# Biotechnological processes: calculation exercises

Dr. Jan B. A. Arends
Jan.arends@ugent.be

# 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<br>Sludge treatment | 13h00<br>Denduyver |
| Nov 12th | Nutrient removal<br>Sedimentation | 13h00<br>Denduyver |
| Dec 2nd | Combined exercise // Overall design<br>8h30<br>HILO auditorium A | |

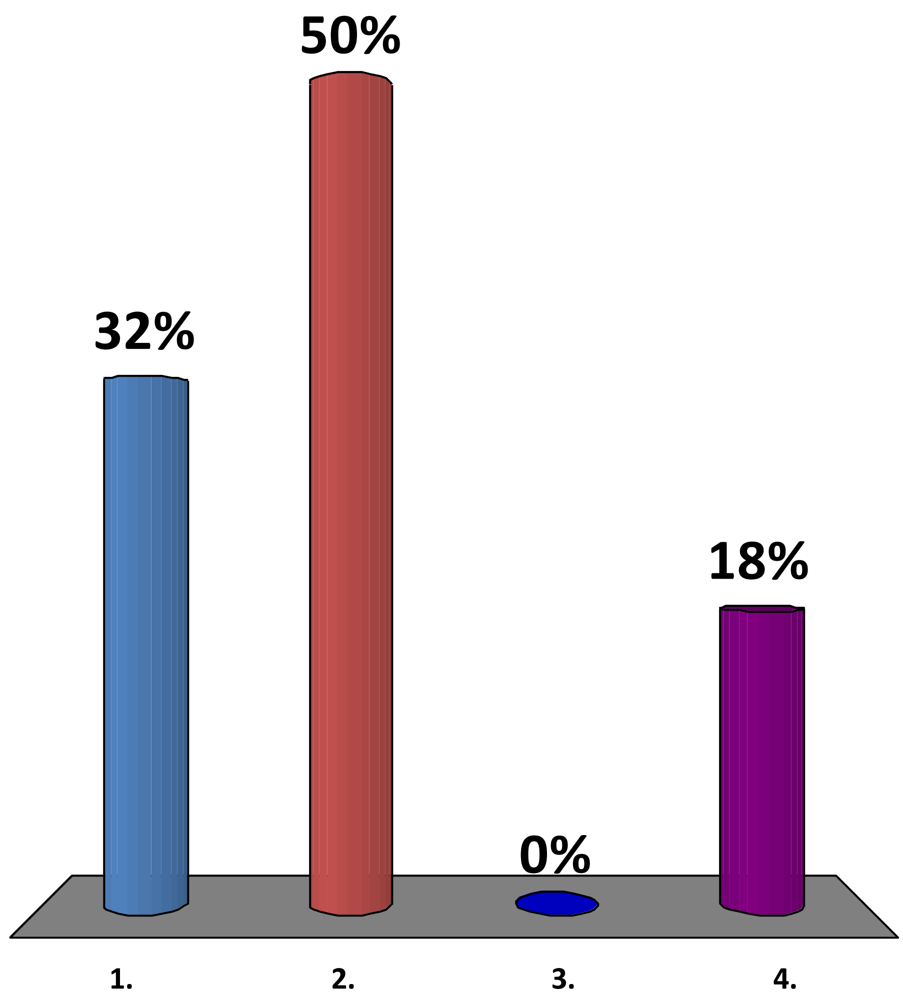
*Filters, aeration, fluidized-bed, oxidation ditch: additional information*

# Part 2: Stoichiometry

Dr. Jan B. A. Arends
Jan.arends@ugent.be

# 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 → C + D

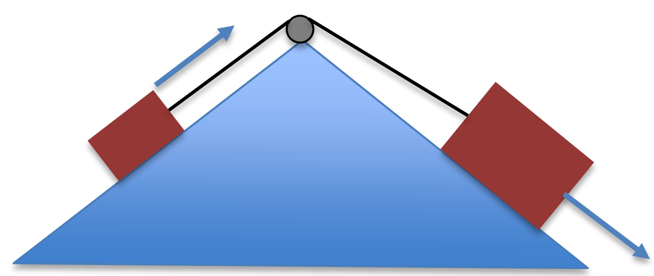
reduction

→ Thermodynamics: thermodynamically feasible

**Driving force : $\Delta G'^0$**

$\Delta G'^0 < 0$ 

$\Delta G'^0 > 0$ 



Different references to calculate $\Delta G'^0$:
- Standard conditions (1 bar, 298.15 K, 1 mol/L, pH = 0)
- <u>BIOCHEMICAL Standard conditions (1 atm, 298.15 K, 1 mol/L, pH = 7)</u>

