
Can wastewater-borne engineered nanomaterials pose a threat to the microorganisms involved in wastewater treatment?

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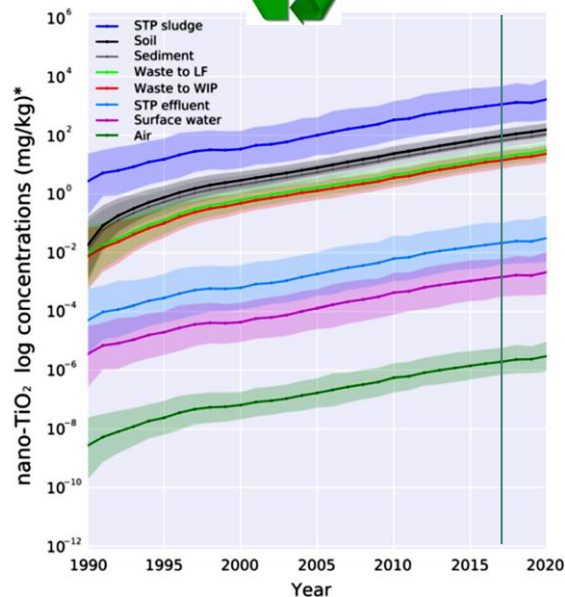
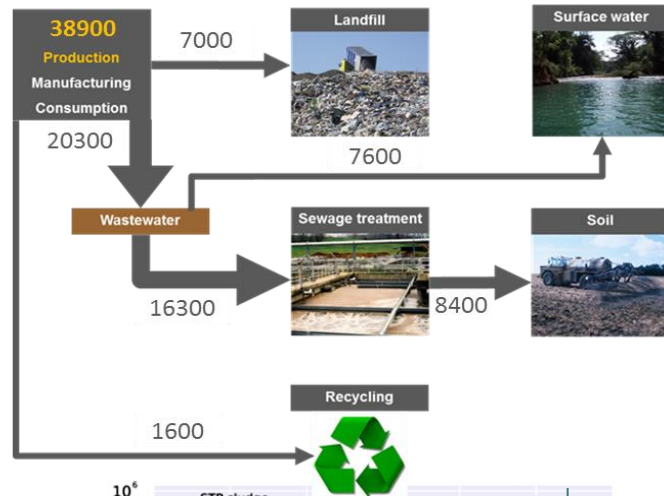
¹NIBIO Norwegian Institute of Bioeconomy Research

²NMBU Norwegian University of Life Science

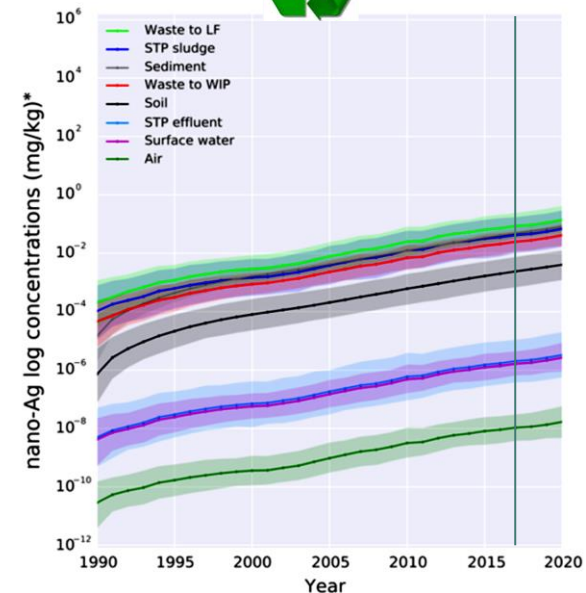
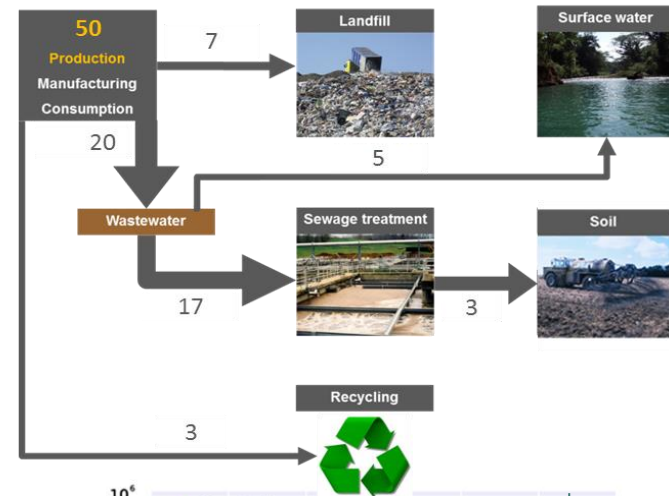
June 19th 2017, 16th ICCE – Oslo

