Understanding the role of *Tetrasphaera* in enhanced biological phosphorus removal

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Introduction

EBPR is an economical, efficient and sustainable way to remove phosphorus from the wastewater.

Alternating anaerobic and aerobic conditions

Uptake and storage of poly-phosphate

Take up simple carbon sources

Candidatus Accumulibacter phosphatis → Most well-known and studied PAO

Full-scale EBPR plants → Other putative PAOs → Genus *Tetrasphaera*
Introduction

Lab-scale
- Volatile fatty acids
- COD consumed in the anaerobic reactor/phase
- Microbiological techniques

Full-scale
- Low concentrations of volatile fatty acids
- Amino acids

Enrichment of *Accumulibacter* (PAOs)

*Tetrasphaera* proliferation

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2 Objective

➢ To gain knowledge on the new PAO genus *Tetrasphaera* by obtaining an enriched culture at lab-scale

➢ Two reactor configurations:

➢ Sequencing batch reactor

➢ Continuous pilot plant system with A²/O configuration
Results: SBR operation

V = 10L
Synthetic influent: 10 mg P-PO$_4^{3-}$/L, 44-113 mg COD/L
Aspartate + Glutamate

Initial time
End of anaerobic phase
End of aerobic phase

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Results: SBR operation

V = 10L
Synthetic influent: 10 mg P-PO$_4^{-3}$/L, 44-113 mg COD/L
Aspartate + Glutamate

Excess of carbon source
Glutamate leakage to the aerobic phase
System failure

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Results: A²/O operation

V = 146L

Synthetic influent: 10 mg P-PO₄³⁻/L, 50 mg N-NH₄⁺/L and 400 mg COD/L

Glutamate

Glutamate as carbon and nitrogen source

P-removal efficiency > 95%

Batch deteriorated glutamate

Excessive NO₃⁻ input in the anaerobic reactor
3 Results: Batch experiments with the SBR sludge

PHA & Glycogen quantification

**Results:**
- **PHA:** 0.69 mmol C/gVSS
- **Glycogen:** 0.47 mmol C/gVSS
- **PAO-enriched cultures:**
  - **PHA production:** 2.67 mmol C/gVSS
  - **Glycogen consumption:** 1.30 mmol C/gVSS

**Graph:**
- **Axes:**
  - X: Time (min)
  - Y: TOC (mg C/L), Phosphate (mg P/L), PHA and glycogen (mmol C/g VSS)
- **Legend:**
  - P (mg/L)
  - TOC (mg/L)
  - Glycogen (mmol C/g VSS)
  - PHA (mmol C/g VSS)
Results: Batch experiment with the A²/O sludge

PHA & Glycogen quantification

- PHA: 0.62 mmol C/gVSS
- Glycogen: 0.06 mmol C/gVSS

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PAO-enriched cultures:
- PHA production: 2.67 mmol C/gVSS
- Glycogen consumption: 1.30 mmol C/gVSS
### Results: Comparison between configurations

#### Anaerobic PHA and glycogen production

<table>
<thead>
<tr>
<th>mmol C/g VSS</th>
<th>SBR</th>
<th>A²/O</th>
</tr>
</thead>
<tbody>
<tr>
<td>COD consumed</td>
<td>3.436</td>
<td>8.87</td>
</tr>
<tr>
<td>PHA</td>
<td>0.692</td>
<td>0.621</td>
</tr>
<tr>
<td>Glycogen</td>
<td>0.476</td>
<td>0.066</td>
</tr>
<tr>
<td>Total=PHA + Glycogen</td>
<td>1.168</td>
<td>0.687</td>
</tr>
<tr>
<td>Carbon recovery ratio</td>
<td>0.34</td>
<td>0.08</td>
</tr>
</tbody>
</table>

Other storage routes should be studied
# Results: Literature comparison

<table>
<thead>
<tr>
<th>Study</th>
<th>Enriched PAO</th>
<th>This study</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Kapagiannidis et al. (2013)</td>
<td>SBR</td>
</tr>
<tr>
<td>$P_{rel}/C_{upt}$ (mol P/mol C)</td>
<td>0.64</td>
<td>0.27</td>
</tr>
<tr>
<td>$\text{PHA}<em>{prod}/C</em>{upt}$ (mol C/mol C)</td>
<td>1.10</td>
<td>0.20</td>
</tr>
<tr>
<td>$\text{Glyc}<em>{prod}/C</em>{upt}$ (mol C/mol C)</td>
<td>Consumption (-0.41)</td>
<td>0.14</td>
</tr>
<tr>
<td></td>
<td>Tayà et al. (2013)</td>
<td></td>
</tr>
<tr>
<td>$P_{rel}/C_{upt}$ (mol P/mol C)</td>
<td>0.34</td>
<td></td>
</tr>
<tr>
<td>$\text{PHA}<em>{prod}/C</em>{upt}$ (mol C/mol C)</td>
<td>1.47</td>
<td></td>
</tr>
<tr>
<td>$\text{Glyc}<em>{prod}/C</em>{upt}$ (mol C/mol C)</td>
<td>Consumption (-0.49)</td>
<td></td>
</tr>
</tbody>
</table>
Results: Bacterial community assessment

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Results: Bacterial community assessment

A²/O pilot plant

- All bacteria
- Specific bacteria

Tetrasphaera
Clone ASM31

Tetrasphaera
Clade II

Tetrasphaera
Clone ASM47

Uncultured
Tetrasphaera

26%
PAO

39%

27%

1%

GAO

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### Results: Bacterial community assessment

<table>
<thead>
<tr>
<th></th>
<th>PAOMix</th>
<th>GAOMix</th>
<th>Tetrasphaera</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SBR</strong></td>
<td>36 ± 1%</td>
<td>21 ± 1%</td>
<td>43 ± 9%</td>
</tr>
<tr>
<td><strong>A²/O plant</strong></td>
<td>26 ± 4%</td>
<td>1 ± 1%</td>
<td>66 ± 5%</td>
</tr>
</tbody>
</table>

Why do we detect the presence of PAO and GAO if we fed the reactor with glutamate for more than 400 days?
Results: Bacterial community assessment

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Further work

We are still operating the A²/O pilot plant with glutamate.

We are working with this enriched-\textit{tetrasphaera} culture in order to better understand this new PAO genus.

We will perform batch assays with different carbon sources and different electron acceptors.

We are waiting for the pyrosequencing results.
Conclusions

• Successful enrichment of sludge in *Tetrasphaera* using glutamate as sole carbon source was obtained for the first time.

• Better results and more stability was achieved with continuous pilot plant with respect to SBR.

• Fermentation products of glutamate did not allow to obtain a highly *Tetrasphaera*-enriched culture.

• The increase of PHA and glycogen during the anaerobic phase only accounted a small percentage of the carbon source consumed.

• Other storage routes should be studied to identify the fate of the carbon source stored under anaerobic conditions.
ACKNOWLEDGMENTS

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Thank you for your attention!
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