# Growth reference system (GRS)

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

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

  $\gamma_x$ = degree of reduction

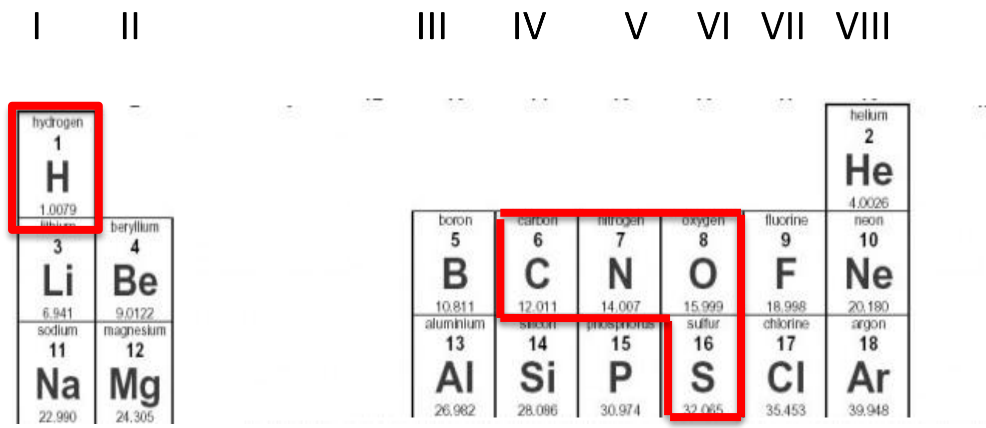
  $\Delta G_{e,x}$ = Gibbs free energy of formation (kJ/mol $e^-$) (Table 2)
- Gibbs energy of formation is 0 for stable end products in **microbial** systems
  - $HCO_3^-$, $SO_4^{2-}$, $NO_3^-$, $H_2O$, $H^+$
- At biochemical standard conditions
  - 1 atm, 298.15K, pH 7, 1 mol/L