Estimated mass flows in the EU in tonnes in 2014

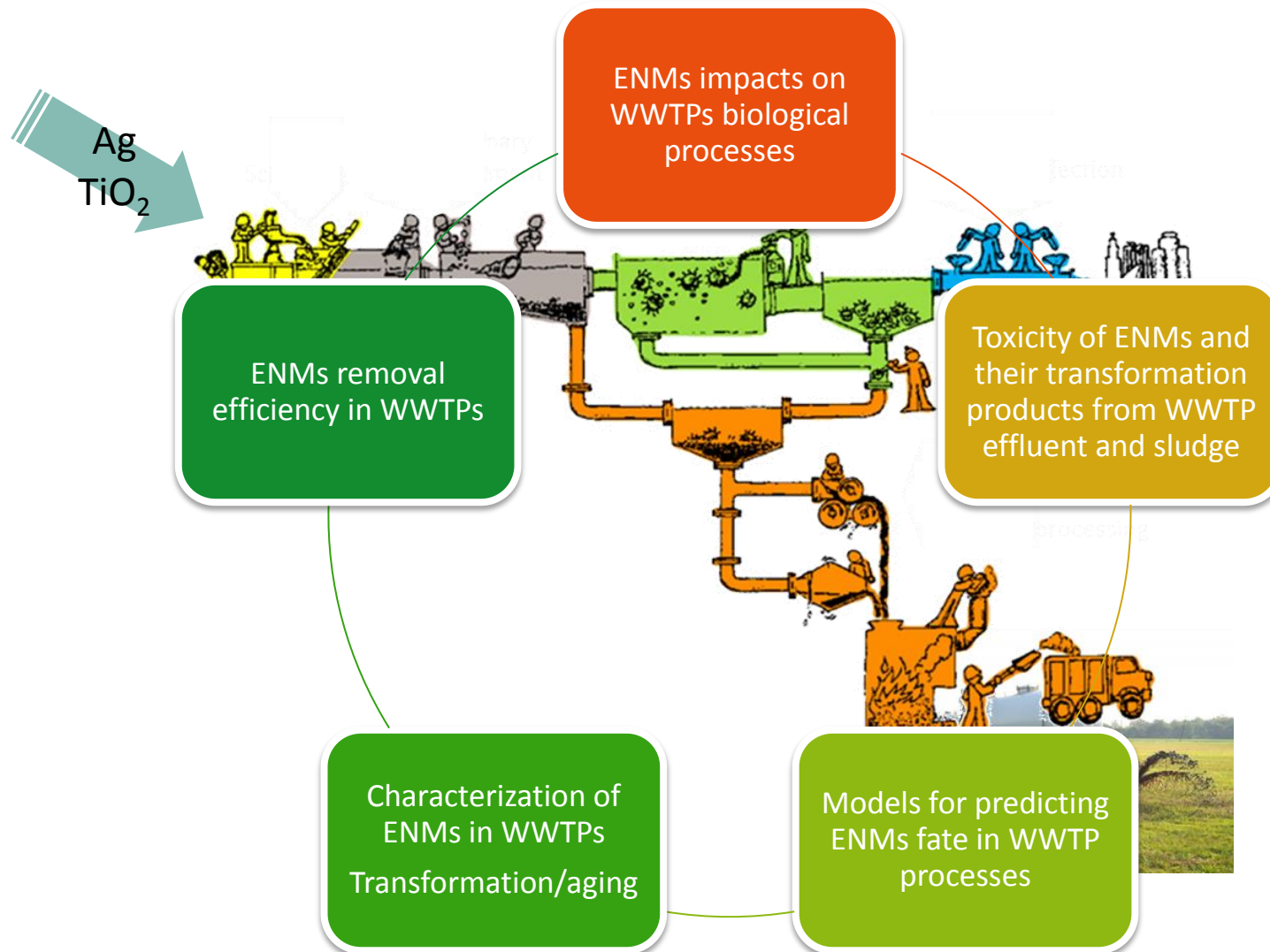
TiO₂ NPs



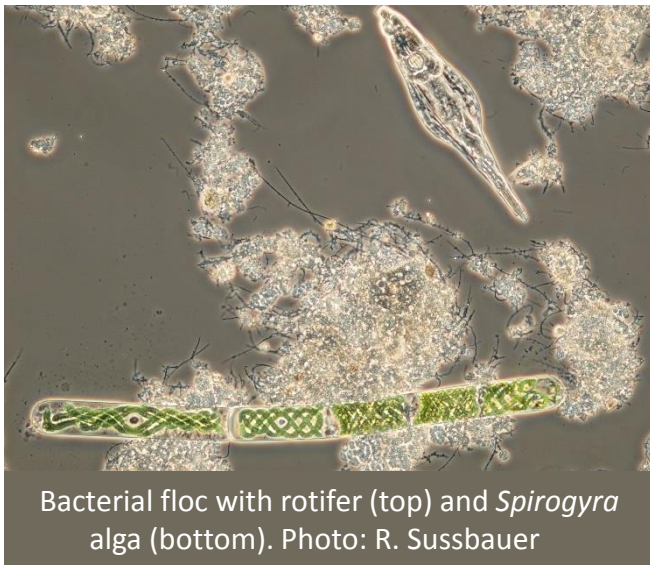
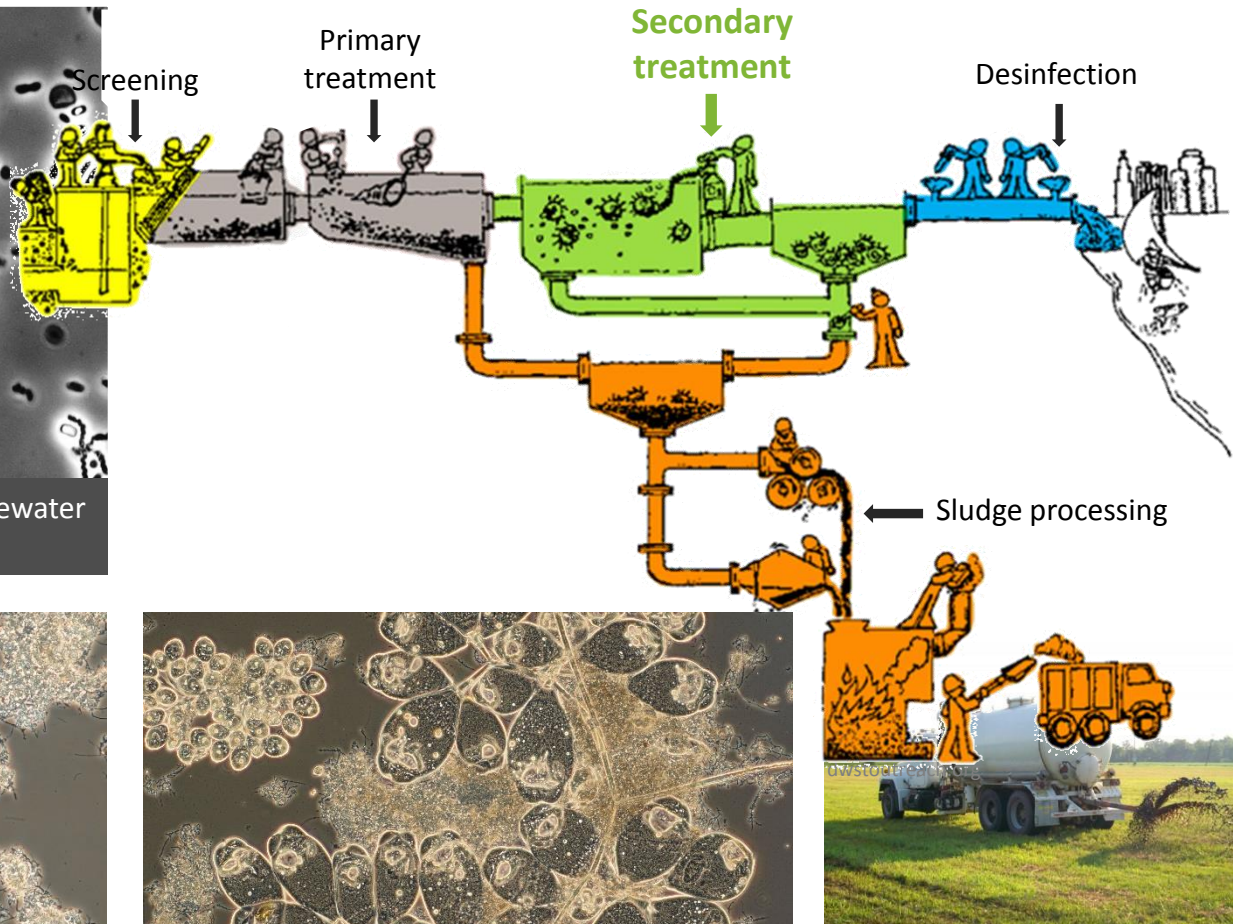
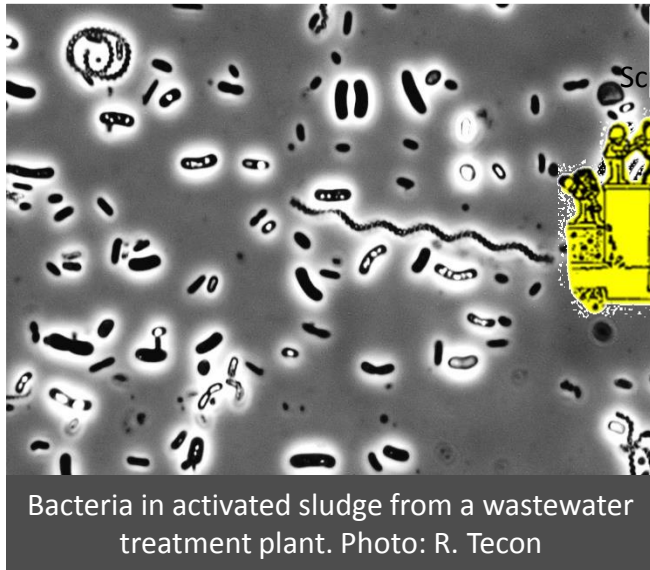
Ag NPs



The NanoWASTE project in a nutshell



