Simultaneous partial Nitritation, Anammox and Denitrification (SNAD) process for treating ammonium-rich wastewaters

Department of Civil, Environmental and Mechanical Engineering
University of Trento - 16 April 2013

PhD Candidate: **Eng. Michela Langone**
Supervisor: **Prof. dr. eng. Gianni Andreottola**

UNIVERSITY OF TRENTO - Italy
Education and Culture DG
Lifelong Learning Programme

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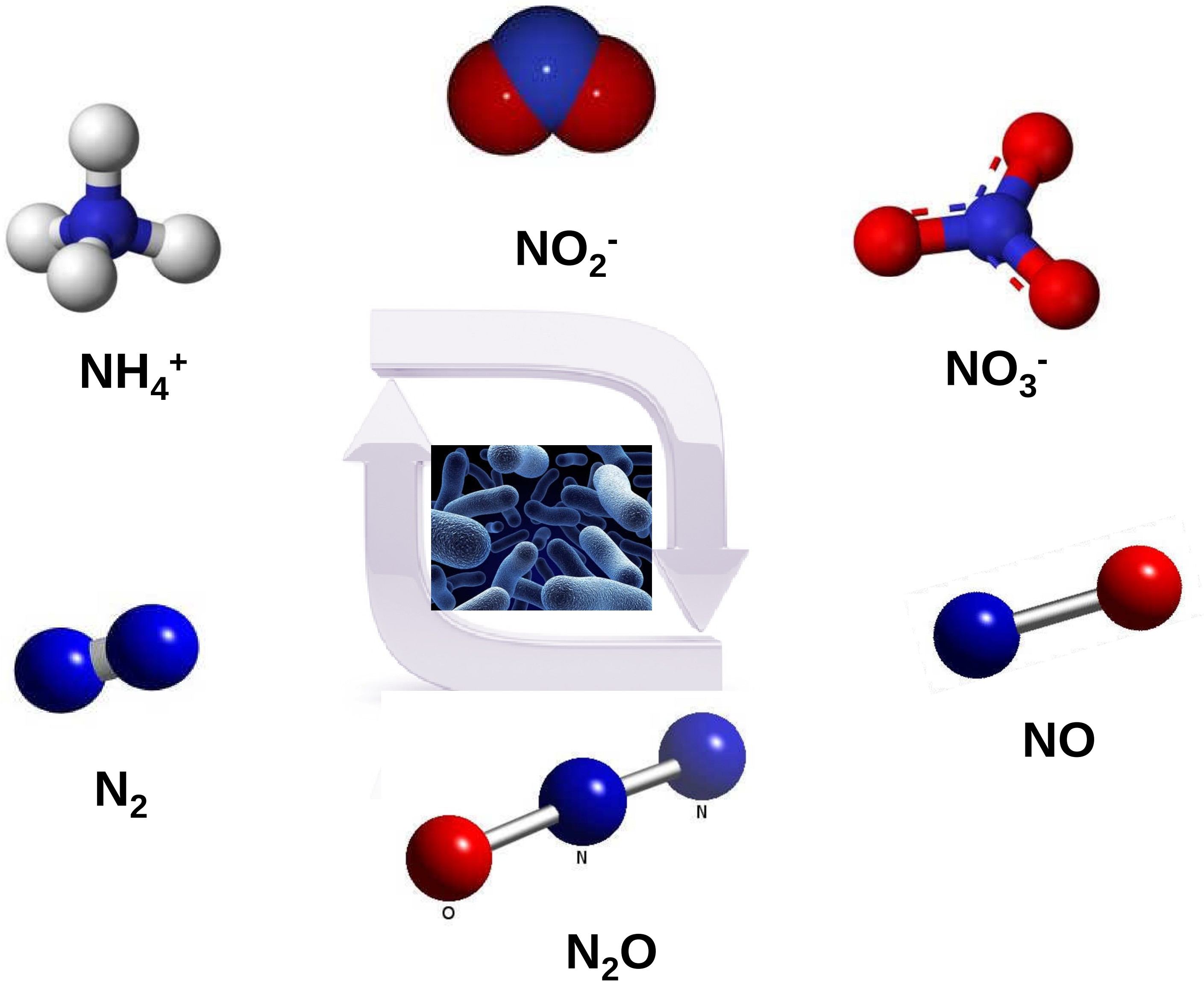
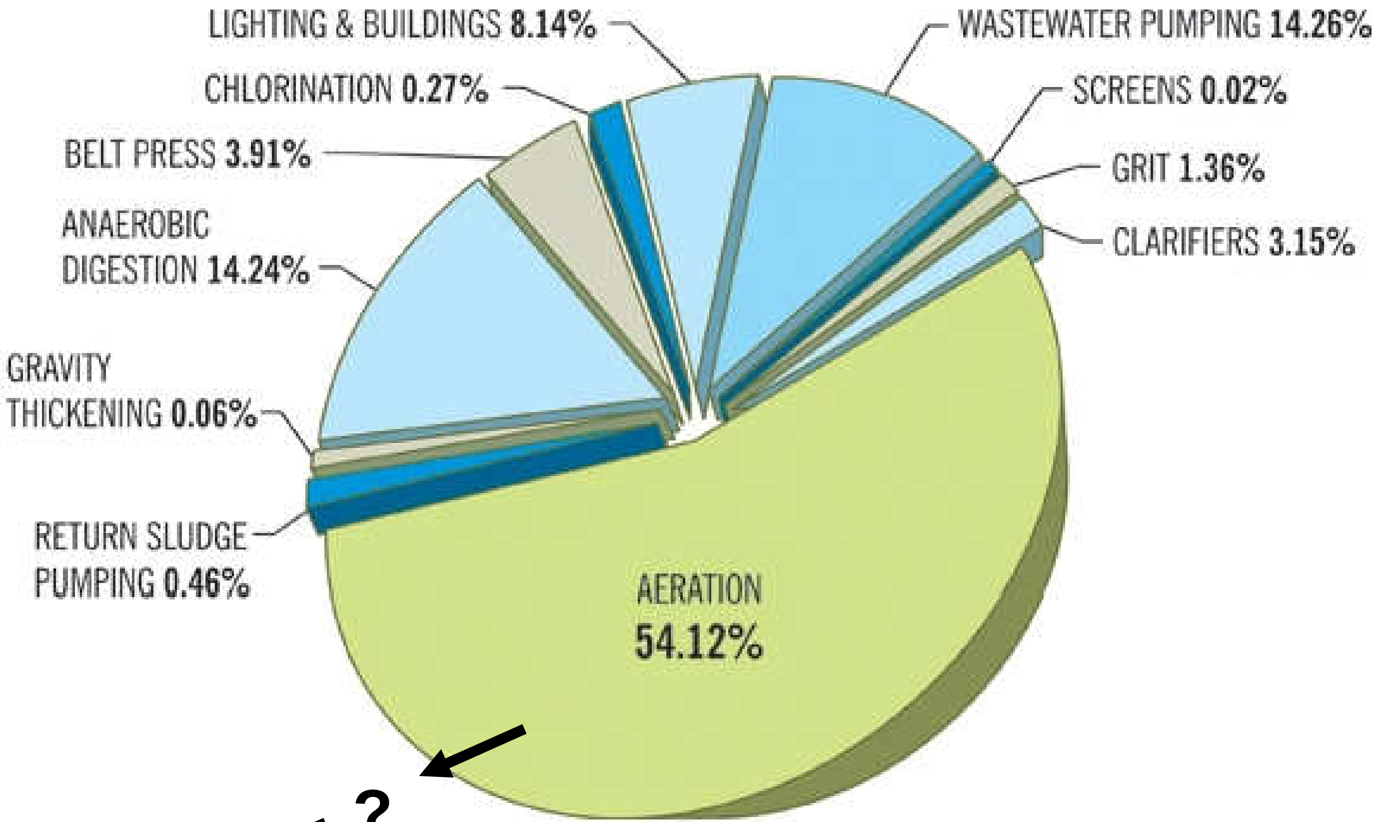

NO_2^-
NH_4^+
NO_3^-
N_2
NO
N
N
O
N_2O



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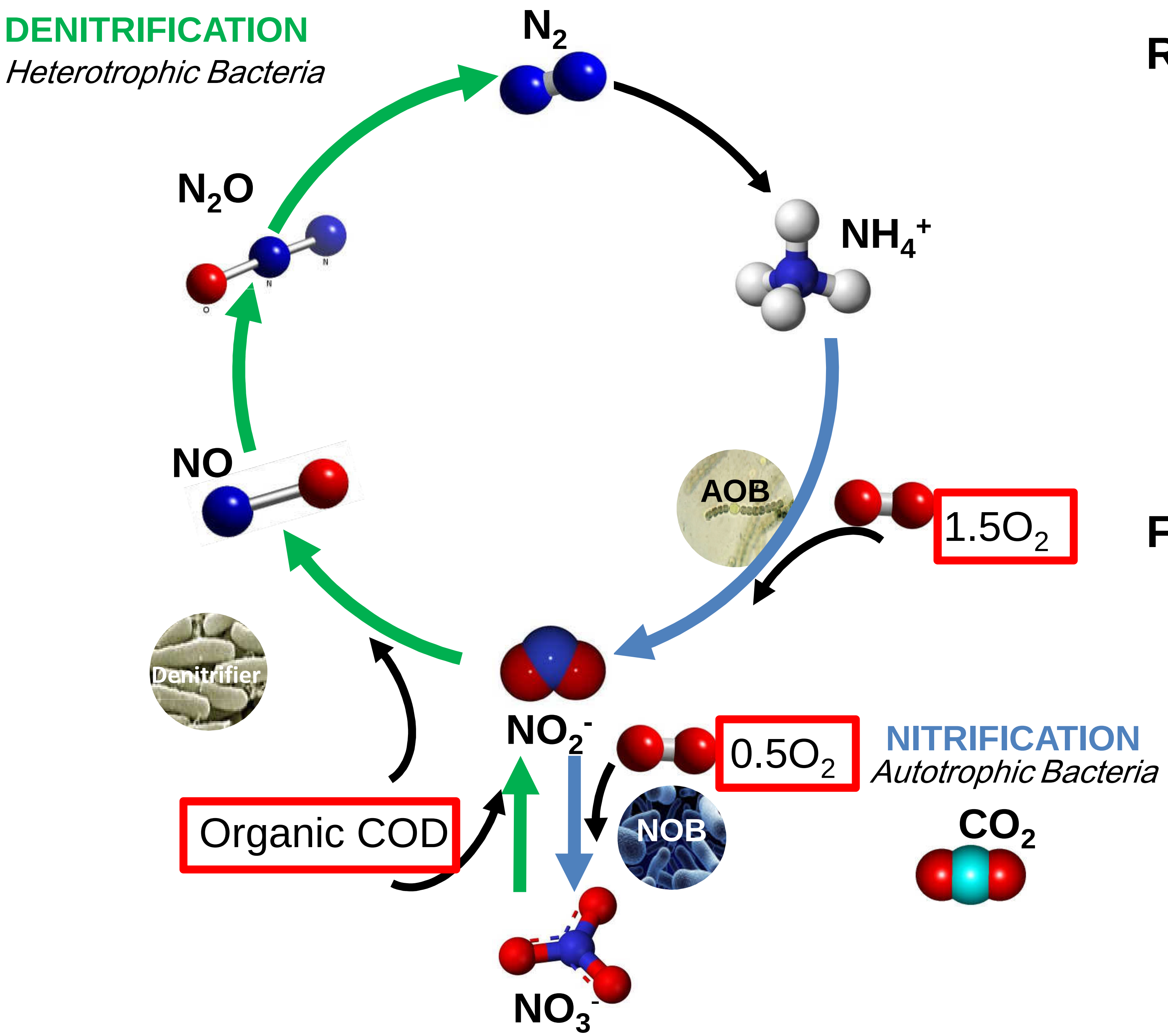
source://www.hazenandsawyer.com

Conventional Biological Processes
Nitrogen Removal

Conventional biological process



Requirements:

- 4.2 $mgO_2/mgN\text{-}NH_4$
- 2.8 $mgCOD/mgN\text{-}NO_3$

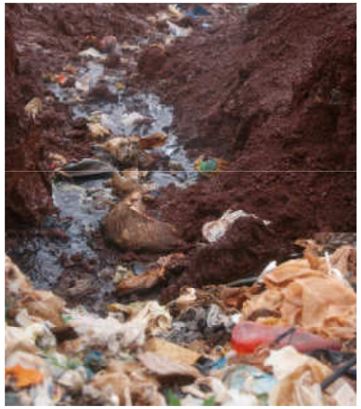
Fields of application:

Urban wastewater :

- Low NH_4^+
- High COD/N → > 4 - 5

Ammonium-rich wastewaters

Old Leachate
urban landfill (Trento)



$N\text{-}NH_4^+ = 1181\ mg\ L^{-1}$

$COD_{biodegr} = 250\ mg\ L^{-1}$

$\mathbf{COD_{biodegr}/N = 0.2}$

Anaerobic digester effluent
urban WWTP (Trento)