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

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

# Degree of reduction ($\gamma$)

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



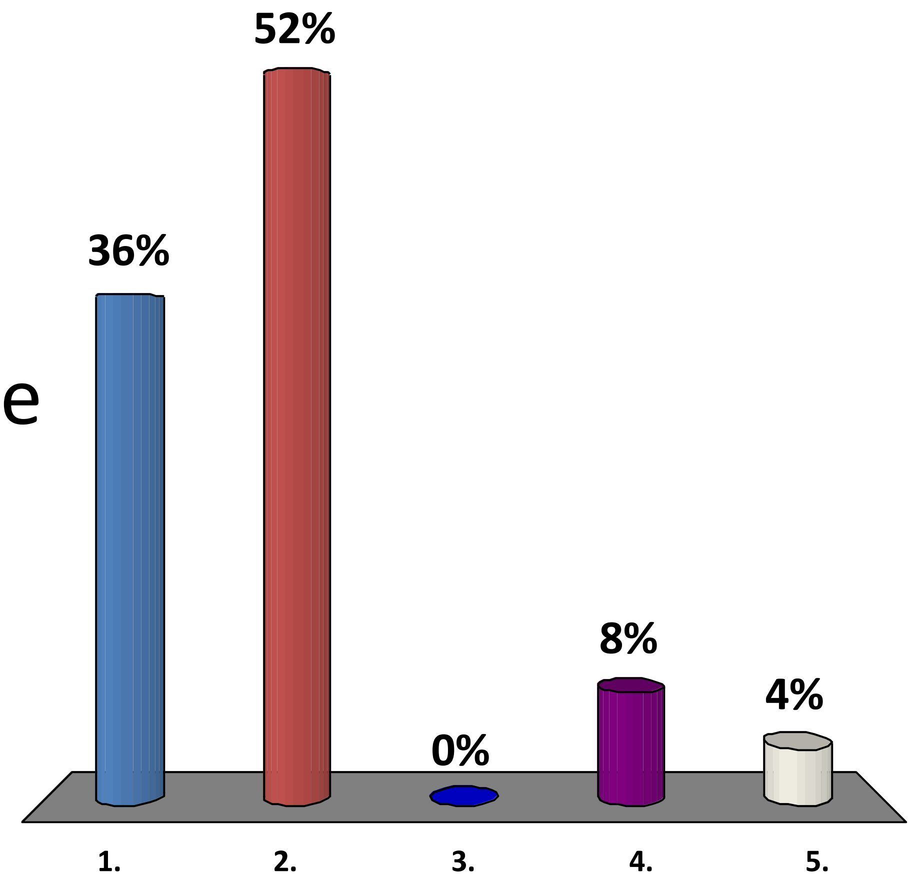

➔Degree of reduction = sum of reduction of all elements in a molecule

$SO_4^{2-}$ →

$NH_4^{+}$ →

# 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 ($\gamma$)

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  - P +5
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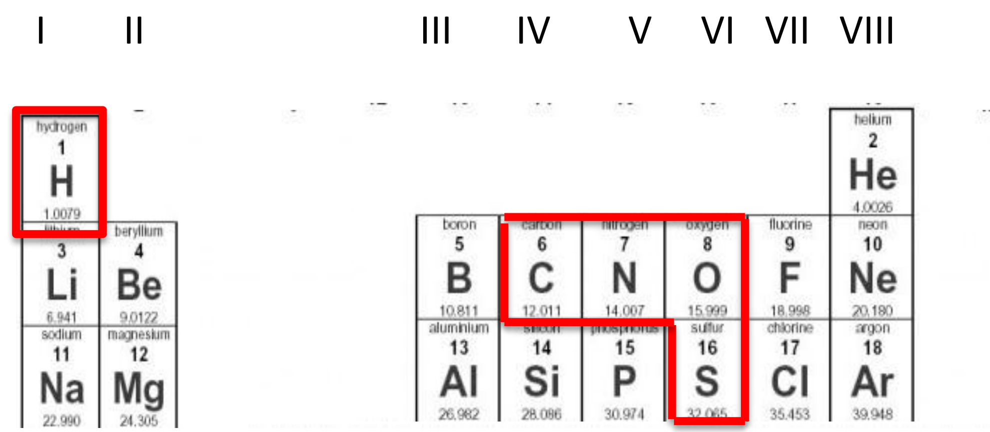



➔Degree of reduction = sum of reduction of all elements in a molecule

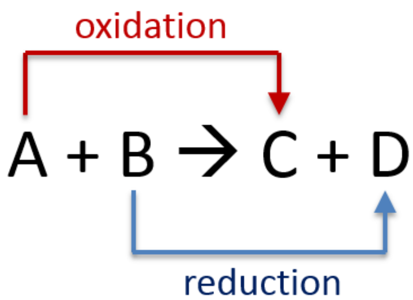
$SO_4^{2-}$ → $\gamma$ = 1 x 6 + 4 x (-2) + 2 x 1 (negative charge) = 0

$NH_4^{+}$ → $\gamma$ = 1 x 4 + 1 x 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 acetate to bicarbonate* (oxidation half reaction)

* $CO_2$ at pH = 7 = $HCO_3^-$

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

# Reaction stoichiometry

## Half reactions

Conversion of oxygen to water (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