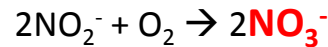
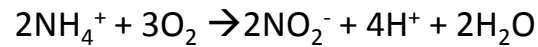
Disturbing useful bacterial processes?



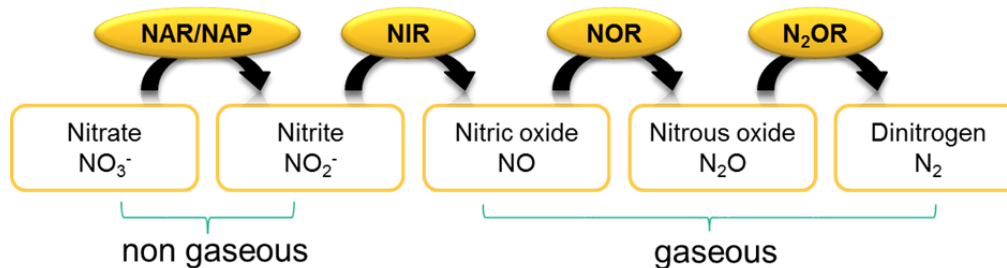
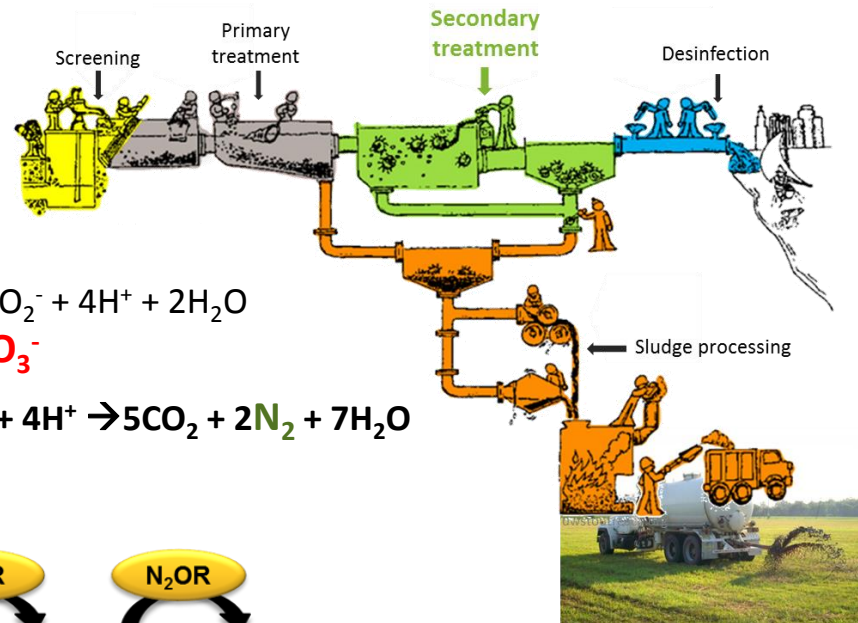
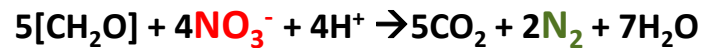
Disturbing useful bacterial processes?



