightarrows 0.5\,N_2 + 3\,H_2O \qquad\quad)}$

$5\,\text{MetOH} + 6\,NO_3^- + H^+ \rightarrow 3\,N_2 + 5\,HCO_3^- + 8\,H_2O$

→5 mol methanol per 6 mol N = 160 g methanol per 84 g N = 1,90 g methanol per g N

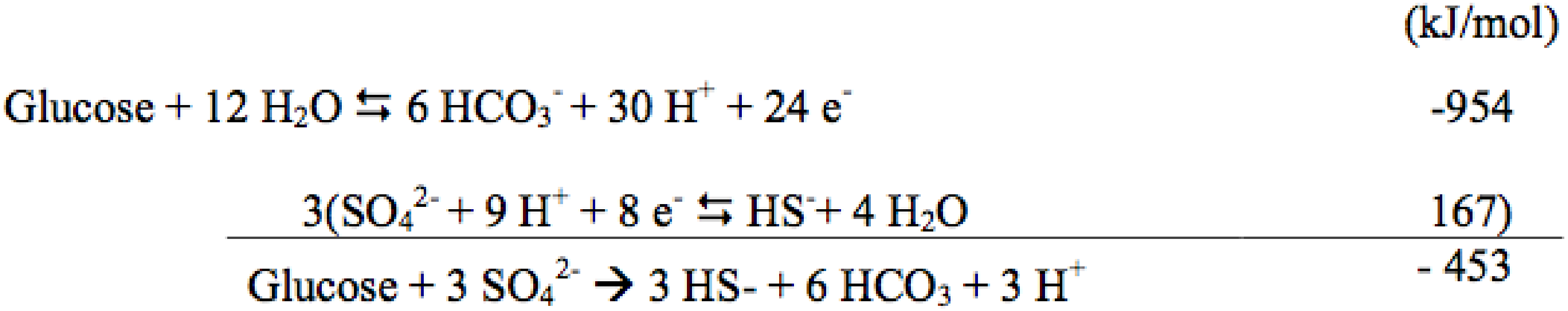
→**€ 0,67 per kg N removed**

→Glucose: € 0,8 per kg N removed

→Methanol = cheaper than glucose

Stoichiometry in WWTP

- Sulfate reduction: reduction to sulfide
- In sewer systems: corrosion due to formation of H_2S → to be avoided!



	(kJ/mol)
$\text{Glucose} + 12\ H_2O \leftrightarrows 6\ HCO_3^- + 30\ H^+ + 24\ e^-$	-954
$3(SO_4^{2-} + 9\ H^+ + 8\ e^- \leftrightarrows HS^- + 4\ H_2O$	167)
$\text{Glucose} + 3\ SO_4^{2-} \rightarrow 3\ HS^- + 6\ HCO_3 + 3\ H^+$	- 453

Biomass production

- Previous reactions = Catabolic

- Need also to include anabolic reactions → bacteria use part of the electron donor (C) for biomass synthesis (also NH_4^+-N)

  - Autotrophic bacteria: HCO_3^-
  - Heterotrophic bacteria: Organic Carbon (e^- donor)

Biomass production

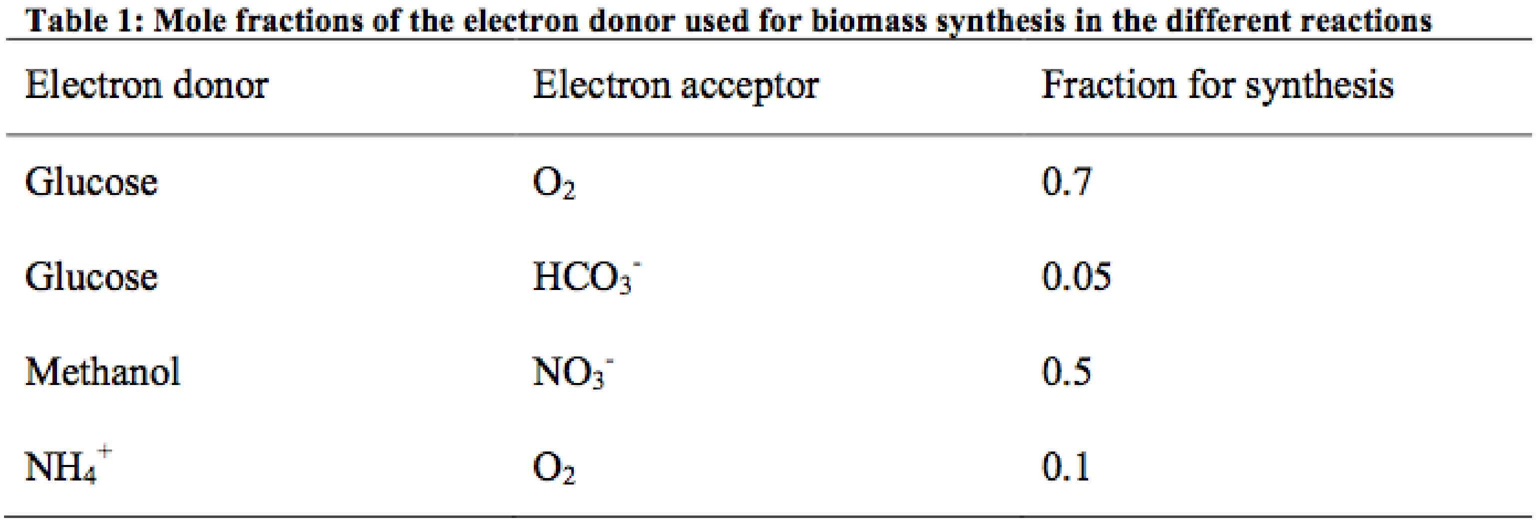


Table 1: Mole fractions of the electron donor used for biomass synthesis in the different reactions

Electron donor	Electron acceptor	Fraction for synthesis
Glucose	O_2	0.7
Glucose	HCO_3^-	0.05
Methanol	NO_3^-	0.5
NH_4^+	O_2	0.1

- Less biomass production in the anaerobic system = big advantage!
- Less sludge production = less sludge to be removed

Wrap-up

At the end of the semester you can:

- Calculate the COD of simple molecules used in WWTP
- Determine the energy gain/input for biochemical reactions using the growth reference system (GRS)
- Comment on sludge production under various conditions in WWTP

When do we meet

Oct 29th	Stoichiometry	
Nov 5th	Removal of organic matter Sludge treatment	13h00 Denduyver
Nov 12th	Nutrient removal Sedimentation	13h00 Denduyver
Dec 2nd	Combined exercise // Overall design 8h30 HILO auditorium A	

Filters, aeration, fluidized-bed, oxidation ditch: additional information