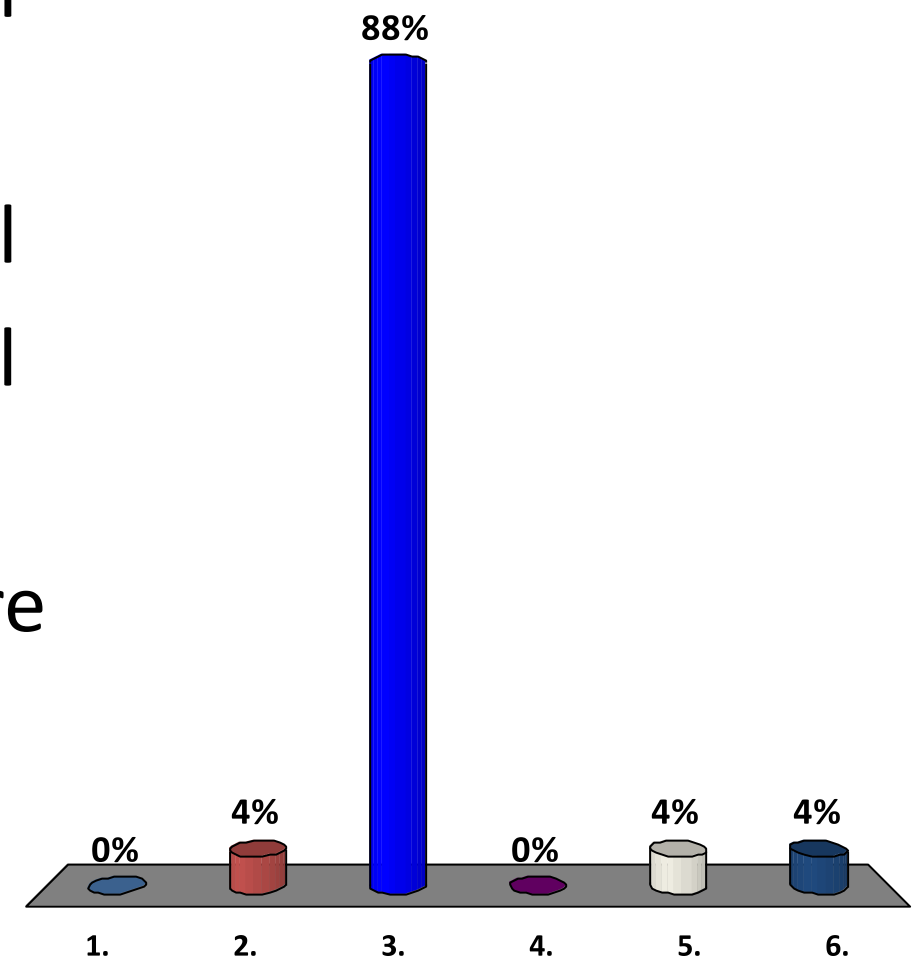
**Example: metanol ($CH_4O$)**

**degree of reduction = 6 $e^-$**

**COD = 6 $e^-$ X 8 gr$O_2$/$e^-$ = 48 gCOD/mol $CH_4O$ = 1.5 gCOD/g$CH_4O$**

# 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)

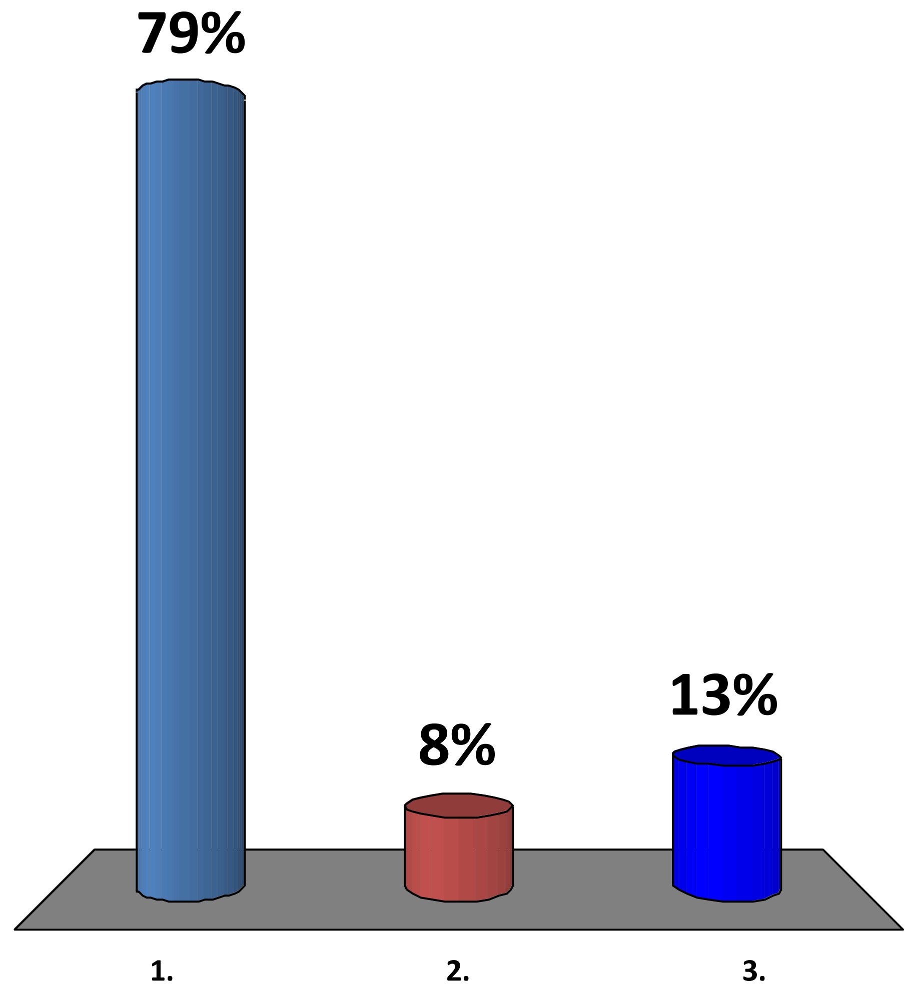
    = 12 mol $e^-$/mol ethanol
  - COD = 12 X 8 = 96 g COD/mol ethanol
  - COD/weight = $\frac{96\ g\ COD/mol}{46\ g\ ethanol/mol}$