Nitrifying bacteria



Denitrifying bacteria



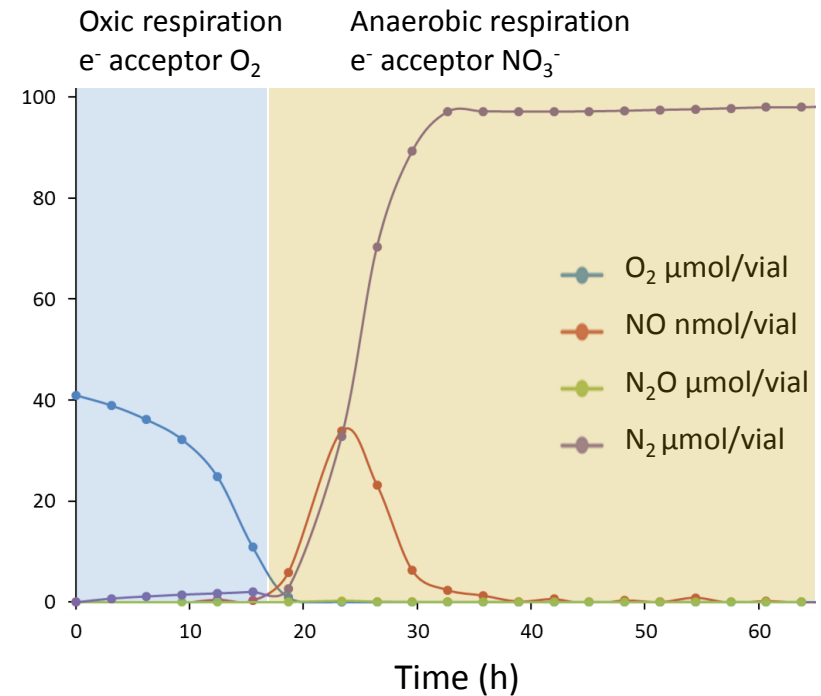
Paracoccus denitrificans
Thauera linaloolentis

Experimental set-up

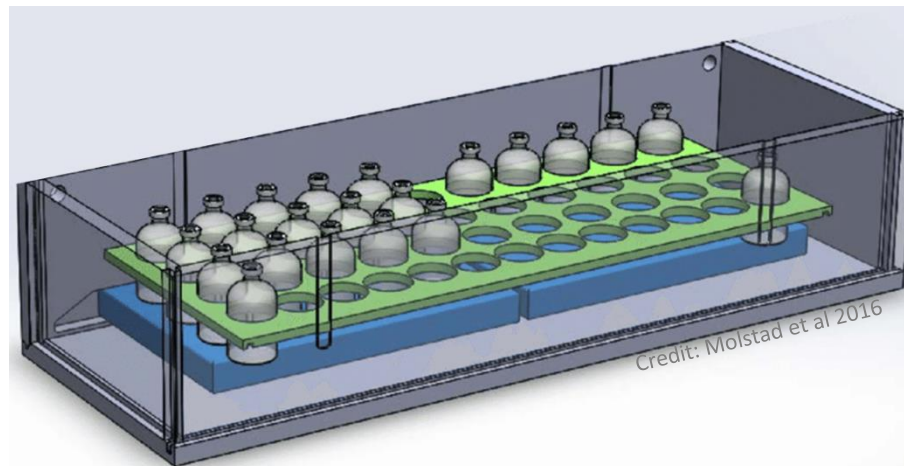


1 mL O₂

40 mL *Thauera* growth medium
 10 mL NP suspension (0.1 µg/L to 10 mg/L)
 100 µL KNO₃ @ 1 mol/L
 1 mL *T. linaloolentis* @ OD₆₆₀ = 0.100



