Innovative fully biodegradable mulching films & fruit protection bags for sustainable agricultural practices LIFE14 ENV/ES/00048

LIFE MULTIBIOSOL

Extracellular biopolymers from granular sludge originating from wastewater treatment systems

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Nitrogen Cycle

- 1880: Winogradski - Zurich

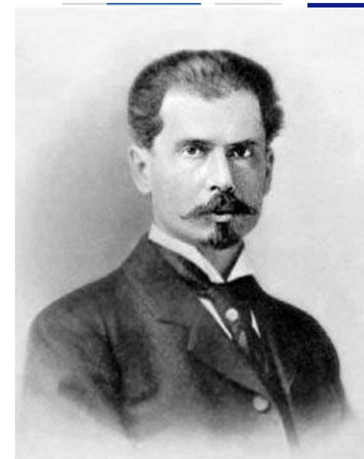
  ➔ Nitrifying bacteria

- ± 1900: Beijerinck - Delft

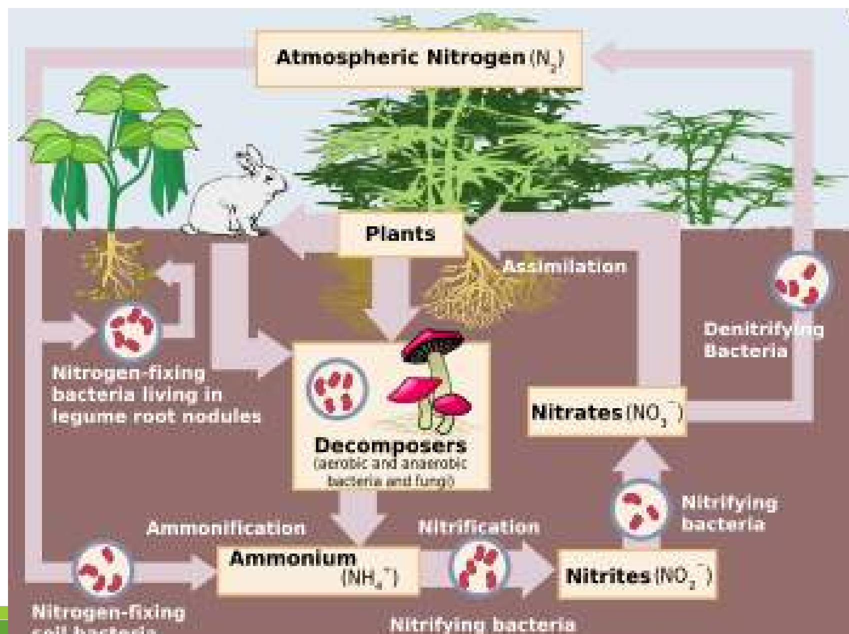
  ➔ Denitrifying bacteria

  ➔ Nitrogen fixing bacteria

Sergei Winogradski
1856-1953

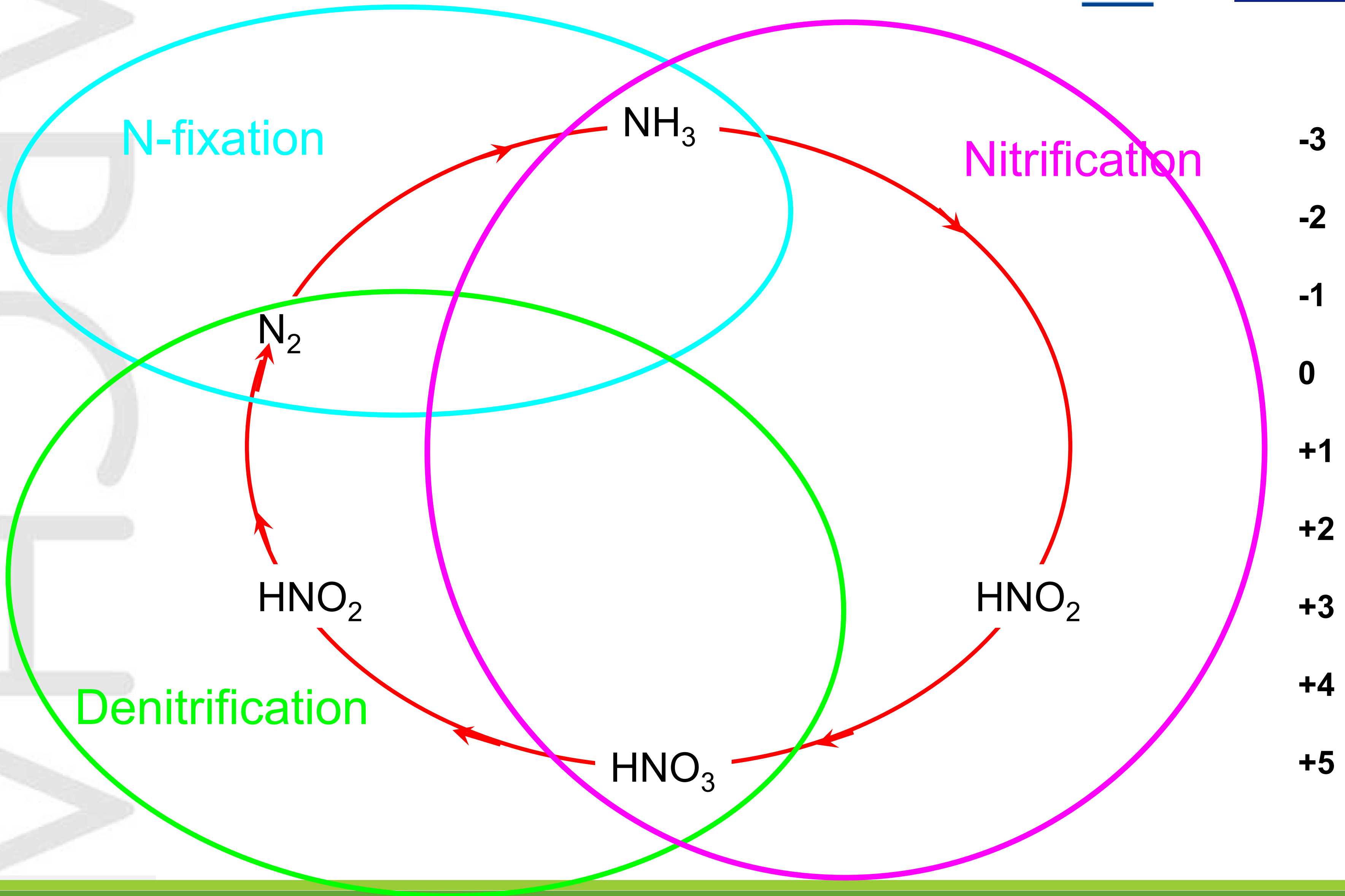


Martinus Willem