$N\text{-}NH_4^+ = 433\ mg\ L^{-1}$

$COD_{biodegr} = 158\ mg\ L^{-1}$

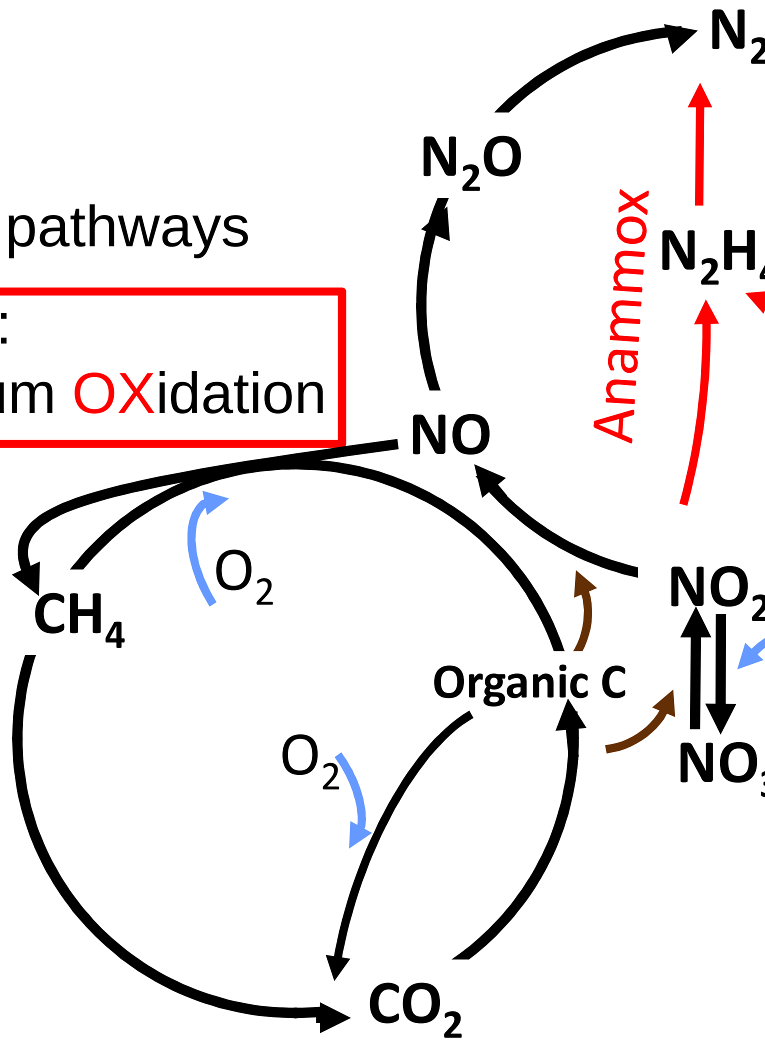
$\mathbf{COD_{biodegr}/N = 0.5}$

✓ Physical – chemical processes

- Ammonia stripping
- Struvite precipitation

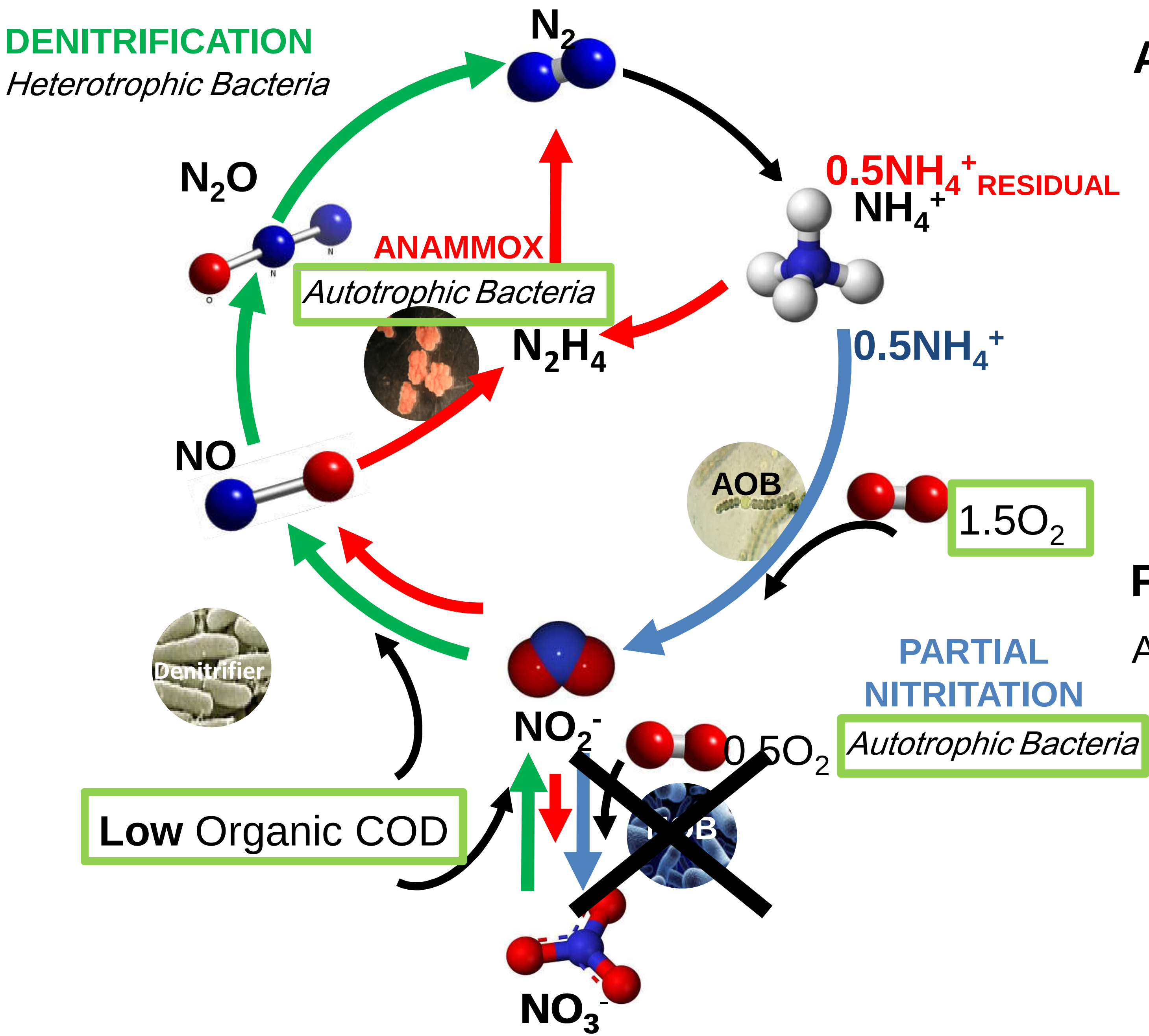
✓ <u>Innovative</u> Biological pathways

- anammox process: ANaerobic AMMonium OXidation
- n-damo process
- DNRA
- Archaea



An innovative biological process:

Simultaneous partial Nitritation, Anammox and Denitrification - SNAD



Advantages:

< 60% oxygen consumption

no external carbon source

Lower sludge production

Fields of application:

Ammonium rich- wastewaters:

- High NH_4^+
- Low COD/N → < 2

Research questions

1. How does the SNAD process work?

2. How can I optimize the performance of the SNAD process?

3. Which are the microorganisms involved in it?

4. Can the SNAD process treat old landfill leachates? And anaerobic digester effluents at moderate temperatures?

5. How to control the SNAD process?

1. How does the SNAD process work?

Main operative conditions

Reactor Configuration

- *Sequential Batch Reactor (SBR) and Granular biomass*
- *Lab scale SBR*

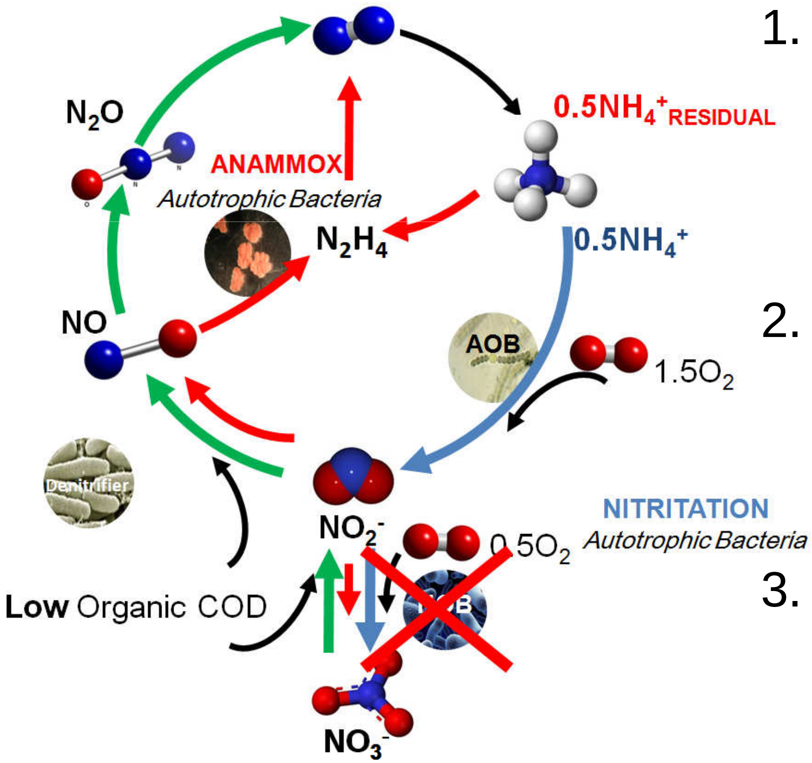
Community composition and spatial distribution

- *Phylogenetic and Morphologic Analysis*

SNAD process treating ammonium-rich wastewaters :

- *Leachate treatment at 30° C*
- *Digester effluent treatment at 25° C*
- *Correlating on-line parameters & “Real time” control strategy*

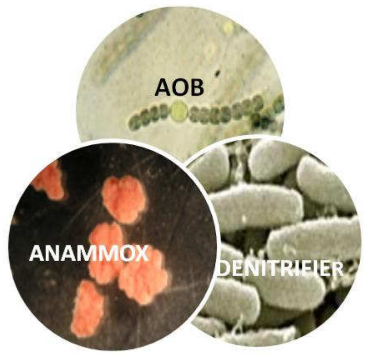
Main operative conditions



1. AOB activity & outcompete NOB → ↓ **Free ammonia**: 5mg/L<NH_3-N<10mg/L; ↑ **Temperature**: T = 30°C; ↑ **Oxigen:** DO < 0.5 mg/L

2. Prevent anammox bacteria inhibition → ↓ **Oxigen**: DO < 0.5 mg/L; ↓ **Nitrite**: NO_2^--N < 30 mg/L; ↓ **Some organic matter** (methanol, etc)