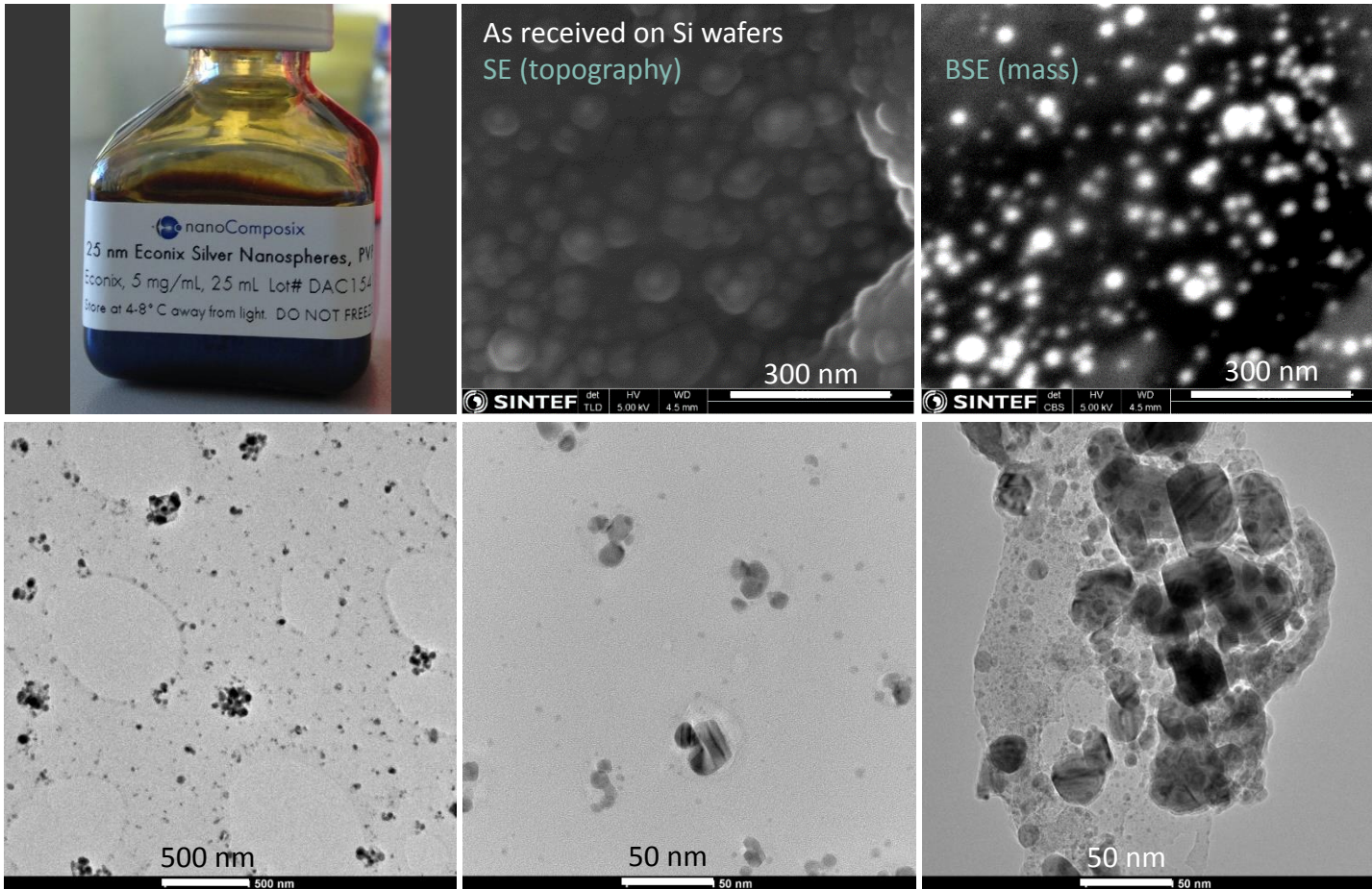
3 replicates/concentration



25 °C, vigorous stirring

Ag nanoparticles

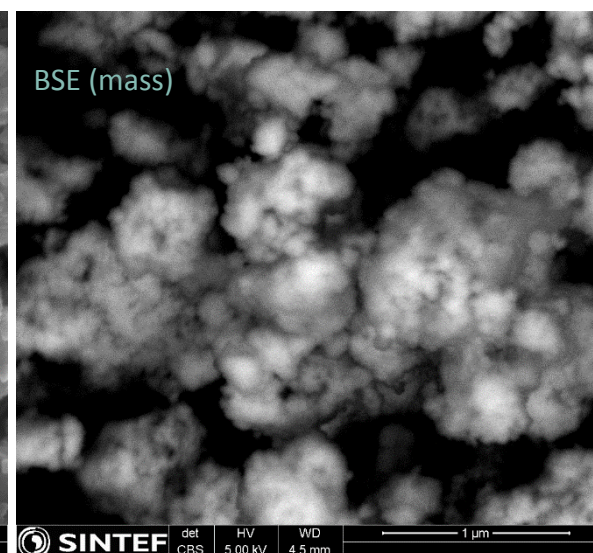
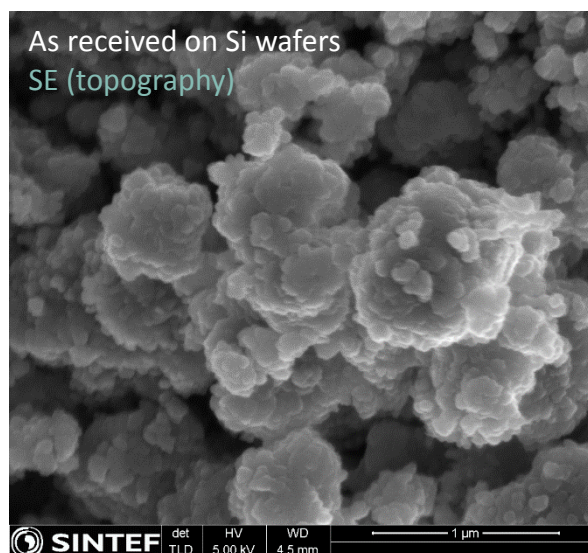
PVP-coated Ag NPs, TEM diameter 21 ± 8 nm, $\zeta\text{-pot}_{\text{H}_2\text{O}}$ -40 mV