Beijerinck
1951-1931

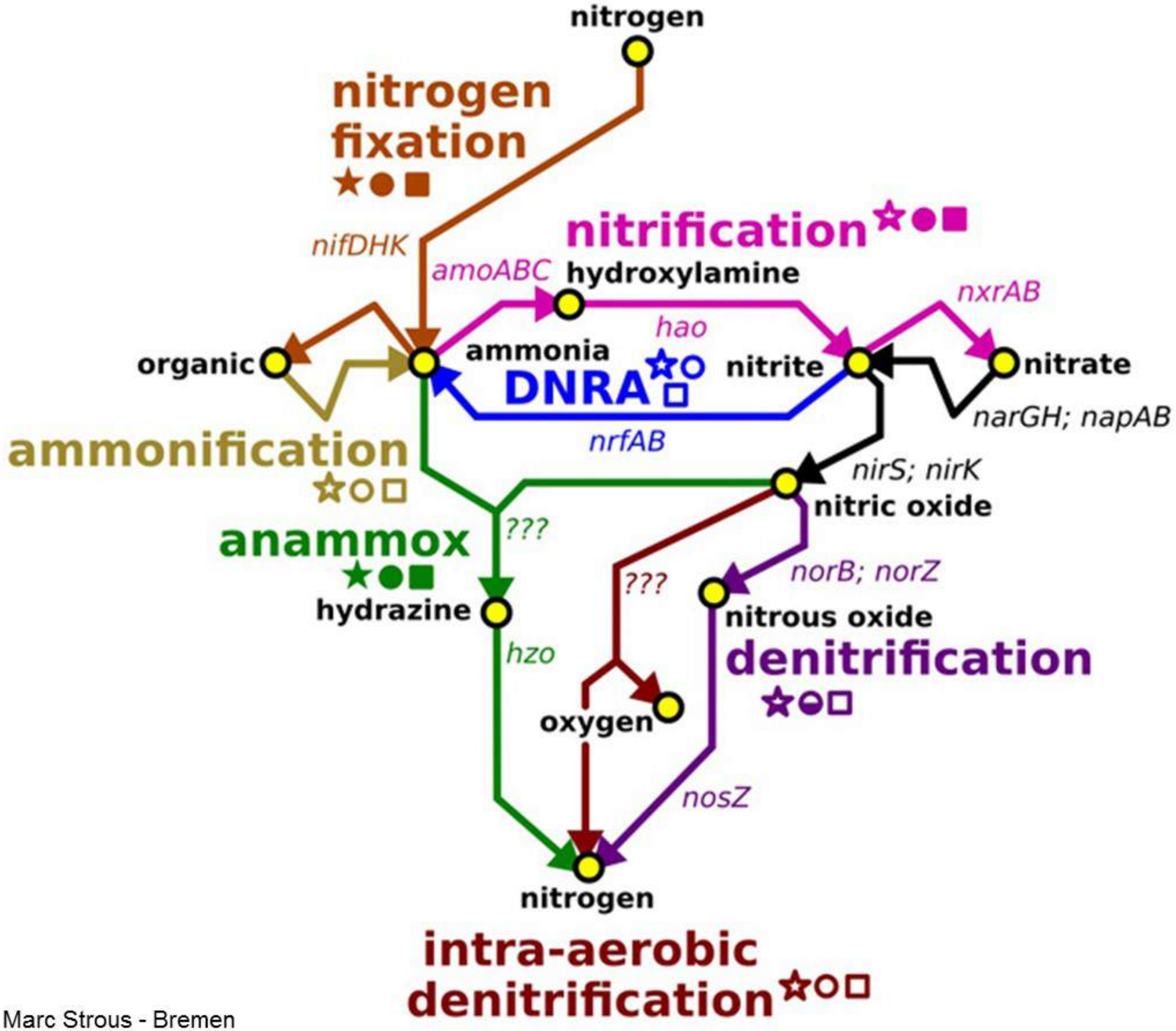


Multibiosol Life+

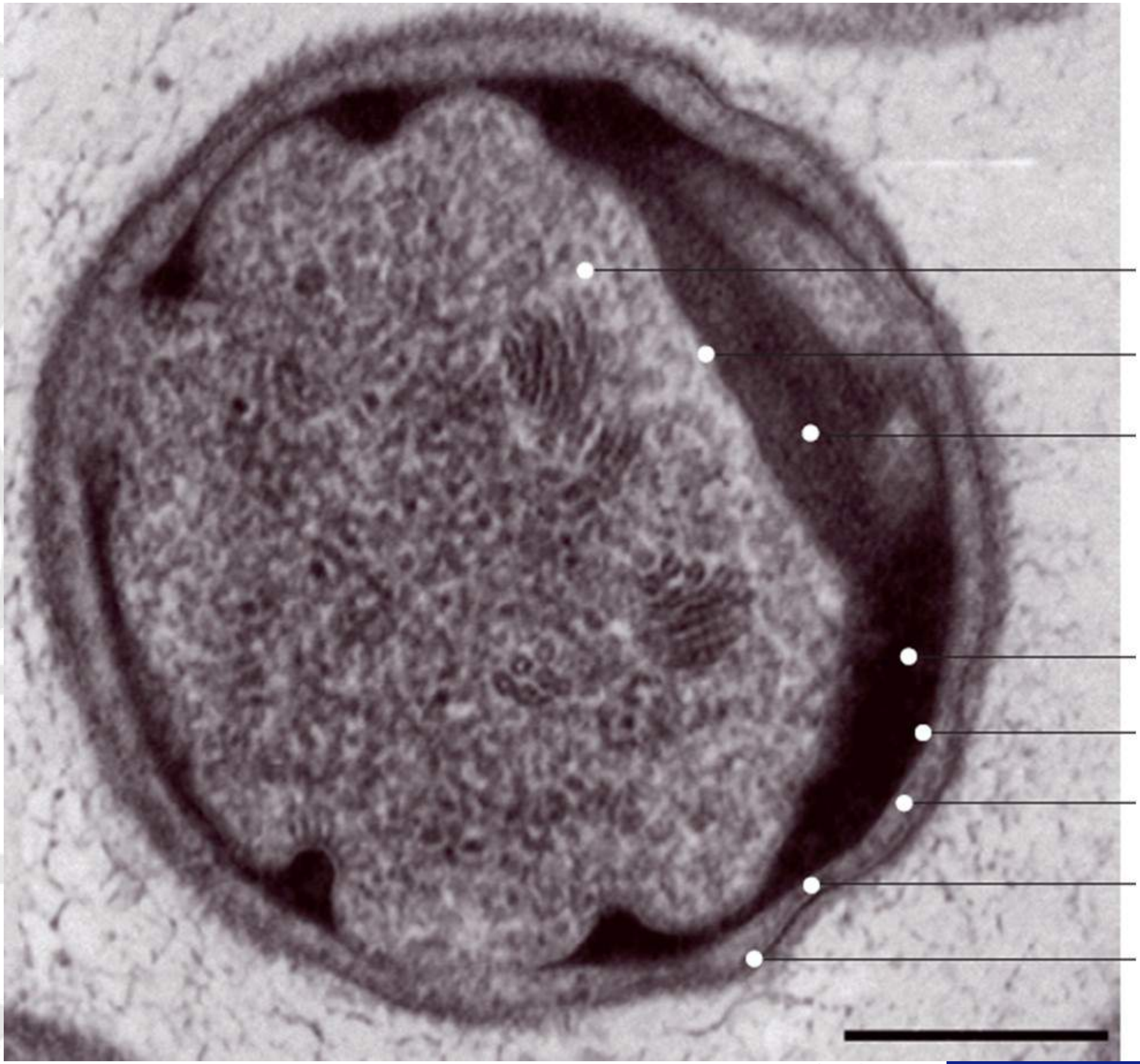
Conventional Nitrogen Cycle



Nitrogen cycle has became a “Nitrogen Web”

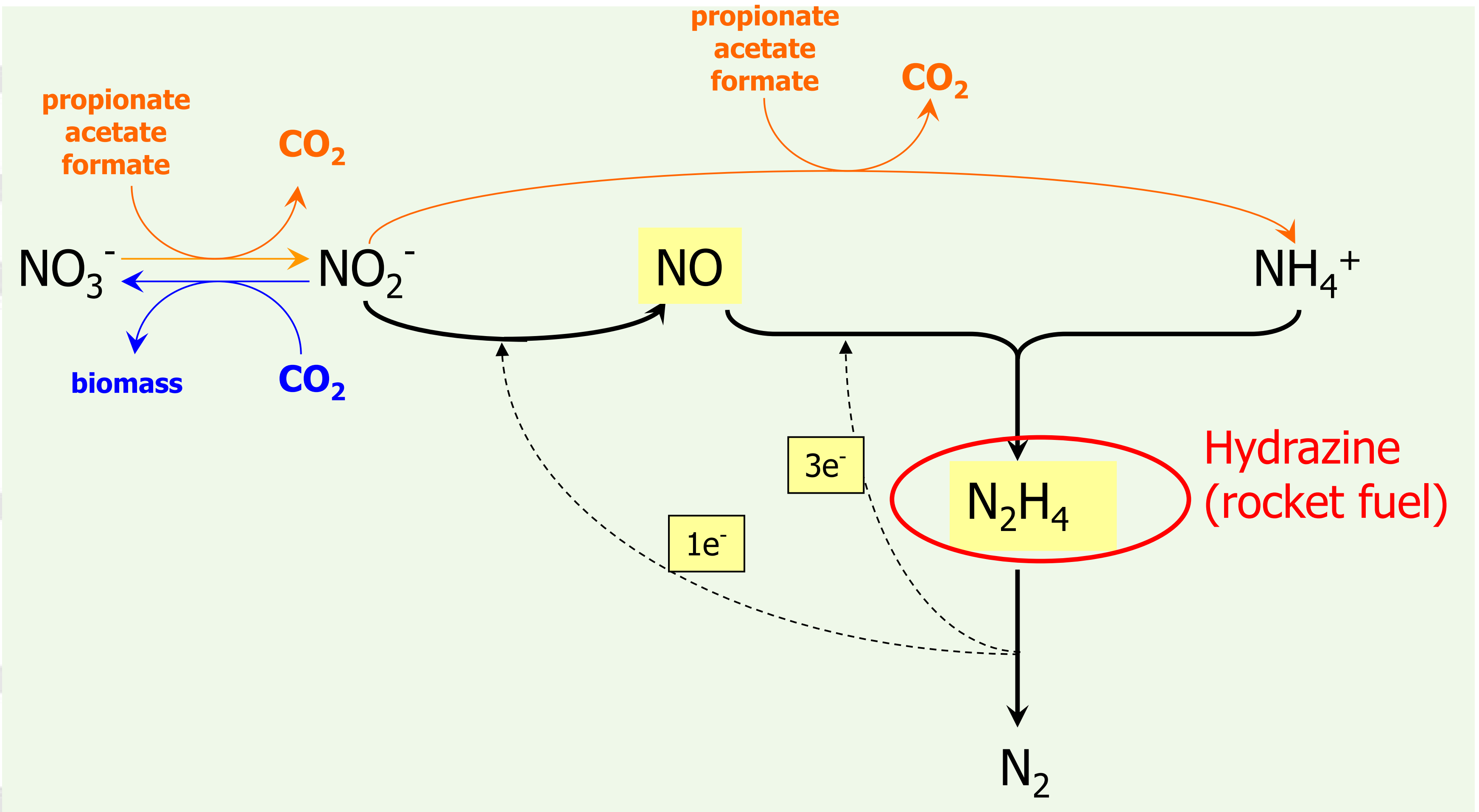


Marc Strous - Bremen



Anammoxosome
Membrana del anammoxosome
Nucleoide
Riboplasma
Membrana introcitoplasmatica
Paryphoplasm
Membrana citoplasmatica
Parete cellulare

Anammox metabolism (autotrophic and mixotrophic*)



*Obligate autotrophy with facultative heterotrophy

CONVENTIONAL AMMONIUM REMOVAL

Nitrification: $2\ NH_3 + (3+1)\ O_2 \rightarrow 2\ NO_3^- + 2\ H^+ + 2\ H_2O$

Denitrification: $2\ NO_3^- + 8g\ COD + 2\ H^+ \rightarrow N_2 + 3g\ SLUDGE$

$2\ NH_3^- + 4\ O_2 + 4\ COD \rightarrow N_2 + 3g\ SLUDGE$

AUTOTROPHIC AMMONIUM REMOVAL

Nitritation: $NH_3 + 1.5\ O_2 \rightarrow NO_2^- + H^+ + H_2O$

Anammox: $NO_2^- + NH_3 + H^+ \rightarrow N_2 + 2\ H_2O$

$2\ NH_3^- + 1.5\ O_2 \rightarrow N_2$

$ Costs $

Missed opportunity

CONVENTIONAL AMMONIUM REMOVAL

Nitrification: $2\ NH_3 + (3+1)\ O_2 \rightarrow 2\ NO_3^- + 2\ H^+ + 2\ H_2O$

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$\Rightarrow 2\ NH_3^- + 4\ O_2 + 8g\ COD \rightarrow N_2 + 3g\ SLUDGE$