3. Co-exixtence anammox & denitrifying bacteria → ↓ **$COD_{biodegr}$/N**: C/N < 1 - 2



2. How can I optimize the performance of the SNAD process?

Main operative conditions

Reactor Configuration

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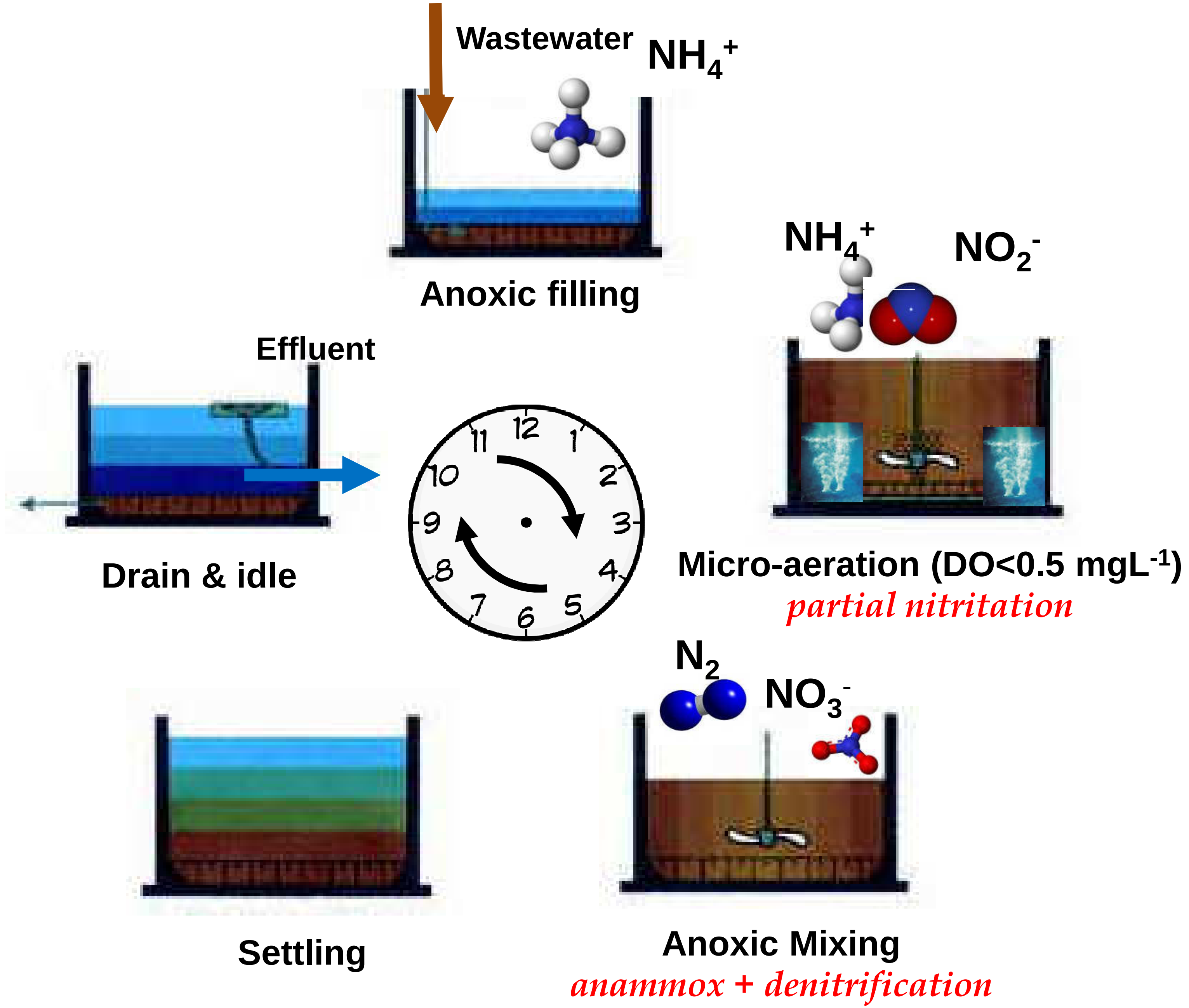
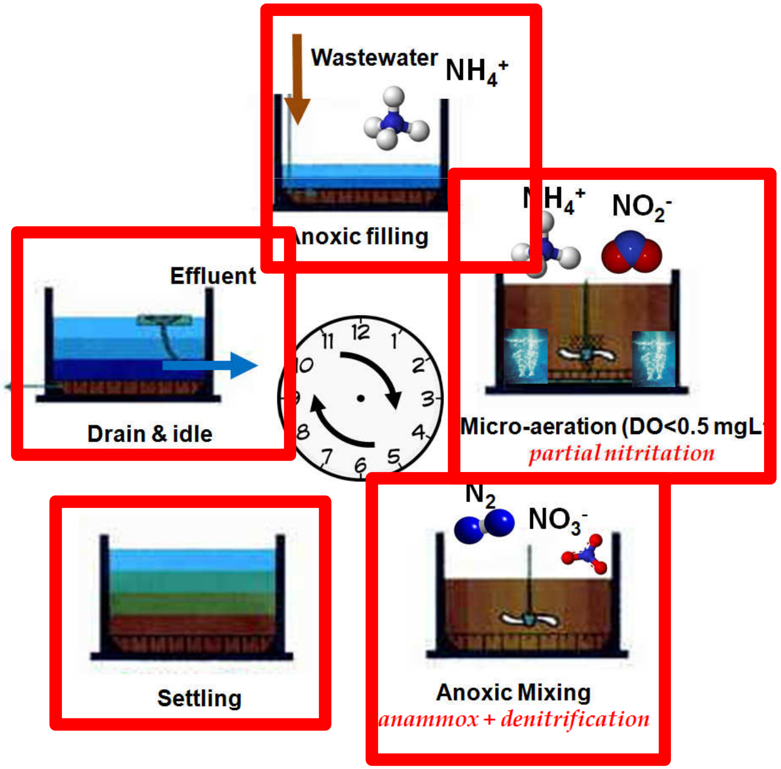
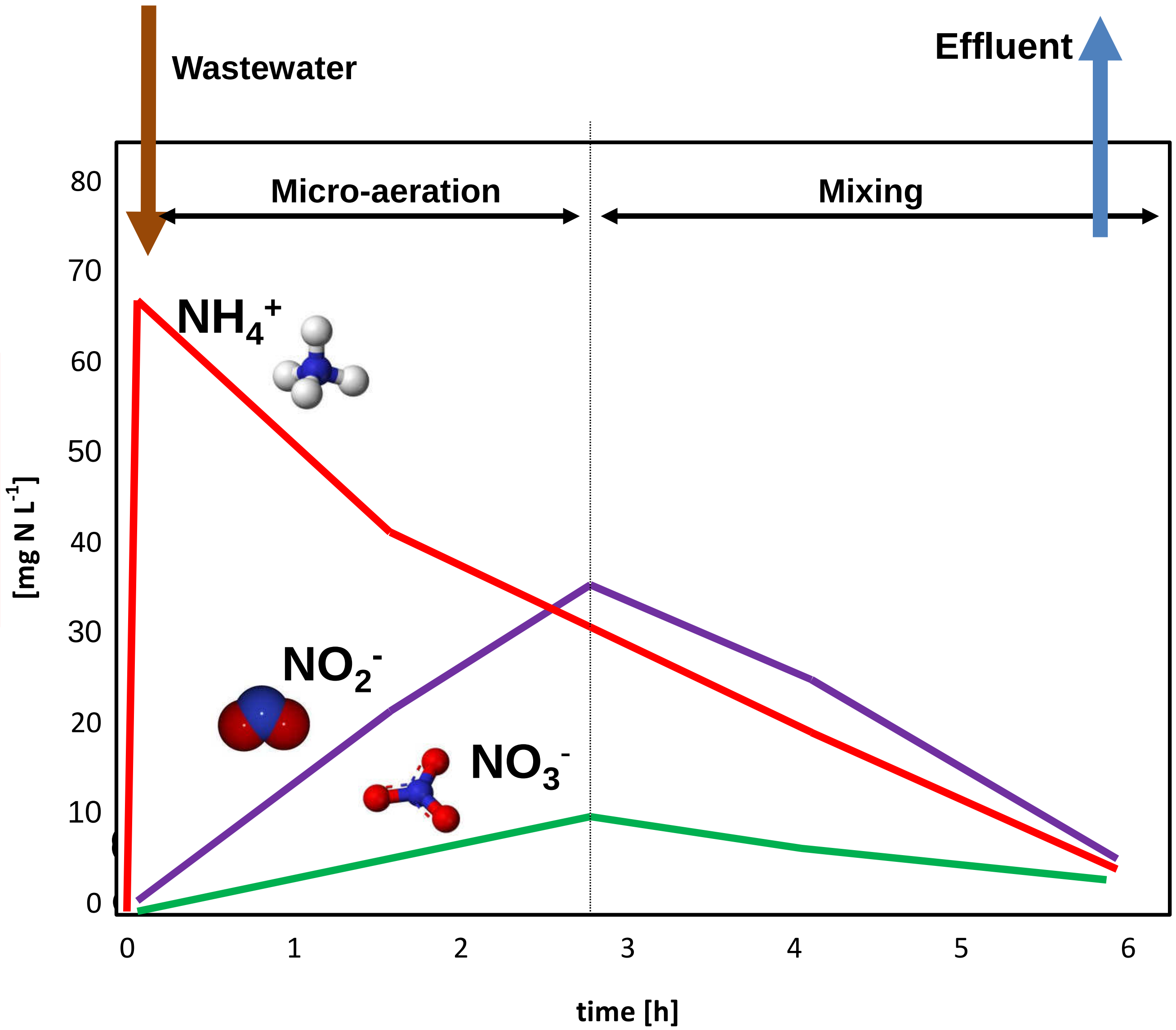
Sequencing Batch Reactor (SBR)



Sequencing Batch Reactor





DIFFERENT MACRO-ENVIRONMENT

Granular Biomass

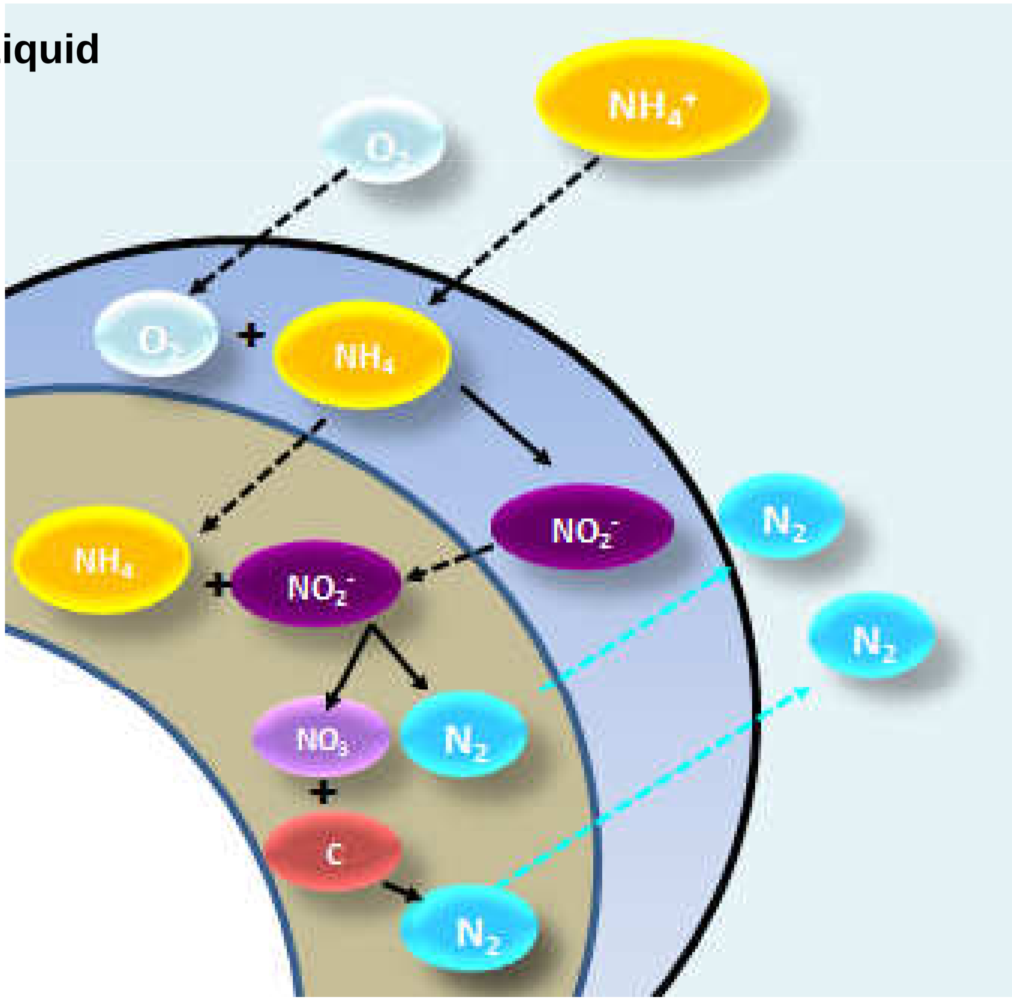
OUTER LAYER
Aerobic

partial nitritation

INNER LAYER
Anoxic

anammox + denitrification

Liquid



Granule

DIFFERENT MICRO-ENVIRONMENT

Lab scale SBR

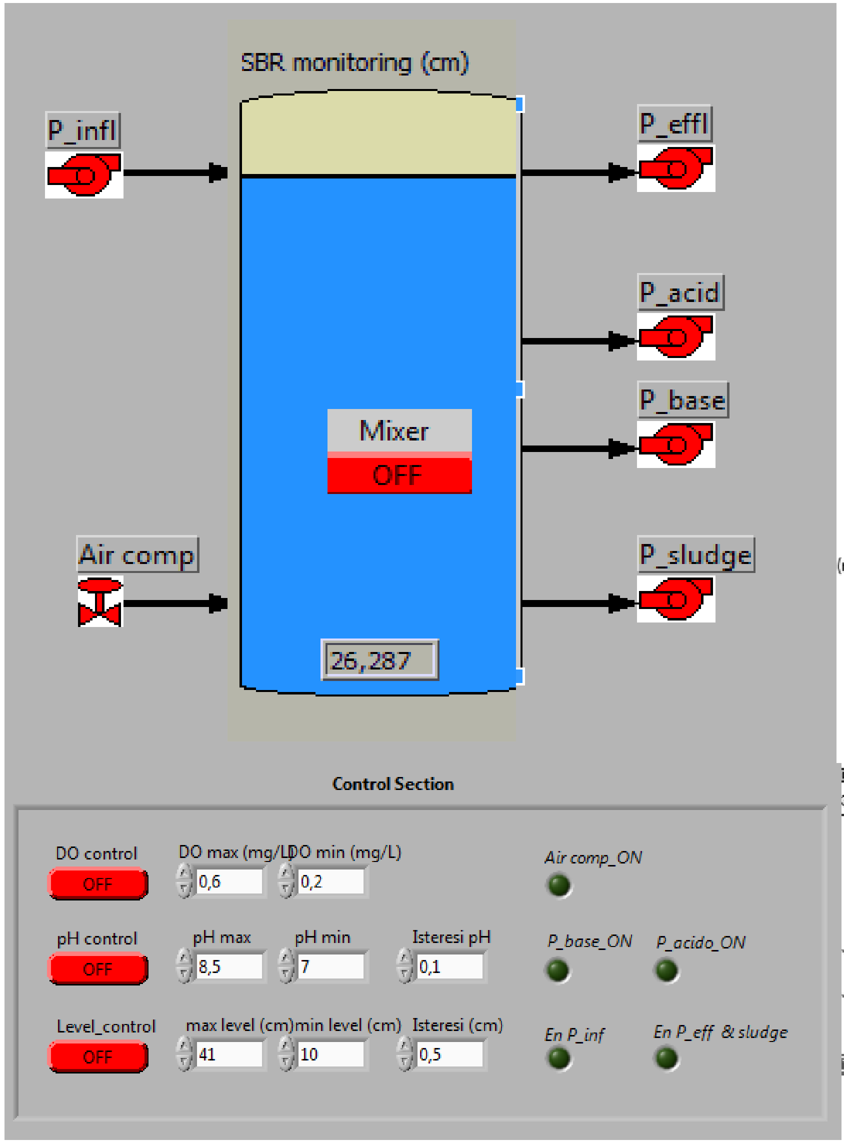
Lab scale SBR
n. 2 SBRs (4 – 8 L)

Inoculum
Zurich WWTP SBR (1400 m^3)