    = 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

    $\gamma$ = 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

$\nu_x$ = stoichiometric coef.

$\gamma_x$ = degree of reduction

$\Delta G_{e,x}$ = Gibbs free energy of formation (kJ/mol $e^-$)

- $\Delta G_r$ : Gibbs free energy of reaction
  - Calculated with conventional values, after production with the reference substances (bicarbonate, nitrate, …)
- $\Delta G_e$ : Gibbs free energy of formation per electron
  - Divide $\Delta G_r$ 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 $\Delta G_r$. 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 $\Delta G_r$ 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°

$HCOOH \rightarrow H_2 + CO_2$

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

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

$\gamma$ 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 $\rightarrow$ does not allow growth

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

(at pH 7)

$\rightarrow$ allows growth

1°

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

(at pH 7)

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

$$\Pi = \frac{a_C{}^{v_C} a_D{}^{v_D}}{a_A{}^{v_A} a_B{}^{v_B}} \approx \frac{[C]^{v_C}[D]^{v_D}}{[A]^{v_A}[B]^{v_B}} = \frac{[HCO_3^-][H_2]}{[HCOOH]} = \frac{1*(10^{-5})}{1} = 10^{-5}$$

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

$$\approx -27kJ/mol$$

→ 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

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

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

$$\Delta G_r = \Delta G_r^{0'} + RT*2.303*(\log[H_2] - 2\log[1]) = \Delta G_r^{0'} + RT*2.303*(-5) of \Delta G_r^{0'} + RT*\ln(10^{-5})$$
$$= 51.476 kJ/mol$$