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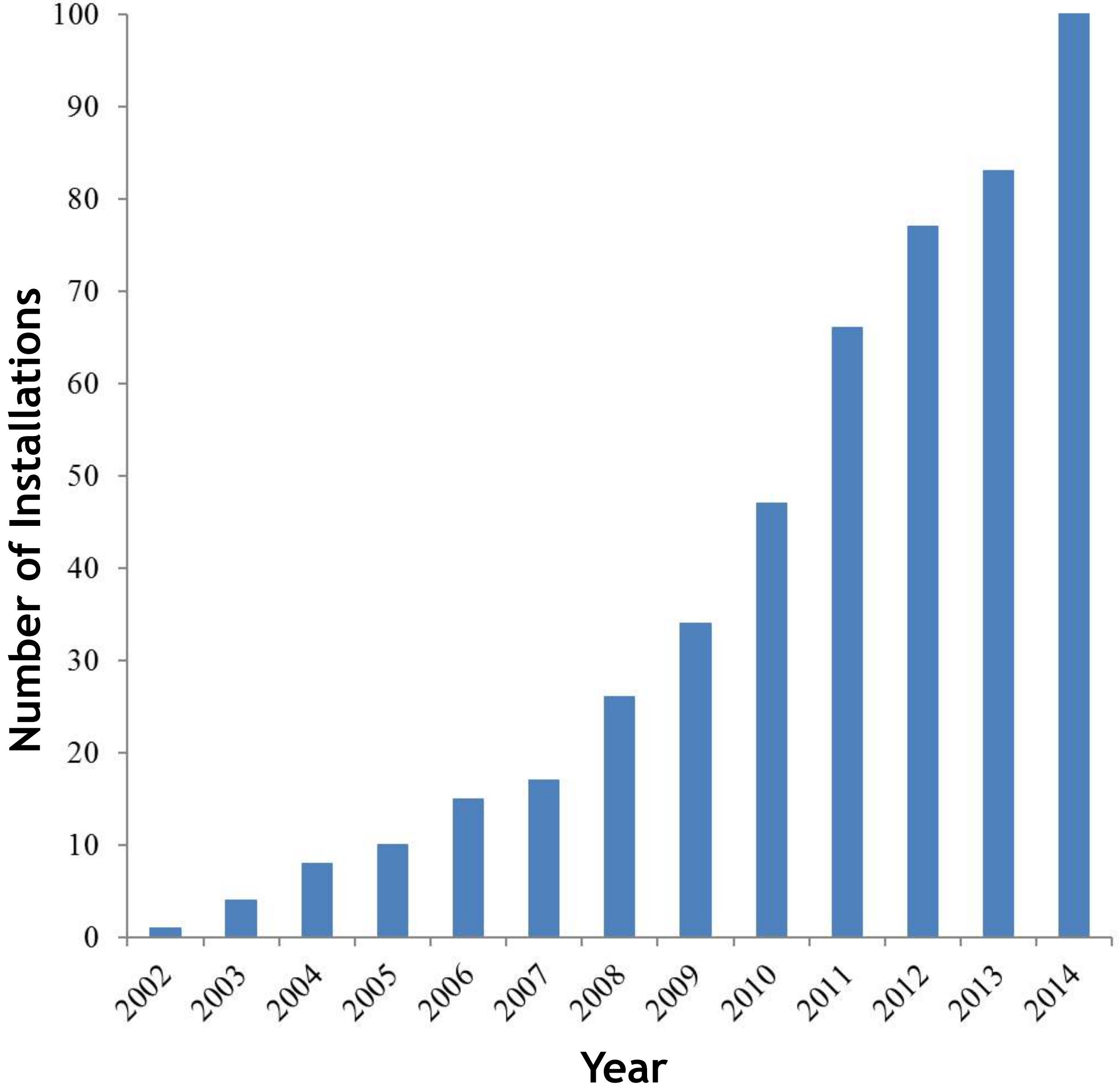
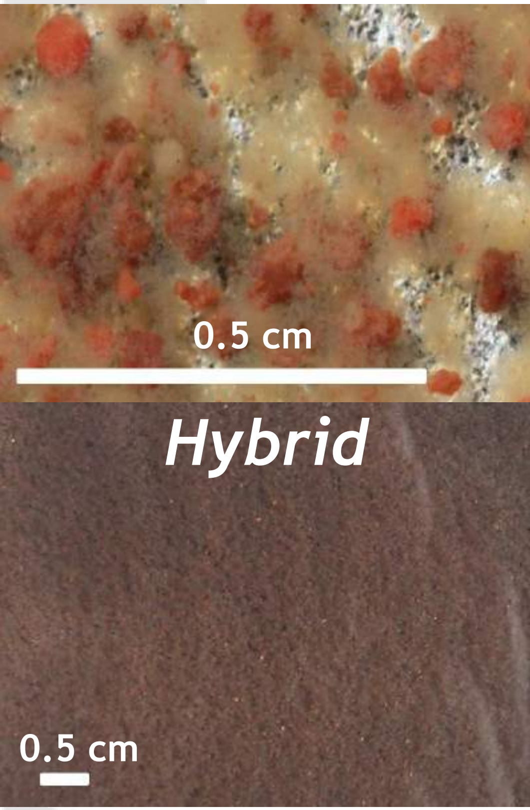
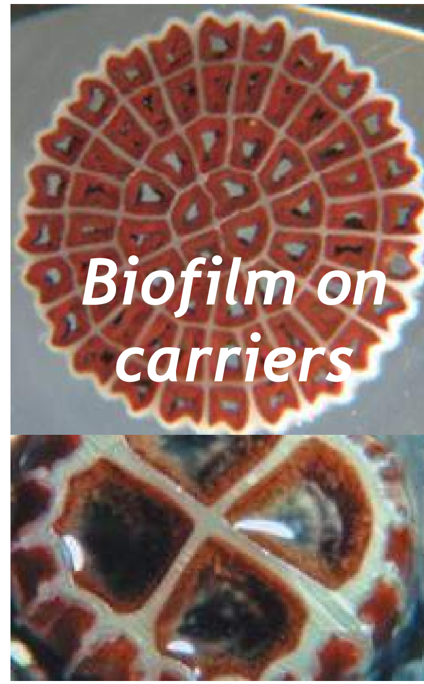
$\Rightarrow 2\ NH_3^- + 1.5\ O_2 \rightarrow N_2$


Number of Installations
100
90
80
70
60
50
40
30
20
10
0
2002
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
2013
2014
Year

Current Anammox Based Technologies
Aggregation status





Anammox EPS: why to study them ?

PN/A is the key process for the development of **energy neutral/producing WWTPs** and spread of **AD** (new biomass sources)

Anammox has slowest growth rate: **minimum SRT** imposed (winter)

Nearly **complete anammox retention**
- Biofilm systems (Granular, MBBR, IFAS..)

Biofilm stability along temperature and HRT daily/yearly fluctuations

High fraction of entire N-load removed via anammox metabolism

Anammox biomass excess sludge

Potential for significant **EPS recovery** before A.D.