TiO₂ nanoparticles

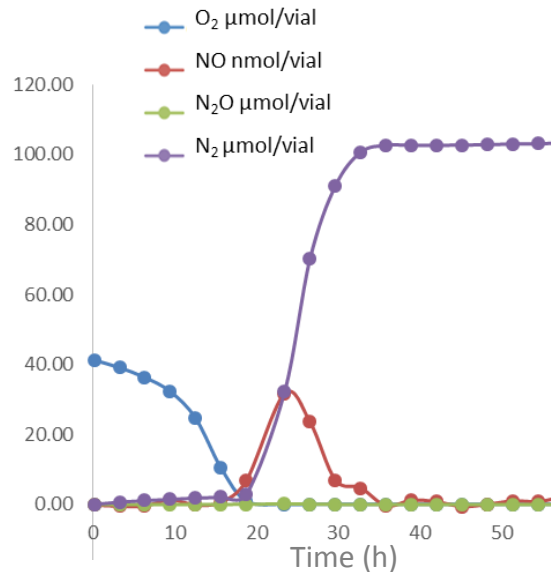
JRCNM01001a, Uncoated anatase, TEM diameter «5-6 nm», ζ -pot_{H₂O} -5 mV

Dispersion in MilliQ water: Sonication on ice, NANoREG protocol for producing reproducible dispersions of ENMs in exposure media



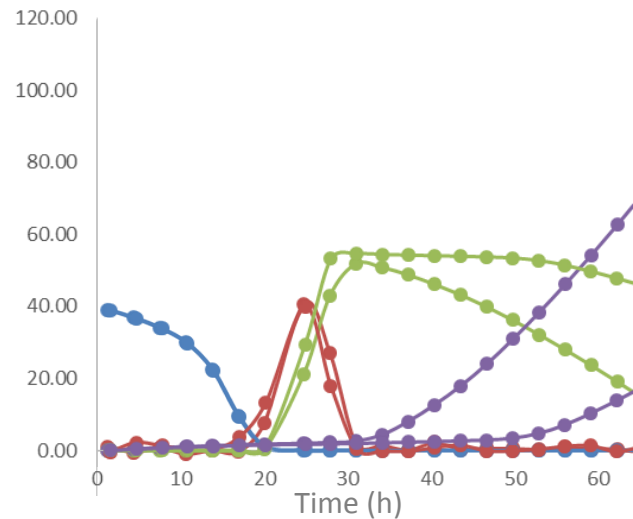
JRC Reference nanomaterial ?!

Denitrification by *Thauera linaloolentis* is impaired in presence of Ag NPs and their transformation products