$\rightarrow$ 51.476 – 78 = <u>-27 kJ/mol</u>

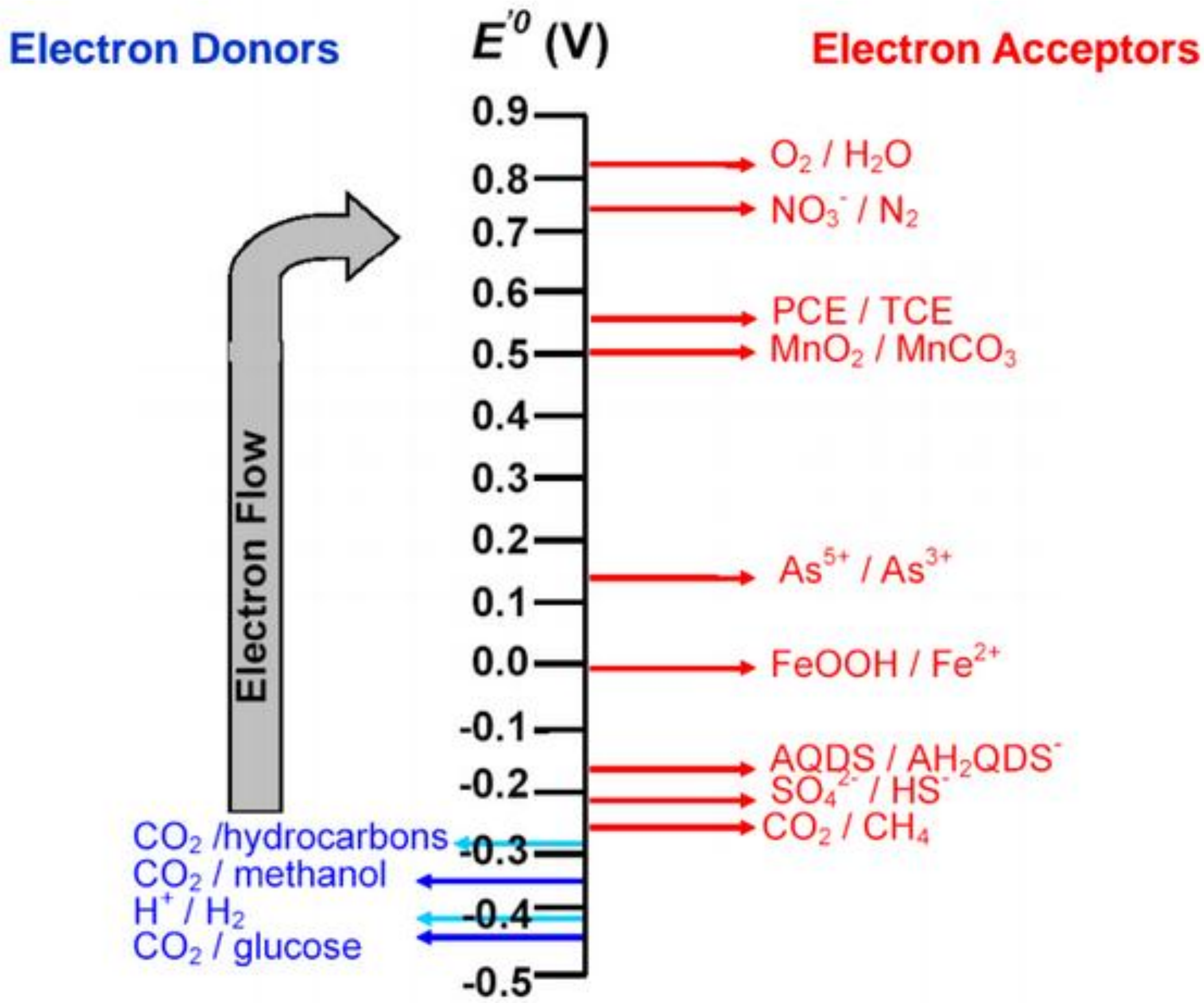
# Stoichiometry in WWTP

- 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) |
|---|---|
| Glucose + 12 $H_2O$ $\leftrightarrows$ 6 $HCO_3^-$ + 30 $H^+$ + 24 $e^-$ | -954 |
| 3X ($HCO_3^-$ + 9 $H^+$ + 8 $e^-$ $\leftrightarrows$ Methane + 4 $H_2O$ | 183) |
| 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