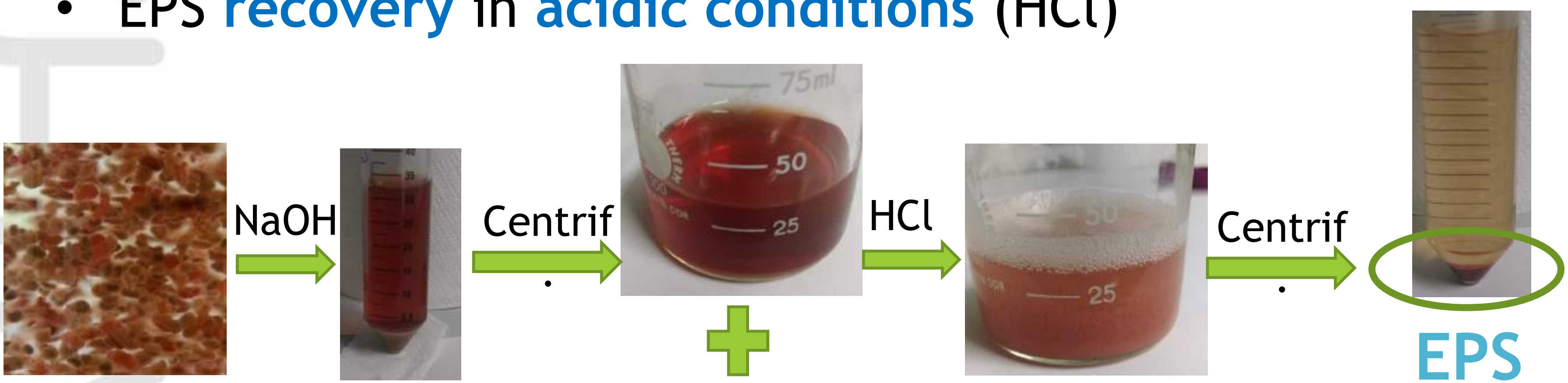
Need for deeper knowledge on the **structural component** of anammox biofilm for both **bioprocess development** and **reuse potentials** exploration

EPS extraction: why alkaline method ?

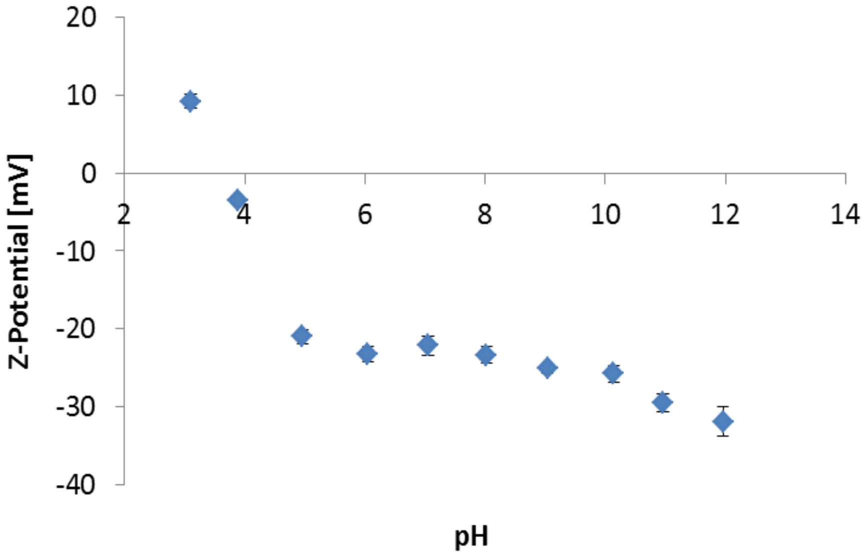
Structural EPS: fraction of EPS forming the tertiary network structure of biofilm

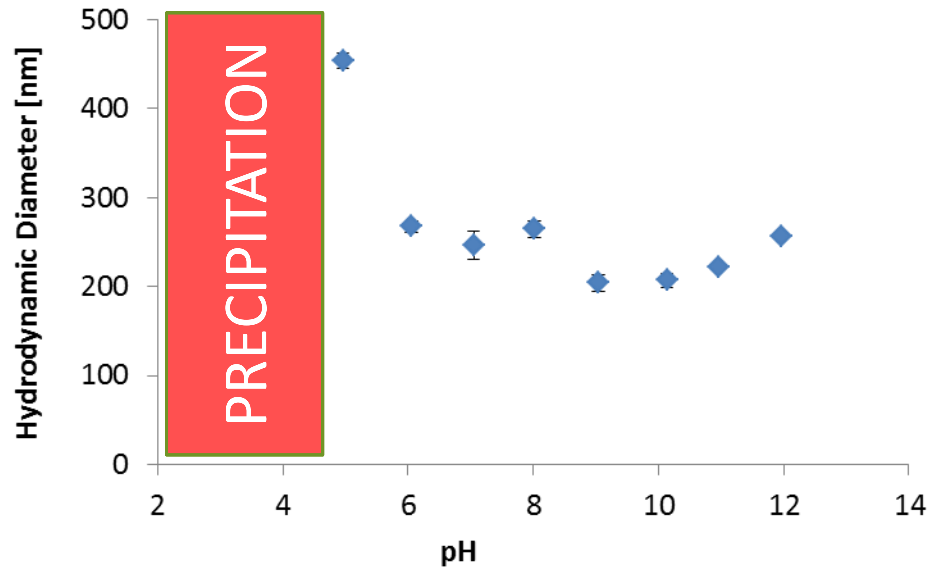
Biofilm matrix should be **solubilized** after structural EPS extraction (Felz et al.,2016)

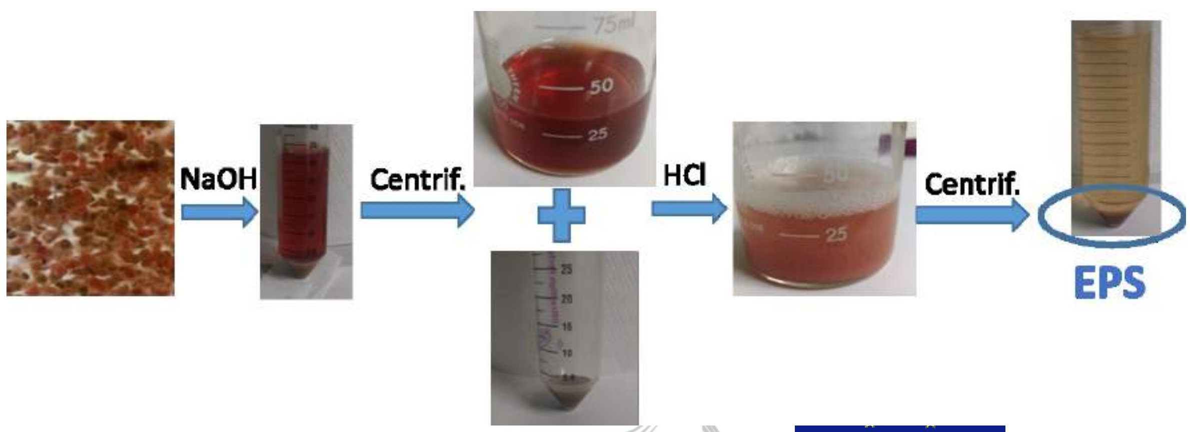
Selected method:
- EPS **extraction** in **alkaline conditions** (NaOH)
- EPS **recovery** in **acidic conditions** (HCl)