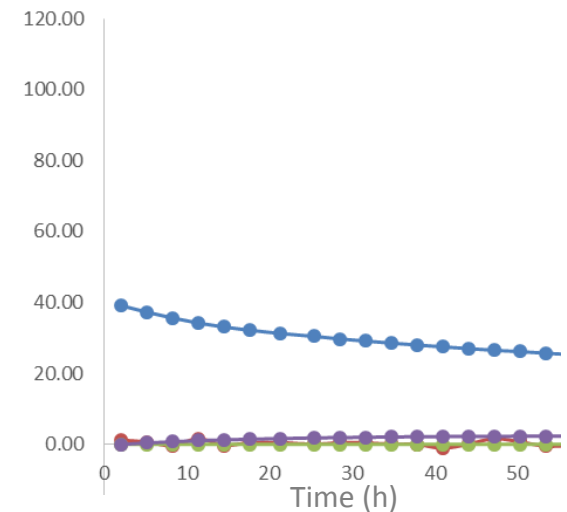
Control
 10 µg/L
 100 µg/L

No effect



500 µg/L
 1000 µg/L

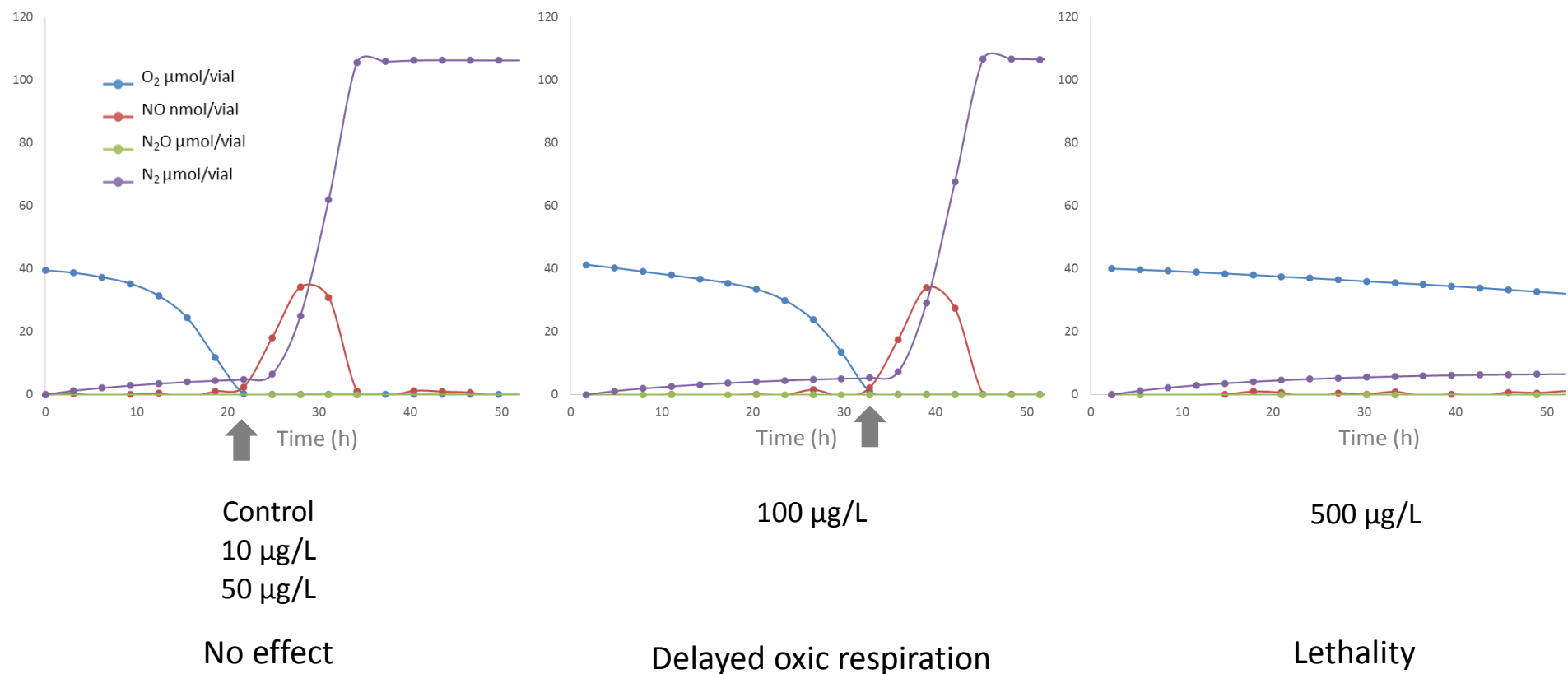
N₂O accumulation
 Inhibition N2OR



5 mg/L
 10 mg/L

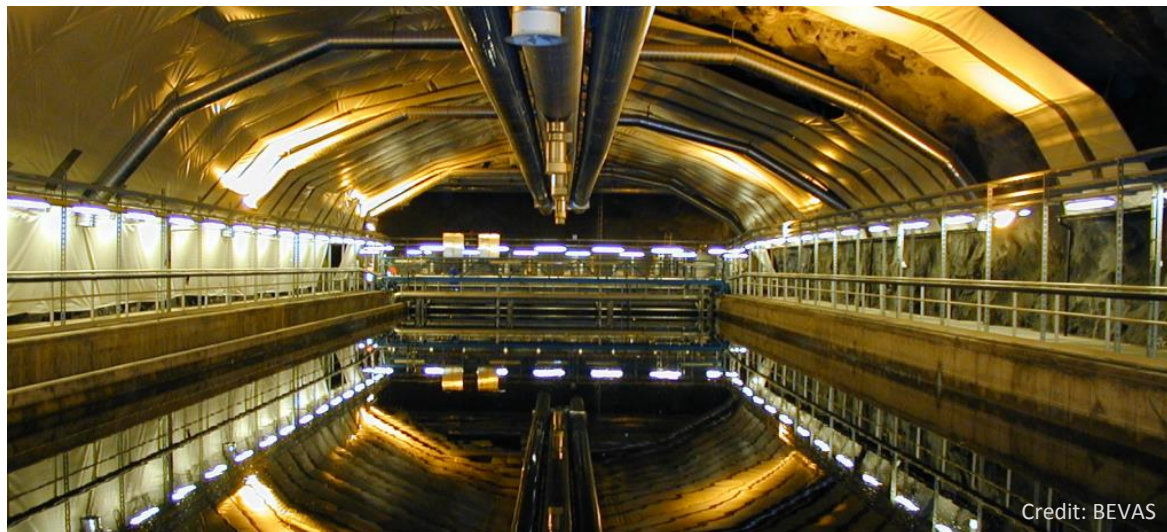
Lethality

Oxic respiration by *Paracoccus denitrificans* is impaired in presence of Ag NPs and their transformation products

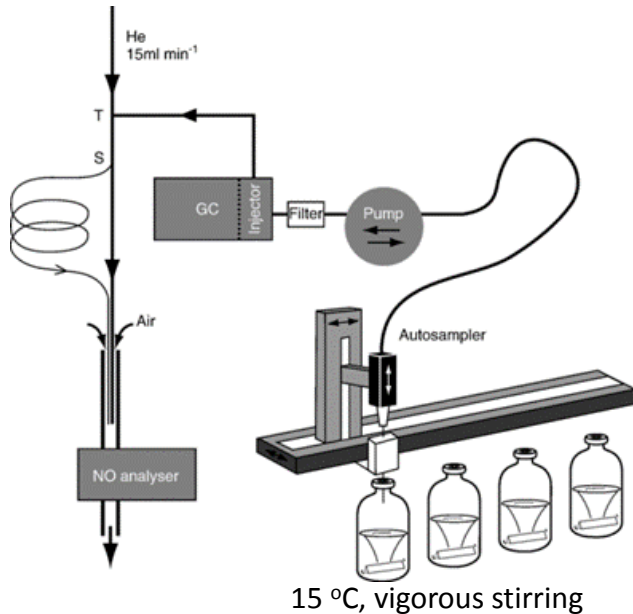


Single bacterial strains – Results overview

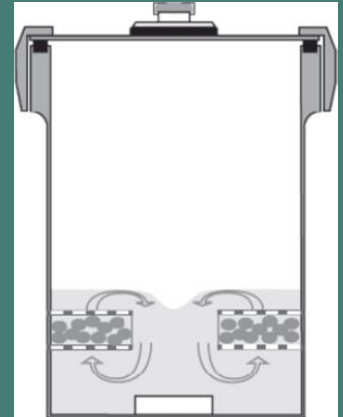
- ✓ Accumulation of N_2O by *T. linaloolentis* exposed to $>100 \mu\text{g/L}$ Ag NPs
- ✓ Delayed oxic respiration of *P. denitrificans* exposed to $>50 \mu\text{g/L}$ Ag NPs
- ✓ No effect of TiO_2 NPs (max tested 10 mg/L)
- ✓ Valuable **mechanistic basis** for investigations at a higher level of ecological complexity, i.e. on indigenous bacterial communities sampled in WWTPs