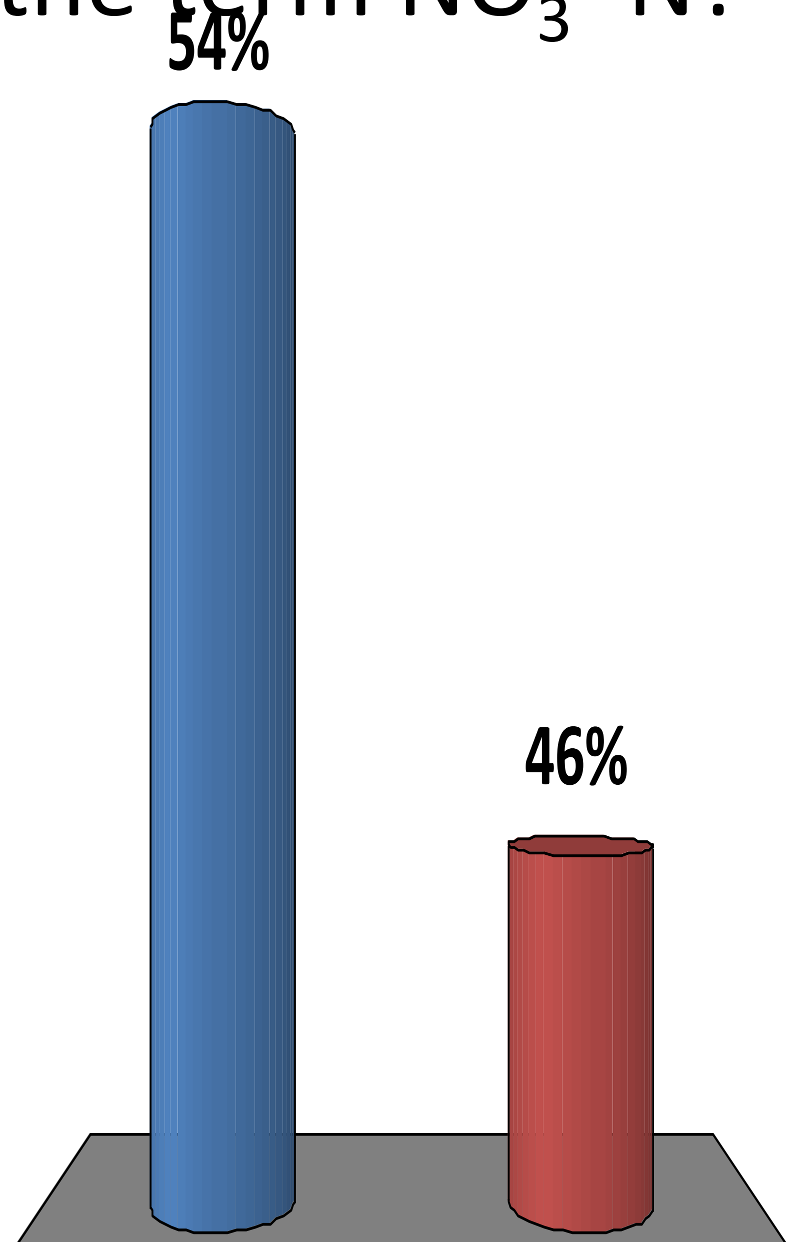
- Nitrogen removal
  - Denitrification: heterotrophic bacteria: need a carbon source!

| | $\Delta G_r^{0'}$ (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

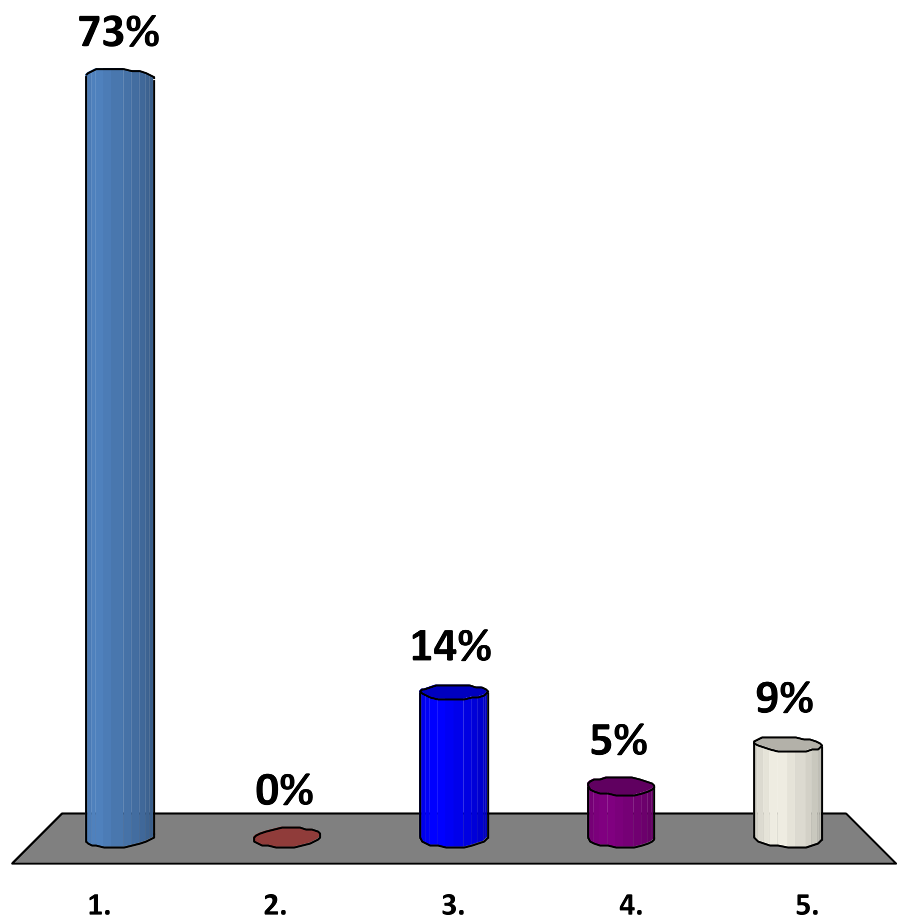


# $NH_4^+$-N, $NO_2^-$-N, $NO_3^-$-N

- 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. ?

