Microbial community stratification in Membrane-Aerated Biofilm Reactors for Completely Autotrophic Nitrogen Removal

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### 1. Introduction

Anaerobic Ammonium Oxidizers (Anammox): A relatively new paradigm in Nitrogen removal from wastewater

**Nitritation by AOB**

**Anammox**

But why using two reactors when one is enough? -> Biofilms

**Advantages**
- Compact configuration.
- Lower NO\(_2\) production (NO\(_2^-\) is produced and consumed in-situ).

**Disadvantages**
- Need of a carrier material.
- Difficulty of control (thickness, O\(_2\) supply, microbial selection...).

### 2. Reactor setup

Our biofilm develops on hollow-fibre bubbleless aeration membranes. More in detail, the setup consists of:

- Substrate bottle
- Gas bag
- Diaphragm pump
- Reactor body and membrane module
- Needle valve
- Needle valve
- Flow cell
- Instrument meters
- A/D Converter
- Computer
- Rotary electric stirrer

### 3. Performance

Through sequential aeration regimes and L\(_{O2}/L_{NH4}\) tuning to 1.73 the reactor performance increased considerably since Anammox inoculation

- Observed removal rates up to 0.77 g-N/L/day (5.5 g-N/m\(^2\)/day, 70% of the N-load). The Anammox process is expected to remove most of it.
- NO\(_2\) produces 30% of the total NO\(_3^-\).

### 4. Materials and methods

Fluorescent In-Situ Hybridization was performed after reactor shutdown (day 470, 0.7 g-N/L/day) to study the microbial community in the reactors

- Membrane
- Bulk liquid

### 5. Microscopic observation & quantification - Conclusions

- Results proved the possibility of engineering biofilm structures for autotrophic nitrogen removal taking advantage of DO gradients inside the biofilm
- AOB grew mainly in aerobic regions, close to the membrane in the biofilm core. Communities developed in areas with high cell densities and tended to grow in radial structures with streptococcal shape
- AnAOB grew in the perimeter of the biofilm structure, where anaerobic conditions prevailed. Since nitrite was produced in-situ by AOB, AnAOB grew in clusters pointing the internal part of the biofilm
- NO\(_2\) developed mainly during the reactor start-up, before Anammox inoculation, and were mainly located in the transient zone of the biofilm, together with other non-identified bacteria (most likely heterotrophic).
- Image quantification using DAIME showed that AOB were the most abundant population while AnAOB only covered 20% of the biofilm area.