Z-pals and DLS: pH effect







Extraction yield

- **Anammox granules** originating from full-scale plant, Rotterdam (2° stage → only AnAOB)
- Variable ash content: 0.33 – 0.76 gVSS/gTSS
- Extraction **Yield**: 0.213 ± 0.046 gEPS/gVS (n.13)
- Total **Proteins**: 0.632 ± 0.013 gPN/gEPS
- Total **Carbohydrates**: 0.275 ± 0.102 gPS/gEPS
  - **PN+PS** > 90% of VSS
  - **PN/PS** = 2.35 ± 1.06

Growth yield = 0.112 gVS/gNH_4-N (Lotti et al., 2014)

→ **Recovery potential**: 25 gEPS per kgNH_4-N removed

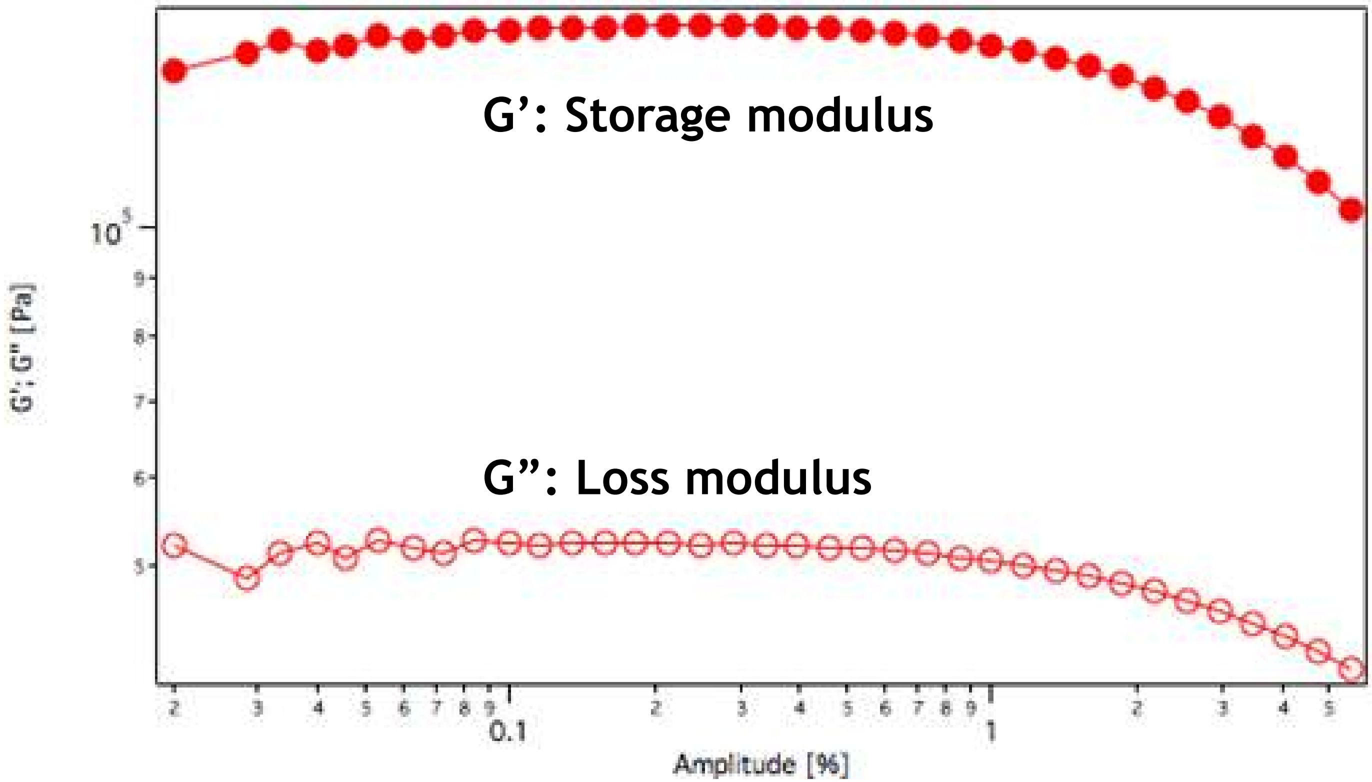
- **Freeze-dried EPS** re-hydrated with Milly-Q water do form hydrogel above a certain **threshold concentration**

→ **Rheological characterization** as function of EPS concentration, wt%

Mineral fraction (precipitates) affects the rheological characteristics of native granules (Lin et al., 2013)