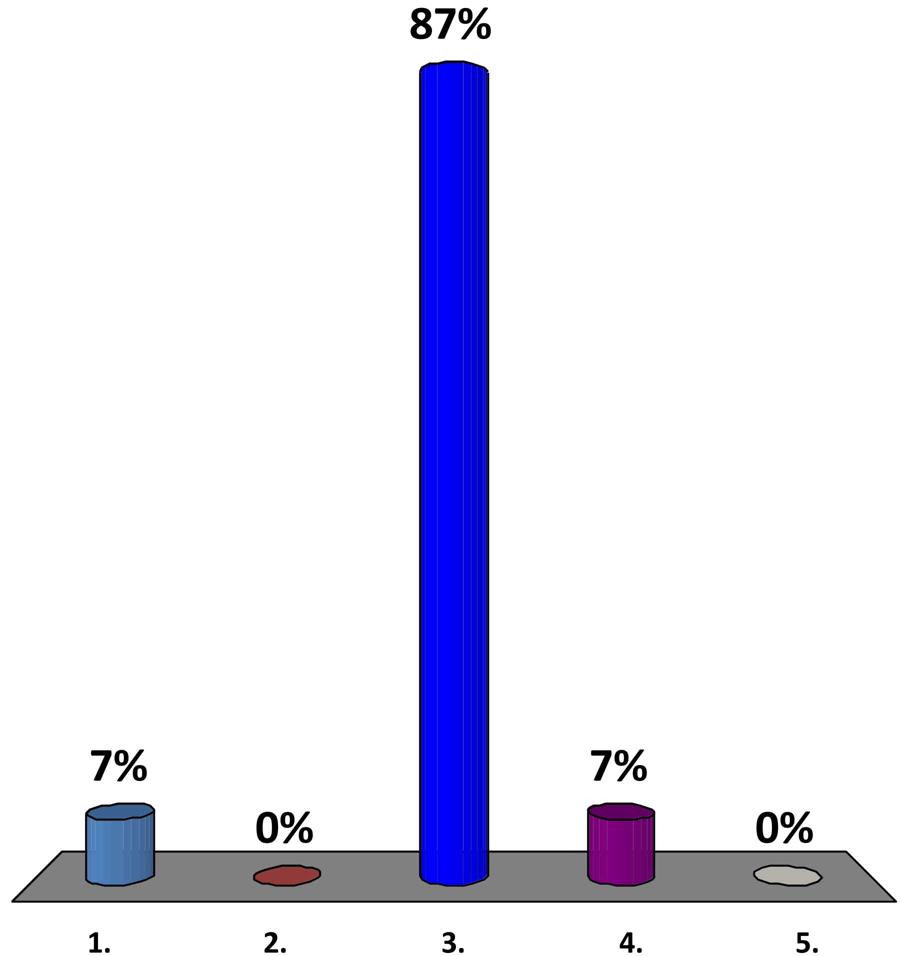


# Exercise 4a

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

$$5\,(\text{Methanol} + 2\,H_2O \leftrightarrows HCO_3^- + 7\,H^+ + 6\,e^-)$$
$$\underline{6\,(NO_3^- + 6\,H^+ + 5\,e^- \leftrightarrows 0.5\,N_2 + 3\,H_2O)}$$
$$5\,\text{MetOH} + 6\,NO_3^- + H^+ \rightarrow 3\,N_2 + 5\,HCO_3^- + 8\,H_2O$$

→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<br>Sludge treatment | 13h00<br>Denduyver |
| Nov 12th | Nutrient removal<br>Sedimentation | 13h00<br>Denduyver |
| Dec 2nd | Combined exercise // Overall design<br>8h30<br>HILO auditorium A | |

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