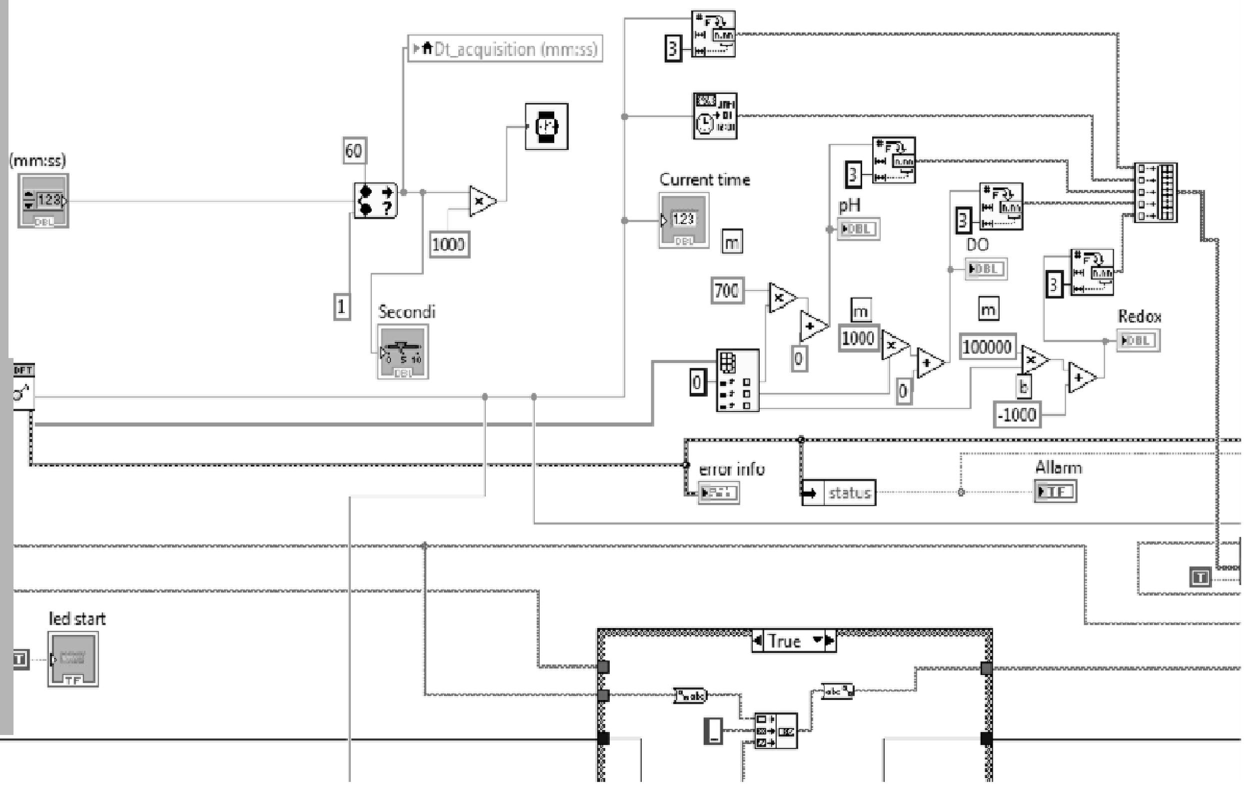
Anaerobic digester effluent at 30℃

(Joss et al 2009)

Design of the software for data acquisition and control of the SNAD-SBR process



1. **Manual Control**
2. **Fixed time Control**
3. **Real time Control**



3. Which are the microorganisms involved in it?

Main operative conditions

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- ***Phylogenetic and Morphologic Analysis***

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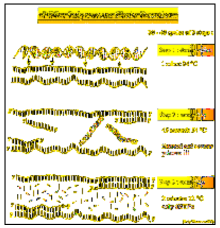
Phylogenetic Analysis on SNAD biomass

Genomic DNA isolation

(CTAB DNA Isolation protocol)

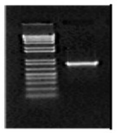
PCR: Polymerase Chain Reaction

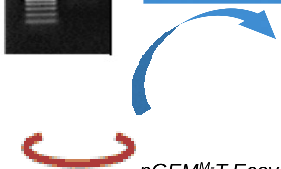
(Amplify 16S or specific enzyme gene by PCR with specific primers targeting the domain bacteria)



Agarose Gel

(Verify PCR products and if necessary purify products)

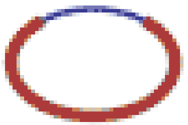




pGEM^{M-}T Easy cloning plasmid vector

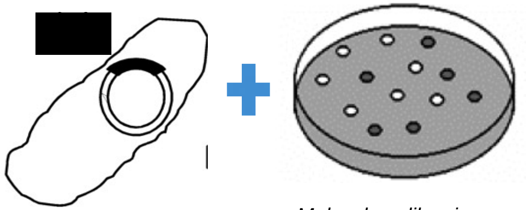
Ligation

(PCR products into the pGEM^{M-}T Easy cloning plasmid vector (promega,USA))



Trasformation

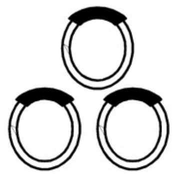
(Transform component Escherichia coli cells with plasmids)



Make clone libraries

Plasmid extraction

(Isolate Plasmid-DNA with Gene JET Plasmid Miniprep kit (Fermentas, Lithuania))

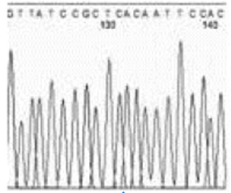


Agarose Gel

(Plasmids were digested with 5U EcoRI enzyme in EcoRI buffer for 1.5 h at 37℃. and examined for an insert by agarose (1%) gel electrophoresis)

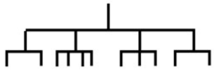
Sequencing

(Sequence DNA plasmid inserts)



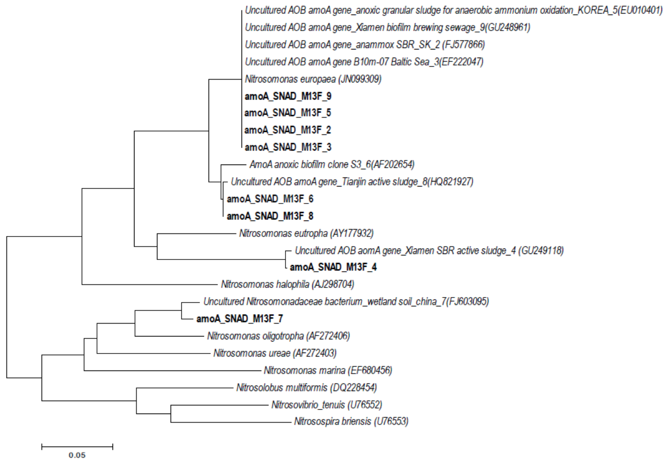
References

(Align library sequences with references from nucleotide BLAST databases to determine phylogenetic affiliations for each sequence)



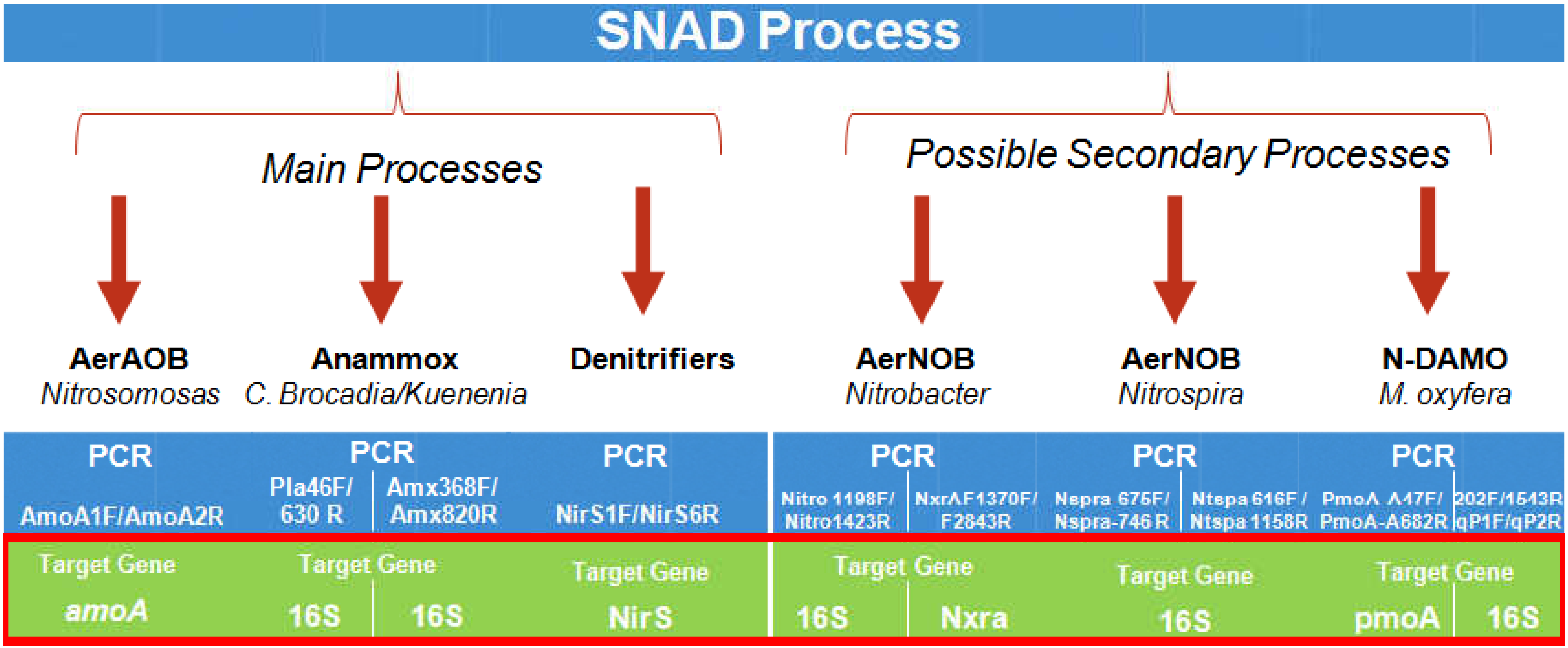
Phylogenetic trees

(Generate phylogenetic trees using neighbor-joining method)



Phylogenetic Analysis on SNAD biomass

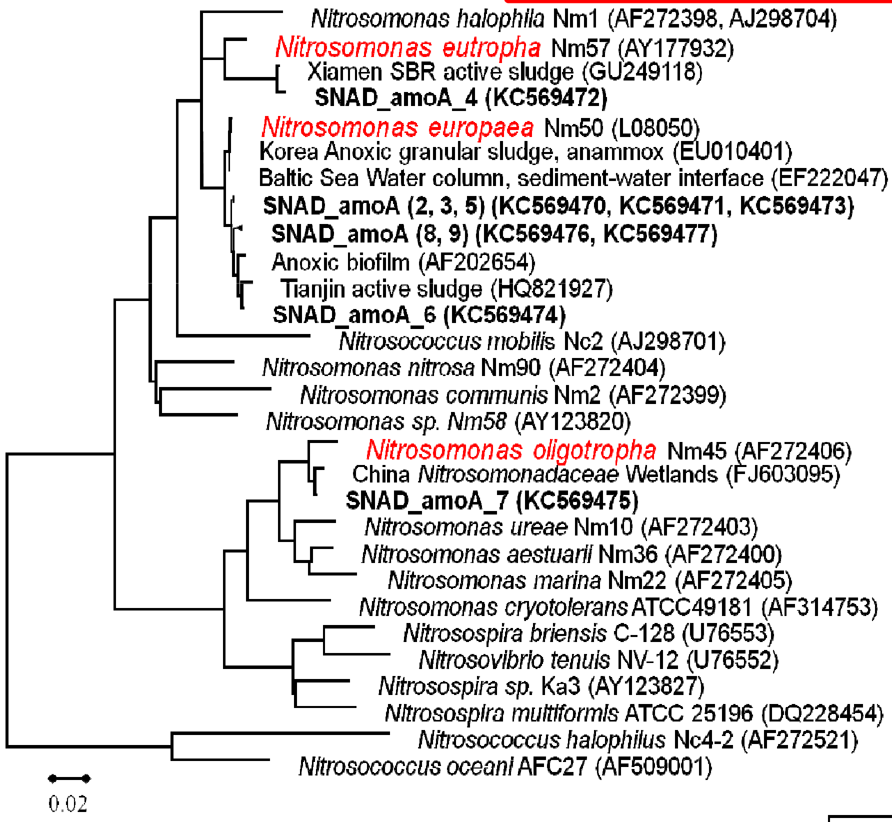
PCR: Polymerase Chain Reaction



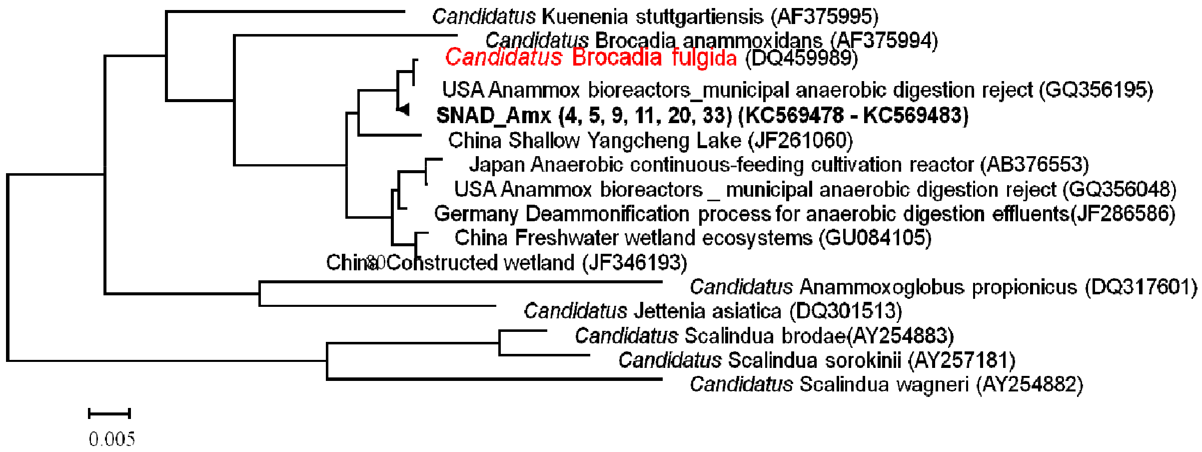
Phylogenetic Analysis on SNAD biomass

Main Processes

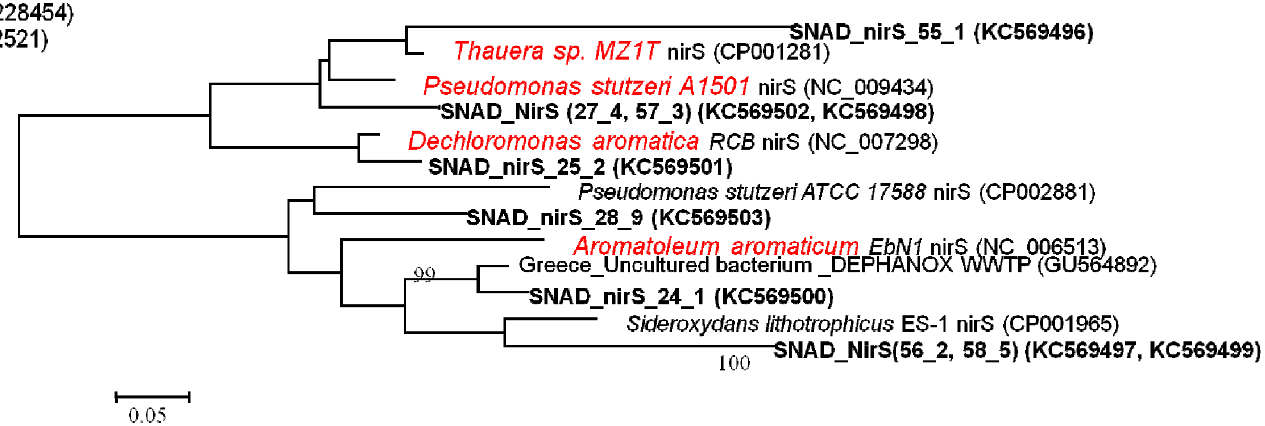
AmoA **gene of aerobic ammonium oxidizing bacteria**