Denitrifying microbial communities from WWTP



Suspended microorganisms

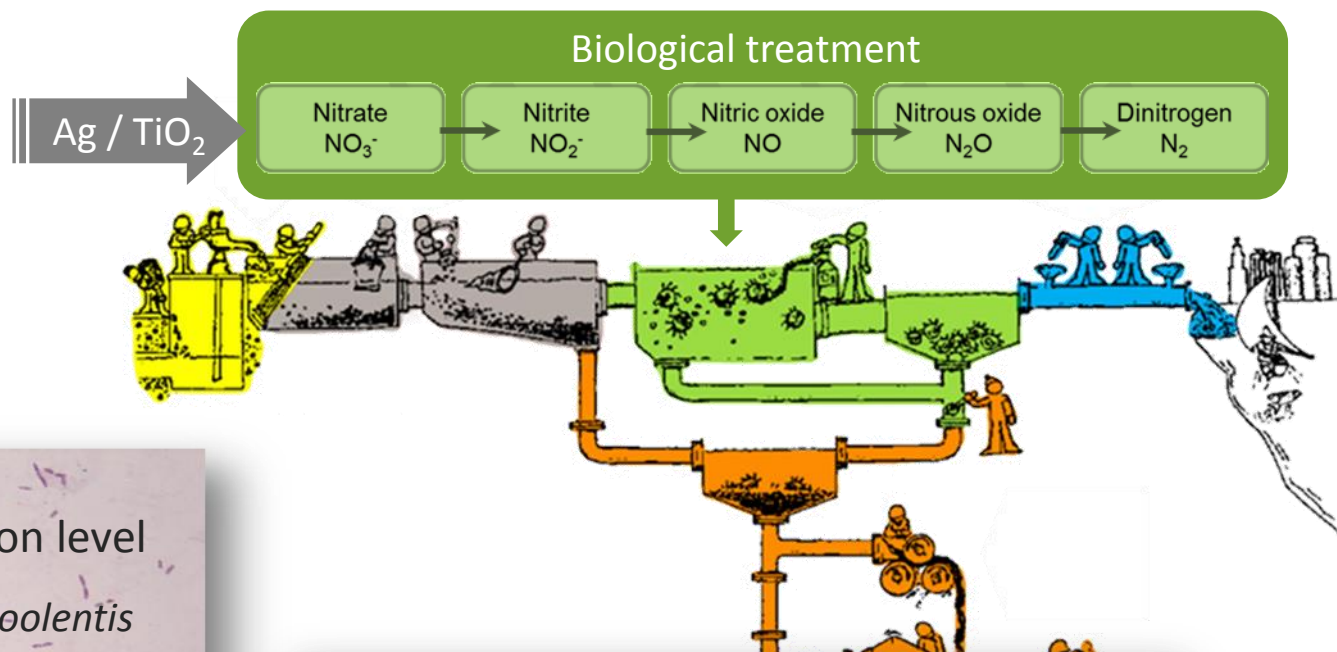


Biofilms on LECA particles

Denitrification not affected by

- ✓ Ag NPs (max tested 1 mg/L)
- ✓ TiO₂ NPs (max tested 10 mg/L)
- ✓ co-exposure Ag and TiO₂ NPs (max tested 1 and 10 mg/L, respectively)

Summary



Single strain – Population level

N_2O accumulation *T. linaloolentis*

Delayed oxic respiration *P. denitrificans*

Mechanisms

Photo: Minet

Activated sludge – community level

Complex structure

Exposure legacy, individual sensitivity

Chemical speciation, bioavailability

Functional redundancy, Resilience

Photo: Cundell

Thank you for your attention

Acknowledgements:

NanoWaste project Partners

The Research Council of Norway

Chemical composition of the growth medium

	g/L	g/mol	mol/L
NH ₄ Cl	0.45	53	0.00849
K ₂ SO ₄	0.05	174	0.00029
MgCl ₂ ·6H ₂ O	0.1	202	0.00050
CaCl ₂ ·2H ₂ O	0.025	146	0.00017
NaHCO ₃	0.04	84	0.00048
CH ₃ COONa	0.2	82	0.00244
peptone	0.5		#DIV/0!
meat extract	0.3		#DIV/0!
Na ₂ HPO ₄	?		#DIV/0!
NaH ₂ PO ₄	?		#DIV/0!
Ag ⁺	0.0001	108	0.00000
pH 7.5			
	mol/L		
NH ₄ ⁺	0.00849		
Cl ⁻	0.00982		
K ⁺	0.00057		
SO ₄ ²⁻	0.00029		
Mg ²⁺	0.00050		
Ca ²⁺	0.00017		
Na ⁺	0.00292		
H ⁺	0.00048		
CO ₃ ²⁻	0.00048		
CH ₃ COO ⁻	0.00244		
Ag ⁺	0.00000093		

Percentage distribution among dissolved and adsorbed species for Ag⁺ = 100 µg/L

Component	% of total concentration Only species > 1%	Species name
Ag ⁺	3.211	Ag ⁺
	50.915	AgCl (aq)
	43.544	AgCl ₂ ⁻
NH ₄ ⁺	98.251	NH ₄ ⁺
	1.575	NH ₃ (aq)
Cl ⁻	99.703	Cl ⁻
K ⁺	99.497	K ⁺
SO ₄ ²⁻	89.365	SO ₄ ²⁻
	3.112	MgSO ₄ (aq)
	1.337	CaSO ₄ (aq)
	5.055	NH ₄ SO ₄ ⁻
Mg ²⁺	95.823	Mg ²⁺
	2.364	MgCl ⁺
	1.805	MgSO ₄ (aq)
Ca ²⁺	96.2	Ca ²⁺
	1.497	CaCl ⁺
	2.281	CaSO ₄ (aq)
Na ⁺	99.523	Na ⁺

Ionic strength 0.0126 mol/L
100 % of components are dissolved