→ **Useless comparing EPS-hydrogel with native biofilm**

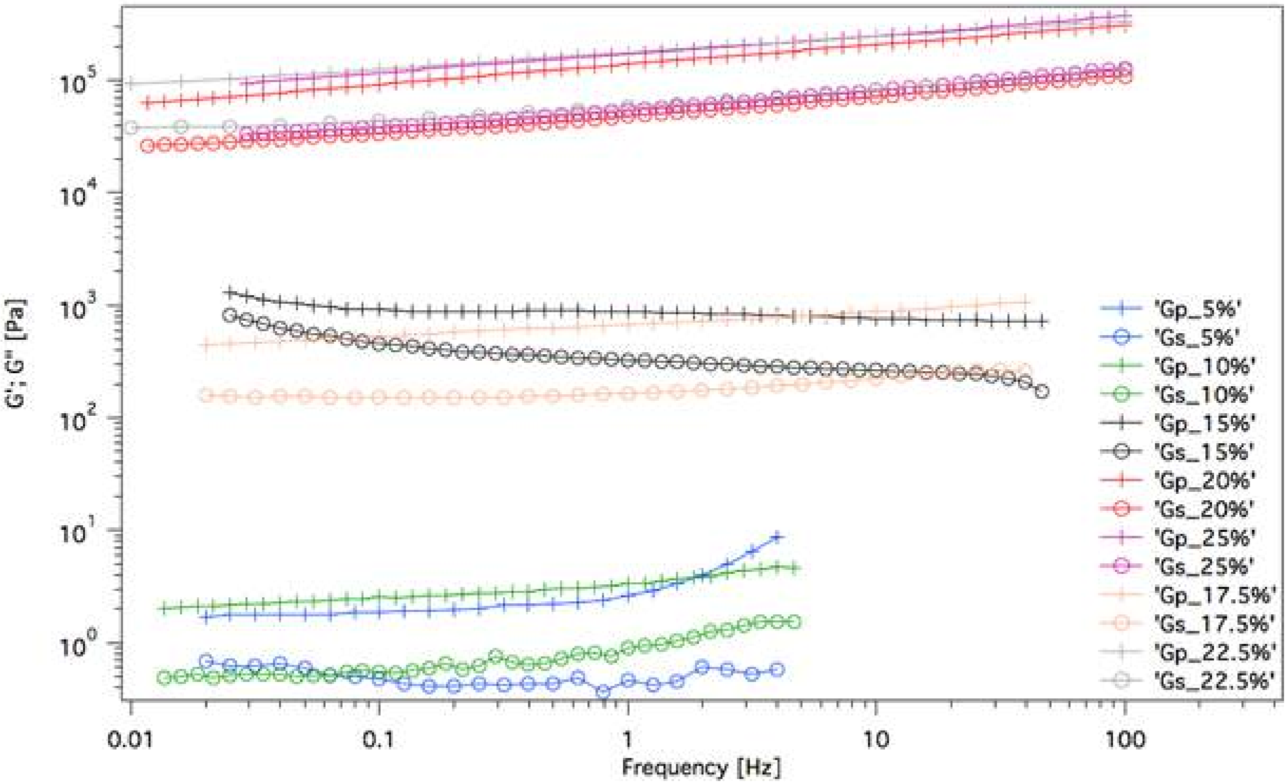
Rheological characterization



- Amplitude sweep to determine the **linear viscoelastic region of deformations (LVR)**

  [oscillation frequency, ω: 1 Hz]

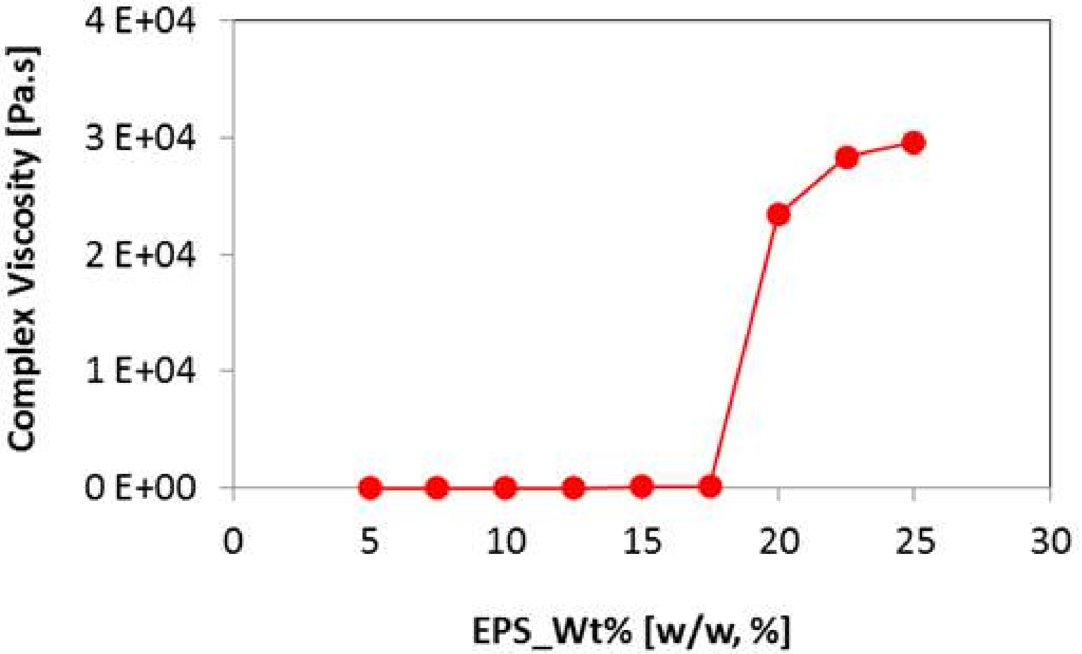
Rheological characterization



- Frequency sweep to measure **G' and G'' moduli** in an oscillation regime in the LVR

  [amplitude strain, γ: 1%]

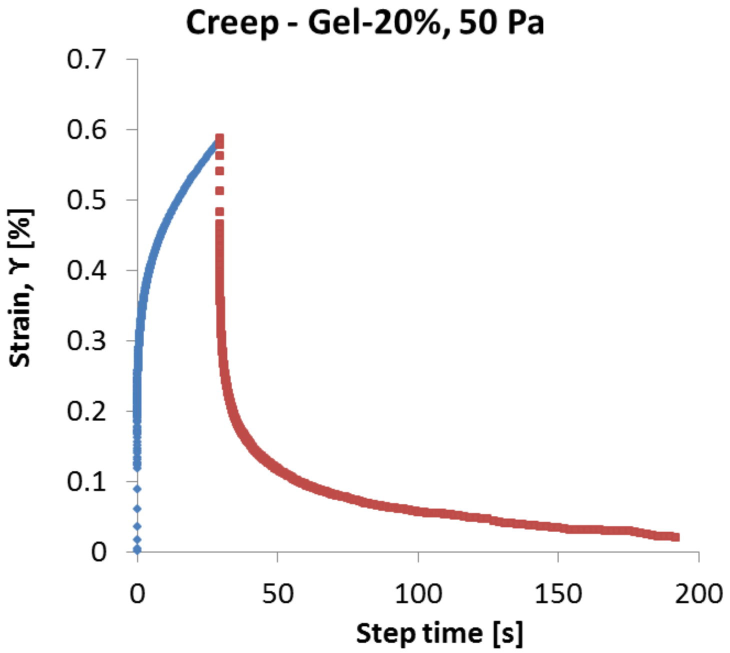
Rheological characterization

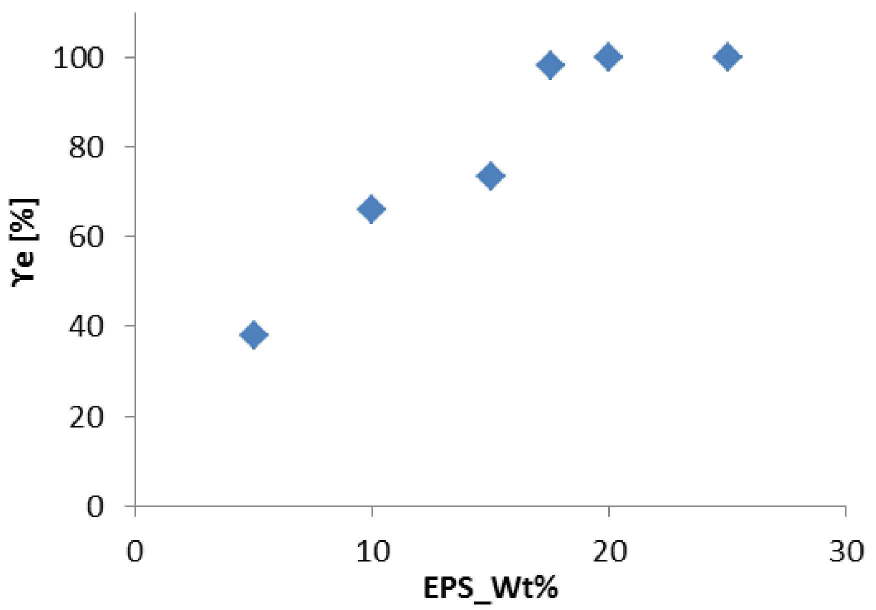


- Trend of the **complex viscosity, *η****, as a function of the EPS network concentration