16 S rRNA gene of anammox bacteria



NirS **gene of denitrifying bacteria**

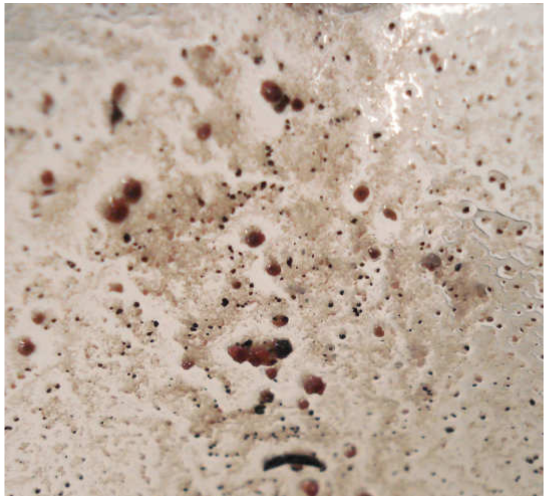


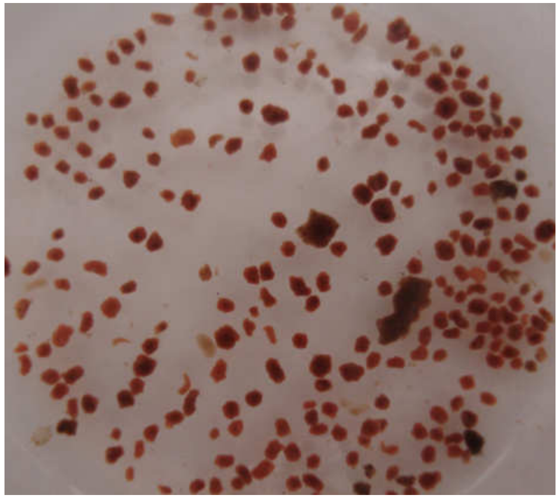
Secondary Processes:

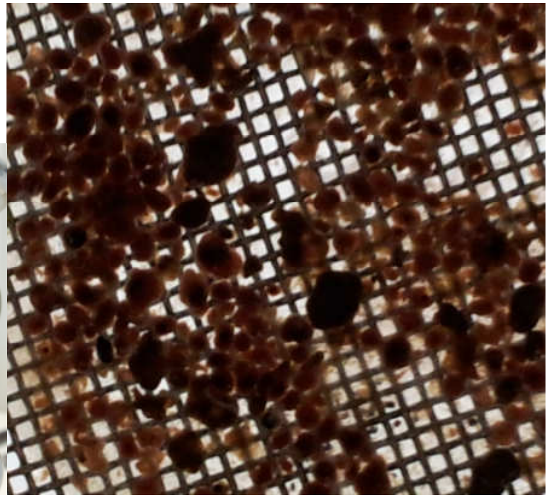
16 S rRNA gene of nitrobacter

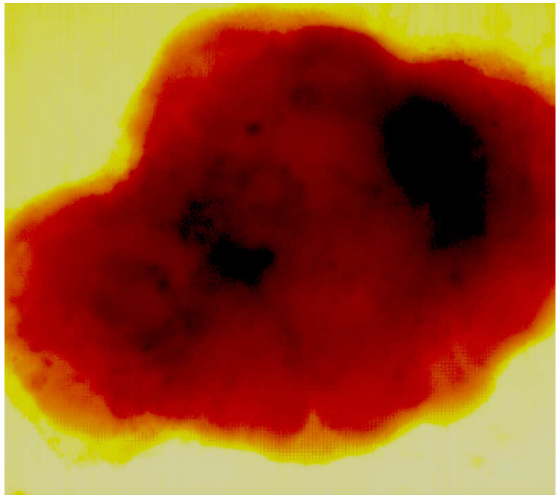
Systematic and Applied Microbiology: Under Review

Morphologic Analysis on SNAD biomass



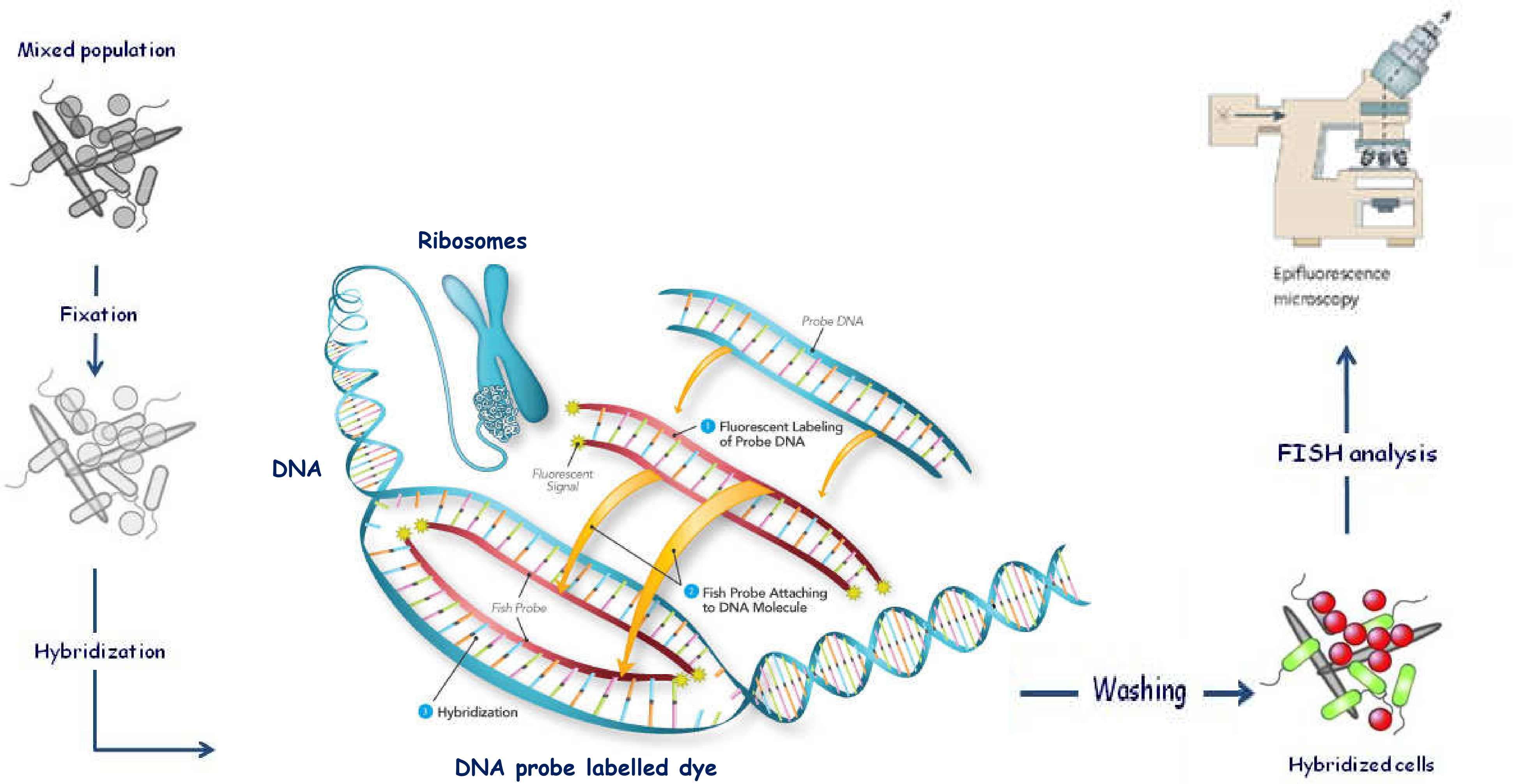






Morfologic Analysis on SNAD biomass – FISH

Fluorescence in situ hybridization



Morfologic Analysis on SNAD biomass – FISH

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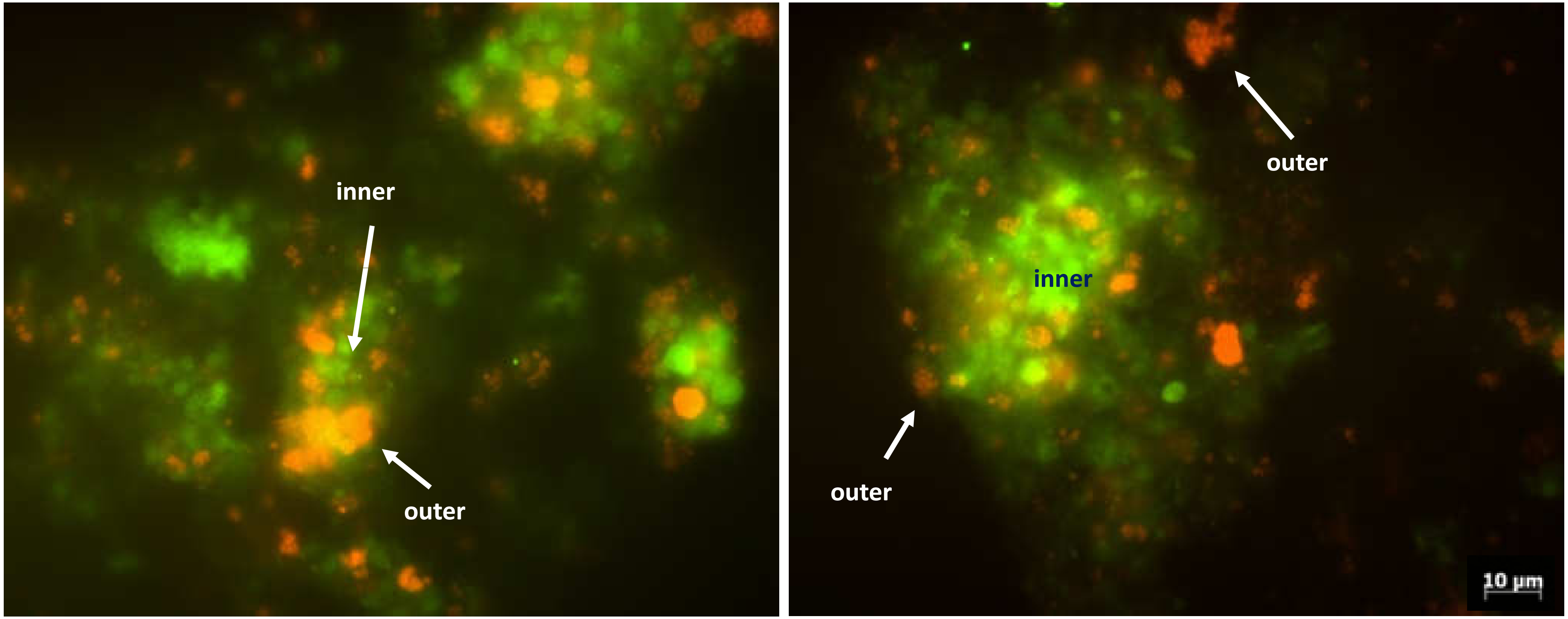


AOB* vs *anammox bacteria

Real Time PCR

New primer set targeting the *hzo/hao* gene of anammox bacteria:

anammox bacteria were present both in brown and red granules

Systematic and Applied Microbiology: Under Review

4.1 Can the SNAD process treat old landfill leachates?

Main operative conditions

Reactor Configuration

- *Sequential Batch Reactor (SBR) and Granular biomass*
- *Lab scale SBR*

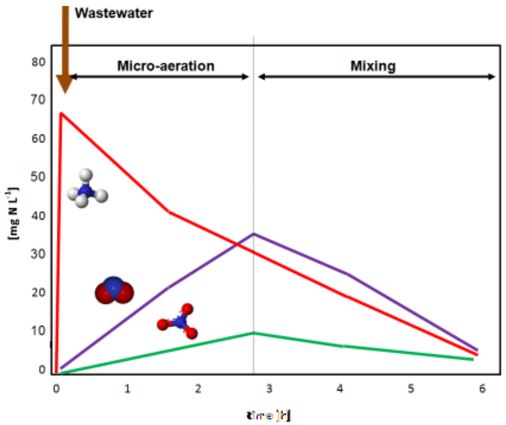
Community composition and spatial distribution

- *Phylogenetic and Morphologic Analysis*

SNAD process treating ammonium-rich wastewaters:

- ***Leachate treatment at 30° C***
- *Digester effluent treatment at 25° C*
- *Correlating on-line parameters & "Real time" control strategy*

Batch tests & old landfill leachate



WORKIN CONDITIONS:

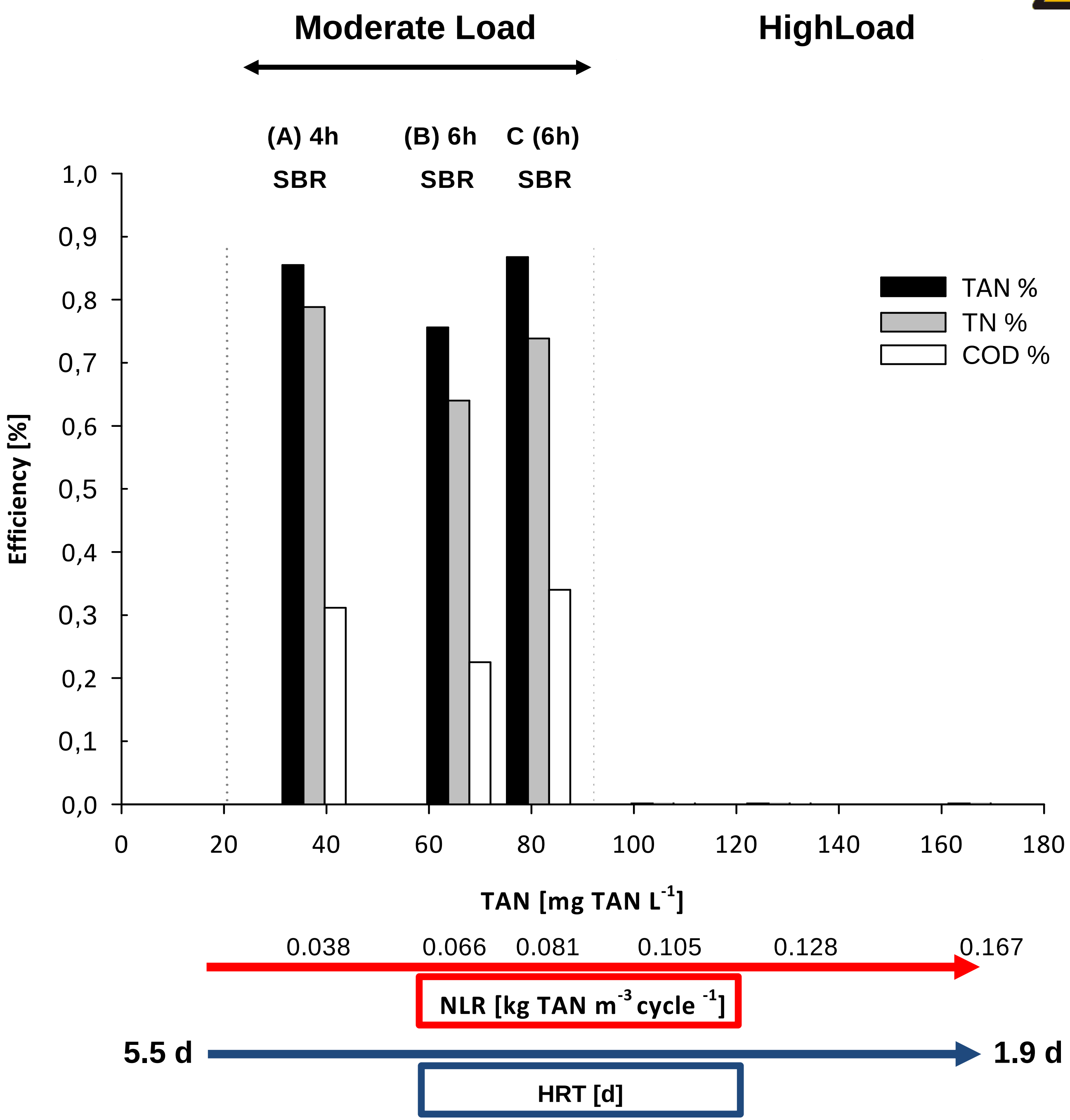
V_{SBR}= 4L

T = 30° C

TSS = 3 – 4 g L^{-1}

DO < 0.3 mg L^{-1}

pH = 7.1 – 8.5



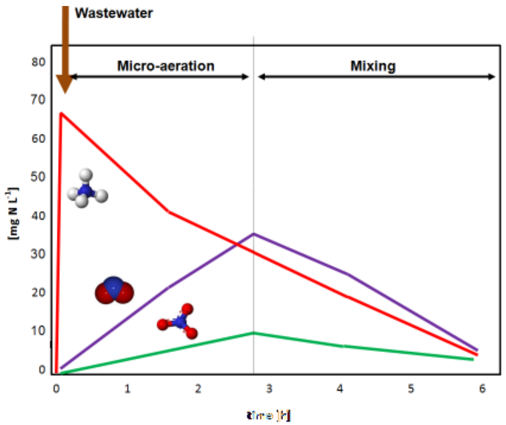
REMOVAL EFFICIENCY:

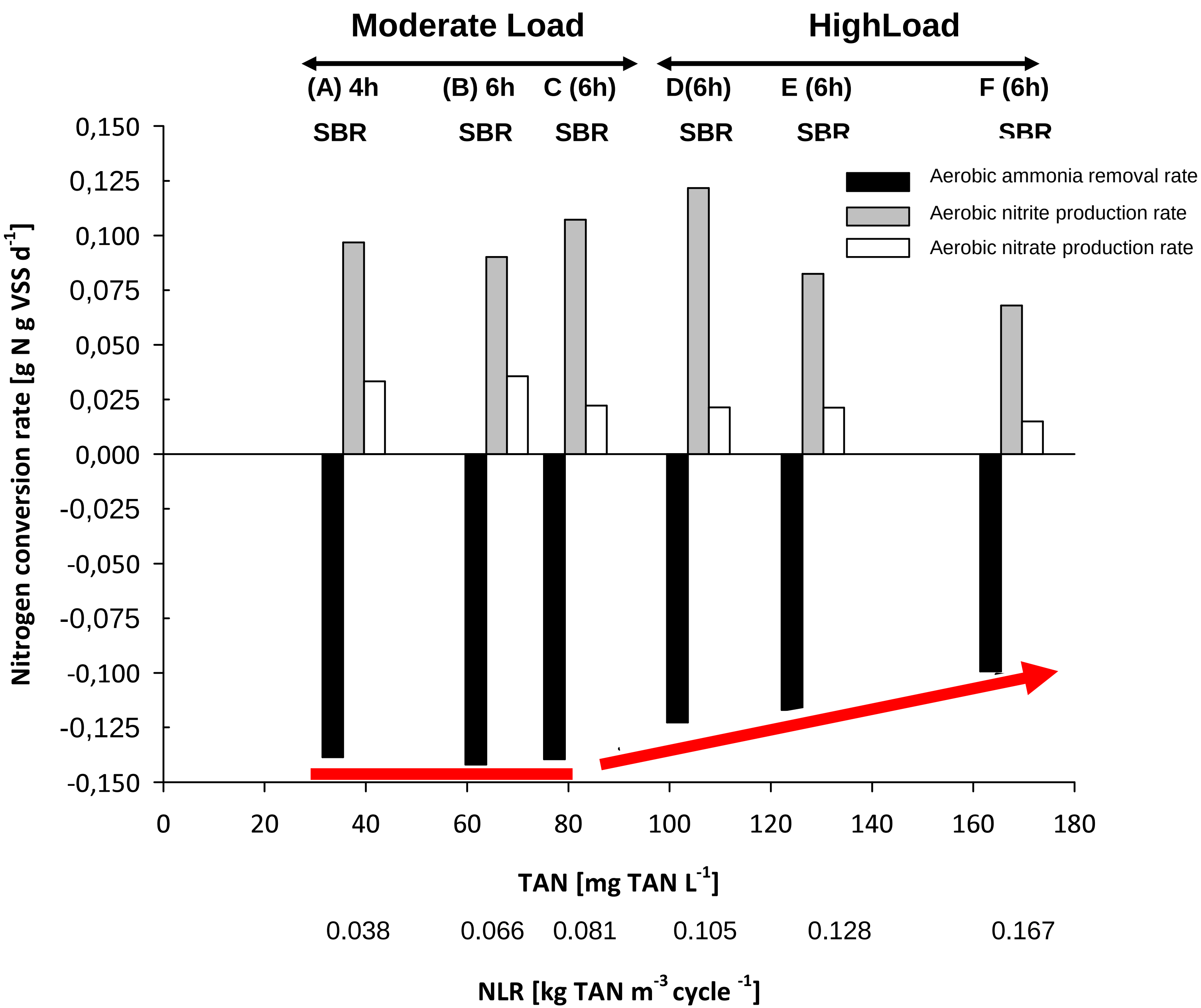
TAN= 76 – 87 %

TN= 64 - 79%

COD= 23 – 34 %

Batch tests & old landfill leachate





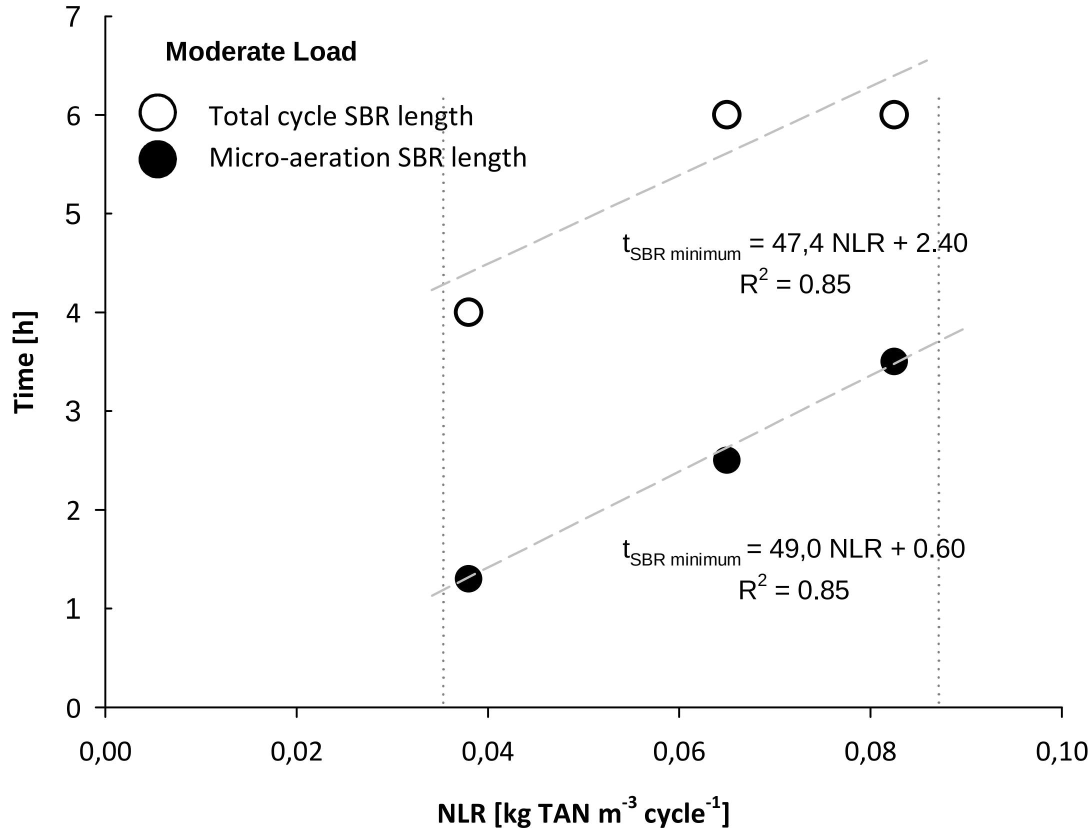
AOB INHIBITION

Batch tests & old landfill leachate - major evidence

1) Feasibility to treat old landfill leachate using a SNAD- SBR system at 30°C

2) NLR = 0.23 – 0.33 kgTAN m^{-3} d^{-1}, HRT= 3.8 - 5.5 d → TAN= 76 – 87 %
TN= 64 - 79%
COD= 23 – 34 %

3) SBR cycle = 4 - 6 hours



Lesson learned:

On the contrary of anammox bacteria, a higher AOB sensibility to:
high load → high FA and high toxic compounds

Water Research: submitted

4.2 And anaerobic digester effluents at moderate temperatures?

Main operative conditions

Reactor Configuration

- *Sequential Batch Reactor (SBR) and Granular biomass*
- *Lab scale SBR*

Community composition and spatial distribution

- *Phylogenetic and Morphologic Analysis*

SNAD process treating ammonium-rich wastewaters:

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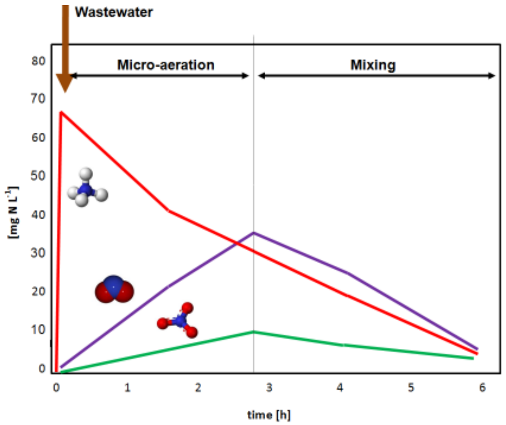
Continuous SBR & digester effluents



CONDITIONS:

V_{SBR}= 8L

T = 30 - 25° C

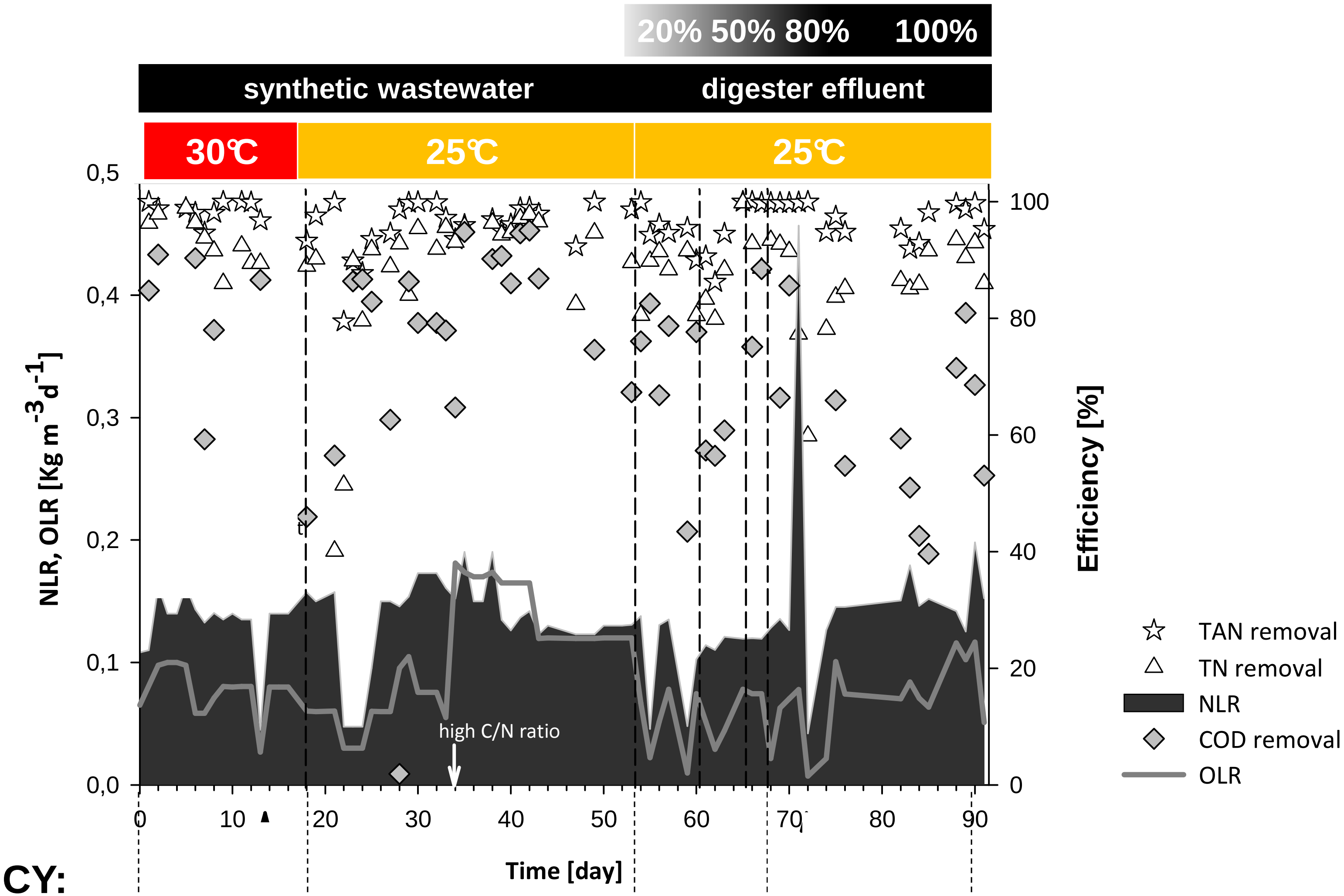
TSS = 3 – 4 g L^{-1}

NLR = 0.13 - 0.15 $kgNm^{-3}d^{-1}$

0.5 < C/N < 0.9

HRT= 3 d^{-1}

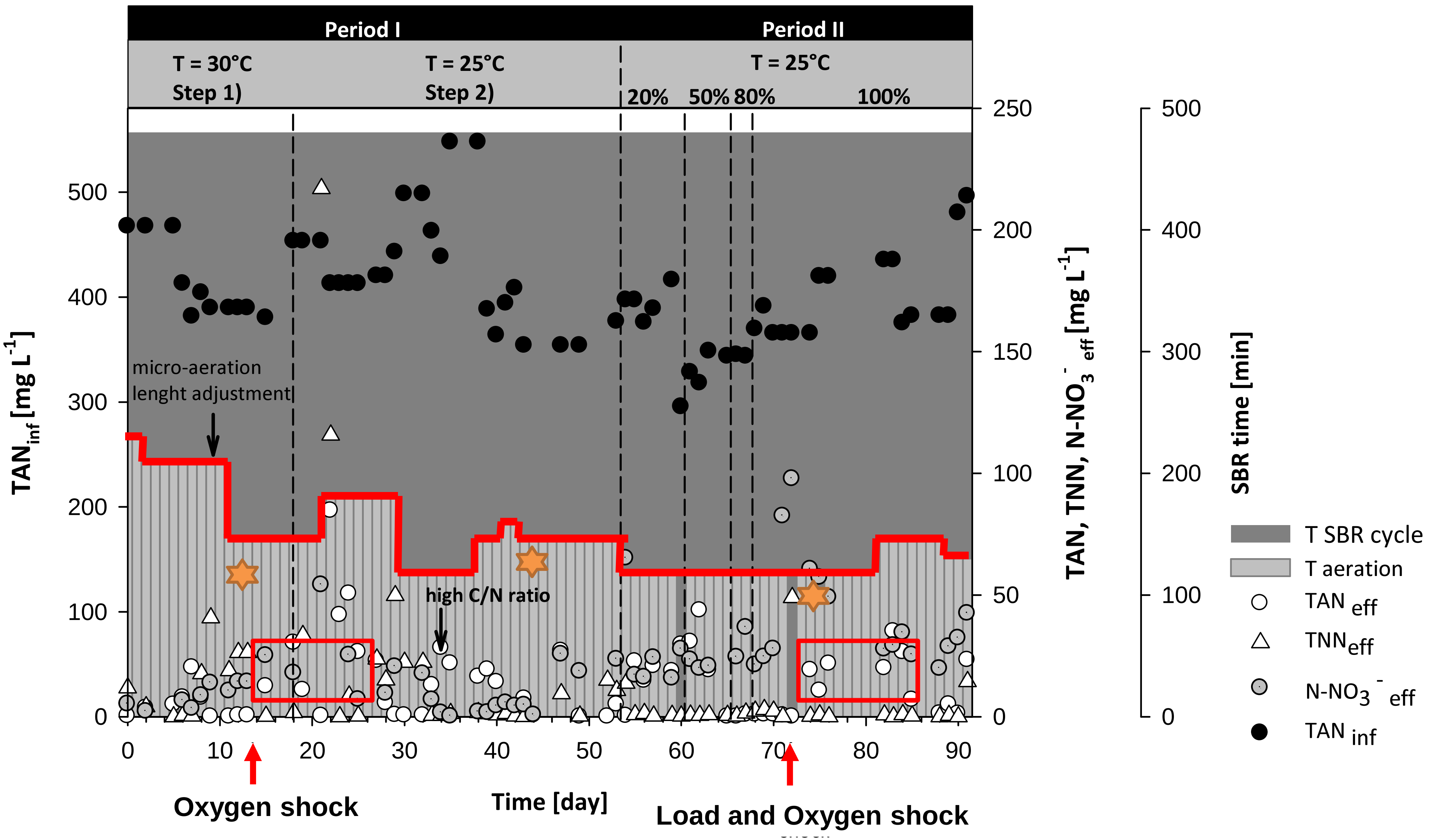
SBR cycle= 8 hours



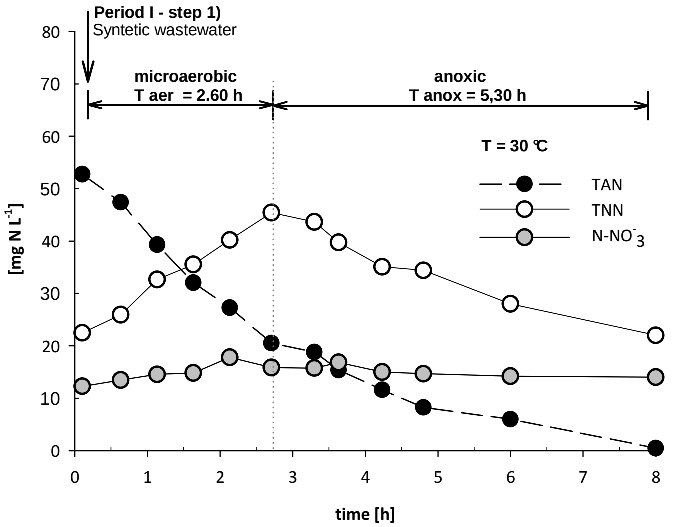
REMOVAL EFFICIENCY:

TAN (%)	98	97	96
TN (%)	93	93	88
COD (%)	81	77	58

Continuous SBR & digester effluents



Continuous SBR & digester effluents



→ ❑ Stoichiometry model

1. Contribution of each process

synthetic wastewater (T= 30°C)

Micro-aerobic	
NITRITAZIONE (AOB)	56%
NITRATAZIONE (NOB)	6%
ANAMMOX	5.2%

\+

Anoxic	
ANAMMOX	23.3%
DENITRIFICAZIONE $NO_3 \rightarrow NO_2$	3%
DENITRIFICAZIONE $NO_2 \rightarrow N_2$	3%

→

digester effluent (T= 25°C)

Micro-aerobic	
NITRITAZIONE (AOB)	38%
NITRATAZIONE (NOB)	29%
ANAMMOX	7.5%

\+

Anoxic	
ANAMMOX	18.7%
DENITRIFICAZIONE $NO_3 \rightarrow NO_2$	2.9%
DENITRIFICAZIONE $NO_2 \rightarrow N_2$	0%

2. NOB reactivation

- ✓ moderate temperature
- ✓ pH decrease in the system, treating digester effluent (from 8.0 to 7.6) → ↓ Free Ammonia (FA) levels

❑ pH, $FA\left[mgNL^{-1}\right] = \frac{TAN}{1+\left(10^{-pH}/K_e^{+NH}\right)}$

Continuous SBR & digester effluents - major evidence

1) Feasibility to treat anaerobic digester effluent using a SNAD- SBR system at 25℃

2) Gradual acclimatation to the moderate temperature and to the anaerobic digester effluent

3) NLR = 0.13 – 0.15 kgTAN m^{-3} d^{-1}, HRT= 3 d → TAN= 96%, TN= 88%, COD= 58%

4) SBR cycle = 8 hours & $t_{micro\text{-}aeration}$ = 2 - 3 hours

5) High robustness of the SNAD- SBR system to oxygen and load shock

Lesson learned:

Treating digester eflluent at moderate temperature:

a tiny pH range control (7.5 <pH < 8.1) → in order to inhibit the NOB

Biorerource technology: Under Review

5. How to control the SNAD process?

Main operative conditions

Reactor Configuration

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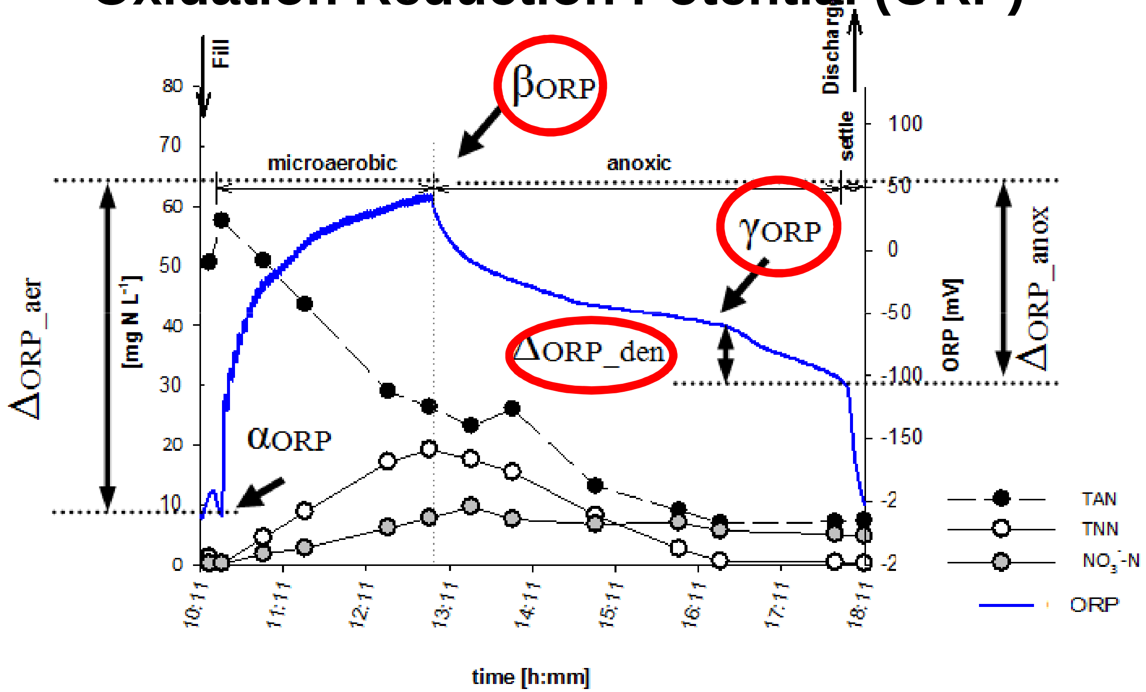
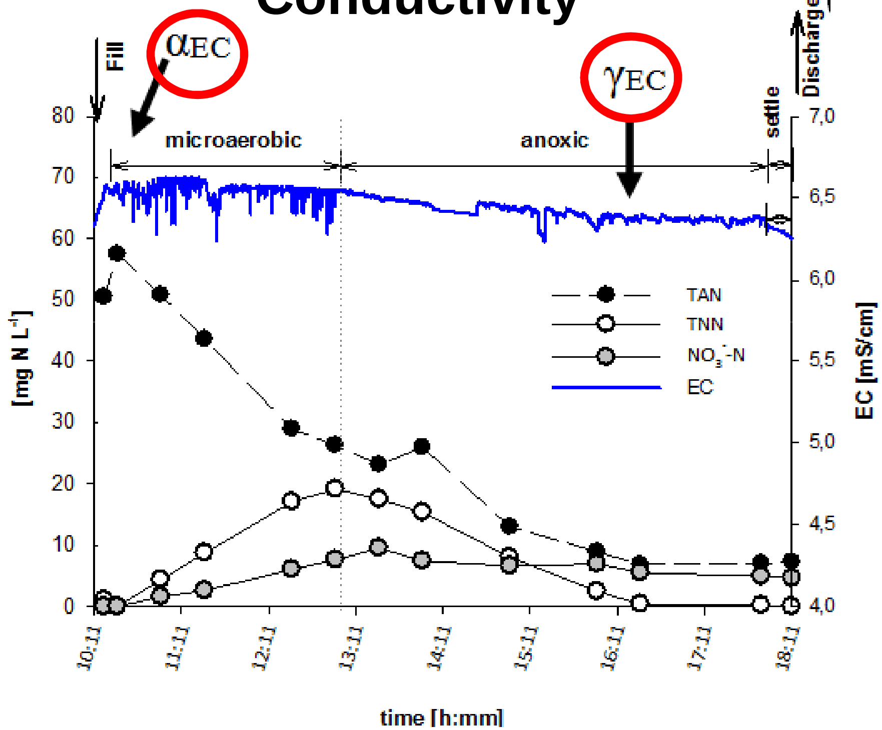
Correlating indirect on-line parameters

Oxidation Reduction Potential (ORP)



Conductivity



1) ORP_{max} ($\beta_{\mathbf{OPR}}$) ~ NO_2^--N_{max} at the end of micro-aerobic phase (NOB inhibited)

2) ORP_{min} ~ (NO_2^--N & NO_3^-- N)$_{min}$ at the end of anoxic phase

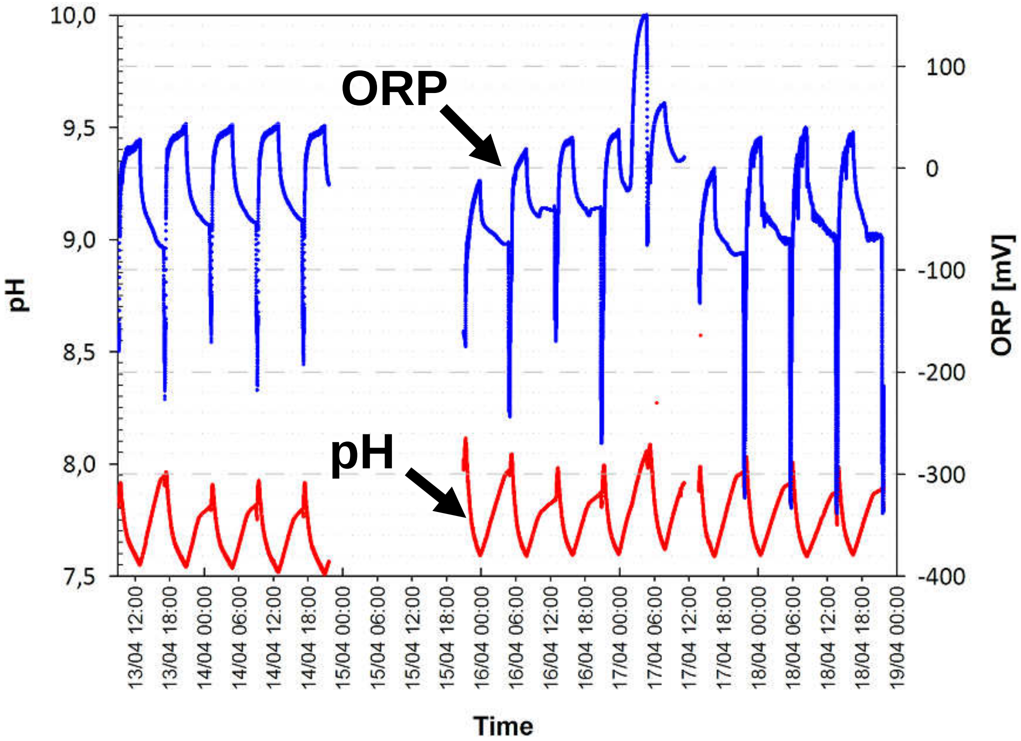
3) EC_{max} ($\alpha_{\mathbf{EC}}$) ~ NH_4^+-N_{max} at the beginning of the SBR cycle

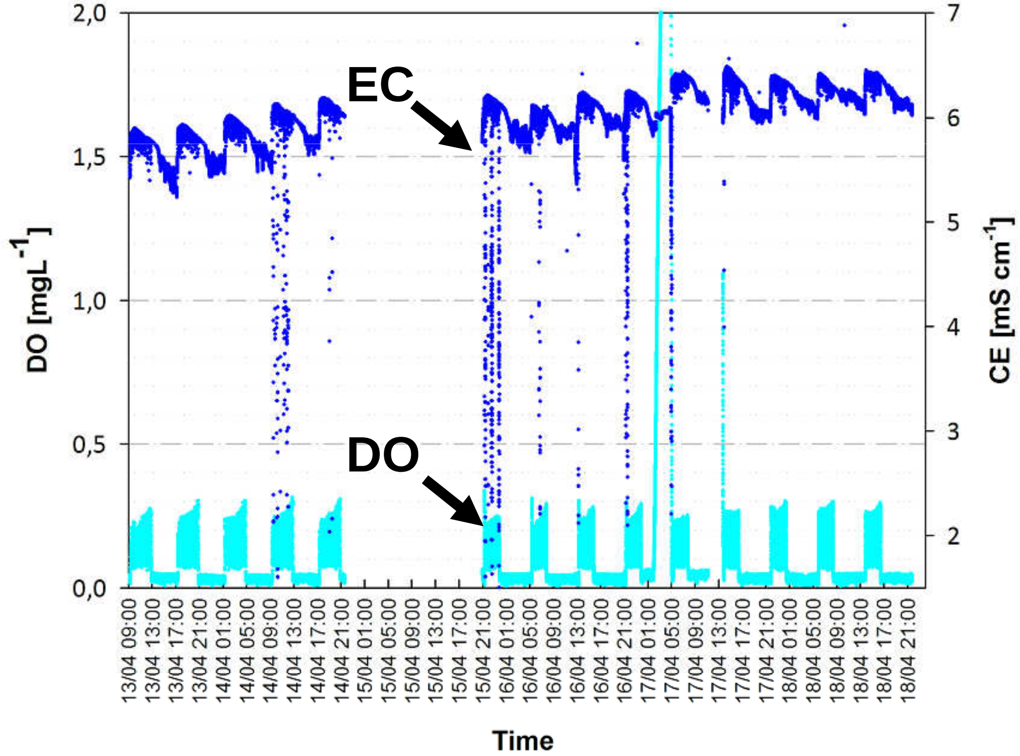
4) EC_{min} ($\gamma_{\mathbf{EC}}$) ~ NH_4^+-N_{min} at the end of the SBR cycle

Biorerource technology: Under Review

Correlating on-line parameters

synthetic wastewater (T=





Biorerource technology: Under Review

“Real time” control strategy

PROTOCOL:

Feeding
start: at the begin of cycle
stop: when maximum conductivity value (α_{EC}, wastewater specific), corresponded to a TAN_{max} was reached

Micro-aeration
start: when $EC_1 < \alpha_{EC}$ was reached or after a fixed delay
OD control (OD < 0.3 mg L^{-1})
stop: when either DEC was removed or a maximum ORP value (β_{ORP}, + 30 – + 50 mV) was reached

Mixing
start: after micro-aerobic phase
stop: when minimum ORP value (g_{ORP}, – 40 mV) was reached

Reaction phase repetitions?
NO: if a minimum conductivity value (g_{EC}, wastewater specific), corresponded to a TAN_{min} was reached at the end of the mixing phase
YES: on the contrary

Settle
start: after mixing phase
stop: after a fixed time

Draw
start: after settle phase
stop: after a calculated time

Control during the whole process
pH control (7.6 < pH < 8.1)
Level control

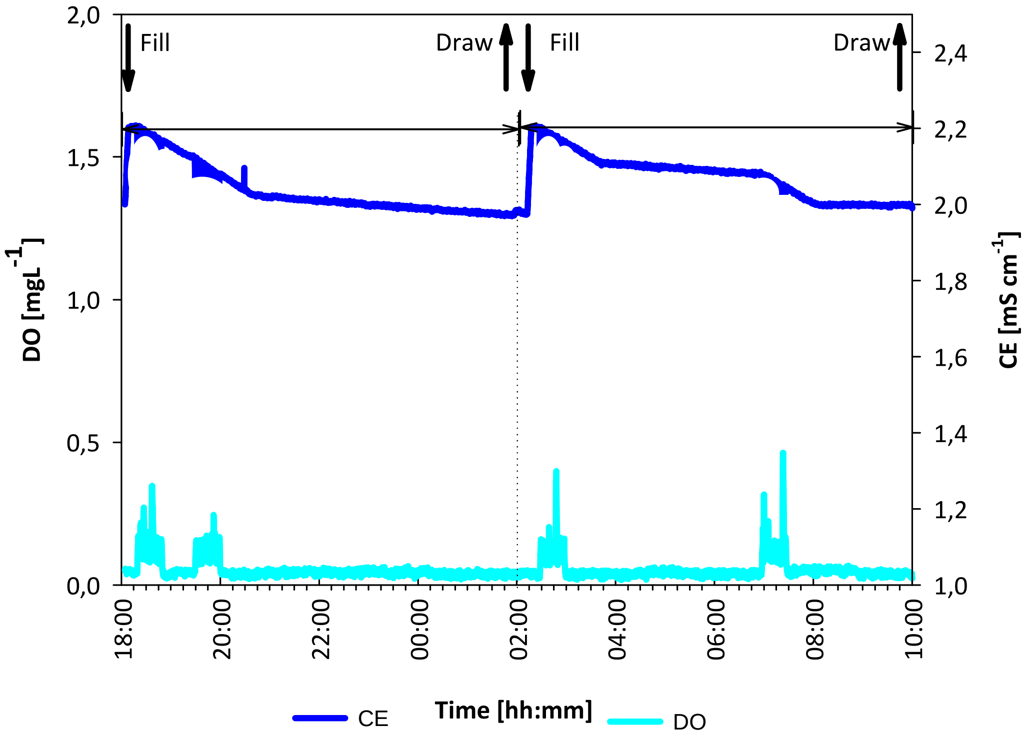
“Real time” control strategy

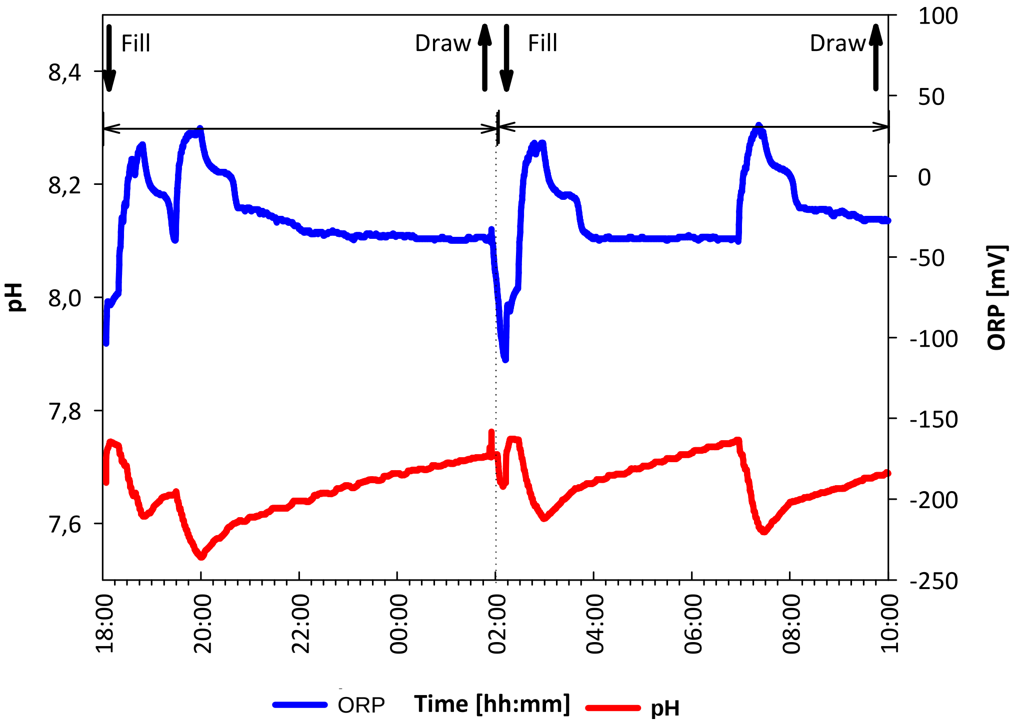
1) EC_{max} (α_{EC}) = 2.2 mS cm^{-1}

2) EC_{mim} (γ_{EC}) = 2.0 mS cm^{-1}

1) ORP_{max} (β_{ORP}) = +30 mS cm^{-1}

2) ORP_{mim} (γ_{ORP}) = - 40 mS cm^{-1}





Advantages:

- ❑ High N removal efficiency (TAN = 96,5% , TN = 88,6%, COD = 58%)
- ❑ Prevent any critical situation:
  - ▪loading shocks
  - ▪anammox inhibition by high nitrite concentrations
- ❑ Low supervision

Conclusions

- **Co-existence** of aerobic ammonia oxidizing, anammox and denitrifying bacteria in a granular-SBR system

- Under steady-state conditions, nitritation, anammox, denitrification **synchronize each other**, establishing a relation depending on the **operative conditions** of the system

- Batch tests showed the **feasibility** to treat **old landfill leachates** using the SNAD-SBR system at a temperature of **30℃**

- The continuous SNAD-SBR system proved to be **effective** in treating urban **anaerobic digester** effluents at a temperature of **25°C.**

- **A "real time" control strategy** based on on-line indirect parameters has been successful applied

Research outlook

- Improving/Enhancing the applicability of the continuous SNAD-SBR system to old leacheates
- Developing a "real time" control strategy based on the use of direct on-line parameters (e.g. NH_4^+, NO_2^-, NO_3^-)
- Application of the SNAD process to the main stream of WWTPs

…during my PhD

Journal Articles
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Journal Reviewer
2011: Reviews in Environmental Science and Bio/technology

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PCR NOB genes Nirs

denitrifiers partial bacteria reactor ORP amoA anaerobic

Anammox organic strategy leachate process activity wastewater digester conductivity

oxygen-limited carbon Ammonium 16SrRNA

co-existence landfill Simultaneous monitoring nitrogen biodegradable

Denitrification nitrifiers effluent Michela

Nitritation SBR Ammonia nitrite FISH

AOB SNAD control C/N

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