➡ **Extended 3-D network** formation occurs above a certain **threshold (≈ 20%)**

Rheological characterization

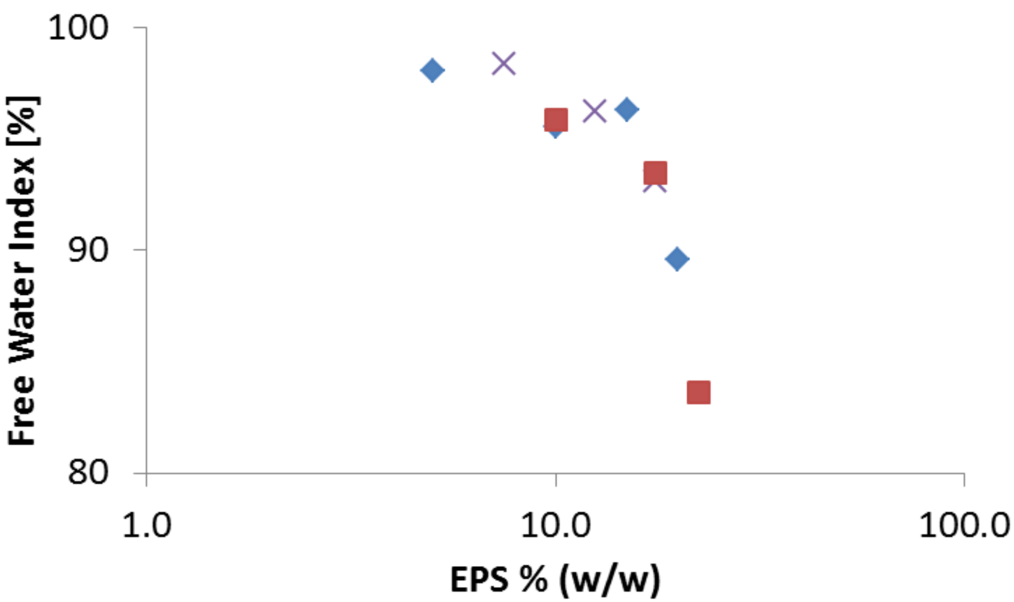




- Creep test: determination of the **Elastic portion of the re-formation, Ye**

Solid-like behaviour with complete elastic recover of the strain above a **threshold (≈ 17.5%)**

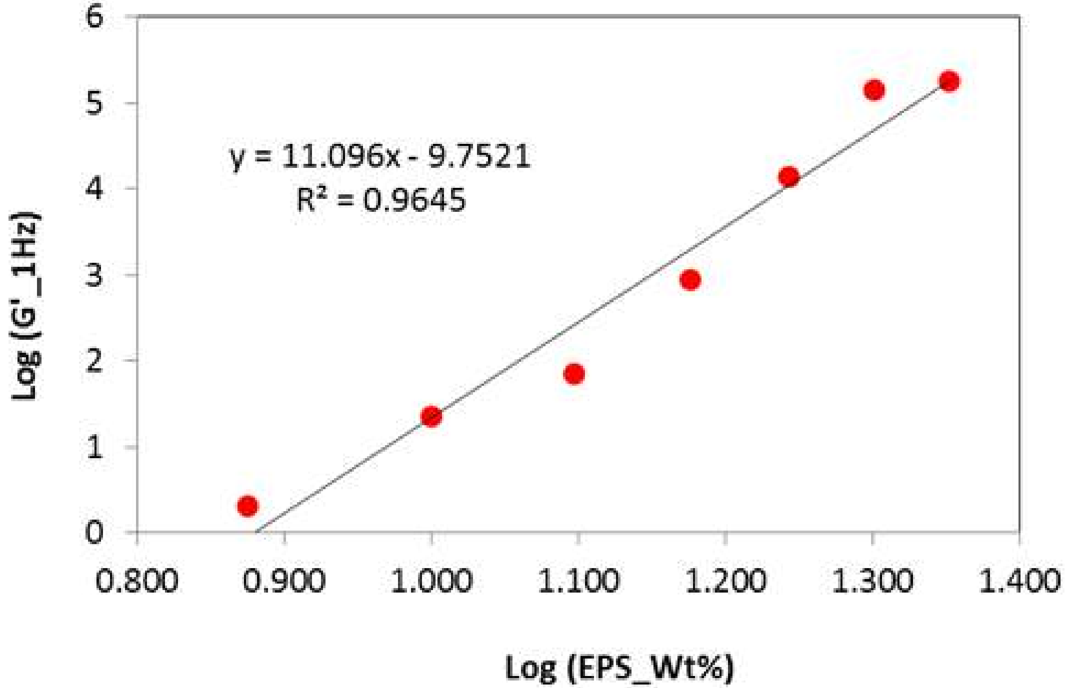
DSC for free/bound water evaluation



- Differential Scannig Calorimetry, DSC, to determine the **Free Water Index** (complement to 100% of ***bound water***)

➡ Higher bound water for higher EPS% with pronounced increase above a **threshold (≈ 20%)**

Seeking for the structural component



Power law fit [Jones and Marquès, 1990, theory for enthalpic elasticity]: **longitudinal fractal dimension** of the interacting objects, D_F, is 2.5

Askew fibrils composing the 3-D network

- Effect of EPS wt% network on the value of the elastic modulus G' at ω=1 Hz. The line represents the best linear fitting [De Gennes, 1976; Guenet, 2000]

Conclusions

- **Hydrogel** can be formed by recovered structural (fraction of) EPS
→ **Good potential for recovery and reuse**

- **Threshold EPS wt% (≈ 20%)** for extended 3-D network formation
[Granule density ≈1.05 gVS/mL (Lu et al.,2012); extraction Yield of 0.21 gEPS/gVS → **could make sense!**]

- Longitudinal fractal dimension indicates **askew fibrils** as the **structural component**

Future work:

- Chemical and physical-chemical characterization of the structural component → **proteomics / AFM-CLSM / DC / FTIRs**
- Correlation with **granulation/biofilm stability** in operating systems
- **Exploring reuse potential in industry**

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