Performance and Microbial Population Dynamics of a Nitritation/Anammox Reactor treating Urine

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Nitrogen elimination from source-separated urine

Source separation of urine is an alternative strategy in wastewater treatment. The basic premise is to separate the recovery or elimination of nutrients from urine from the remaining wastewater. Urine separation is particularly interesting for decentralized treatment, especially for low income countries. Various treatment strategies for source separated urine are currently under investigation at Eawag.

Where nitrogen recycling is not economically desirable, the nitrogen load has to be reduced before discharging wastewater into the environment. A cost and energy-efficient treatment process is the combination of nitritation and anammox in a single reactor (PNAA).

PNAA sequencing batch reactor

In this project we studied a sequencing batch reactor for nitrogen elimination from source separated urine by combined **nitritation and anammox (PNAA process)** (Fig. 1). Due to the high organic load of urine, heterotrophic denitrifying organisms also play an important role.

- **Is the temporal dynamics of the microbial community related to reactor performance?**
- **What are the main drivers of microbial community composition in the system?**
- **Characterize the populations of organisms involved in PNAA.**

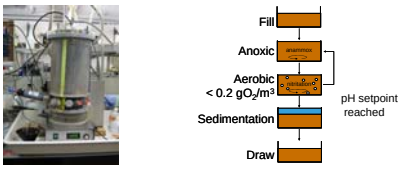

Figure 1: The PNAA sequencing batch reactor

Methods

The microbial community in the reactor was sampled intensively during the start-up phase (switching from sludge supernatant to diluted urine), and at reduced frequency to monitor the long term development. Microbial populations were studied based on DNA extracted from sludge samples followed by ARISA and DGGE analysis (general bacteria and anammox: 16S rRNA genes), sequencing of excised DGGE bands, and screening and sequencing of clone banks. From February 2008 onwards a number of chemical and physical parameters was determined every 2-3 days (on average).

General bacterial population

The general bacterial population showed a marked transition in the apparently dominant phylotypes during the start up phase. A strong shift occurred again before the beginning of long term monitoring. Gradual changes in the population continued during long term operation (Fig. 1). The initially low diversity increased during the first months of operation (Fig. 1, insert). In 2008/2009, three phases are visible in the microbial community development (green, red, purple indicators, Fig. 2)

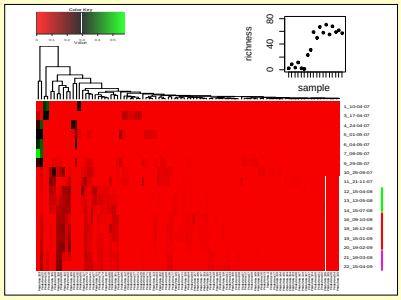

Figure 1: A heatmap representation of ARISA peak area data. Peaks with similar behavior over time are clustered together. Insert indicates richness over time

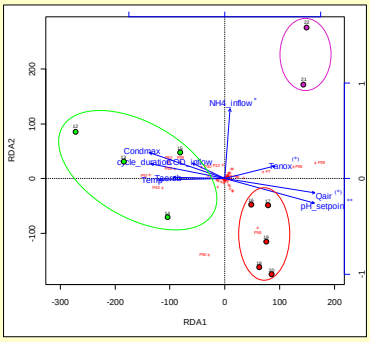

*Figure 2: Biplot of Redundancy Analysis of 2008/2009 ARISA community data constrained by process control variables. Significance by permutation test: ** p<0.01, *p<0.05, * p< 0.1. Color codes represent results from cluster analysis and correspond to phases of reactor performance (Fig. 3)*

Performance

By successive increases of the pH setpoint (resulting in shorter cycle duration) and aeration, nitrogen elimination rate could be greatly improved by ~400% (Fig. 3, upper panel).

The increase was not sustainable, however: nitrite accumulation and decreased ammonium elimination (Fig. 3, middle panel) led to a prolonged decrease in performance.

The main phases of reactor performance (increase, decrease, recovery) are mirrored by changes in the microbial community (Fig. 3, bottom panel). The microbial community shifted to a new stable state – in ecological terms the population experienced a regime shift. The pH setpoint and the inflow ammonium concentrations were the most significant variables explaining the development of the microbial community (Fig. 2).

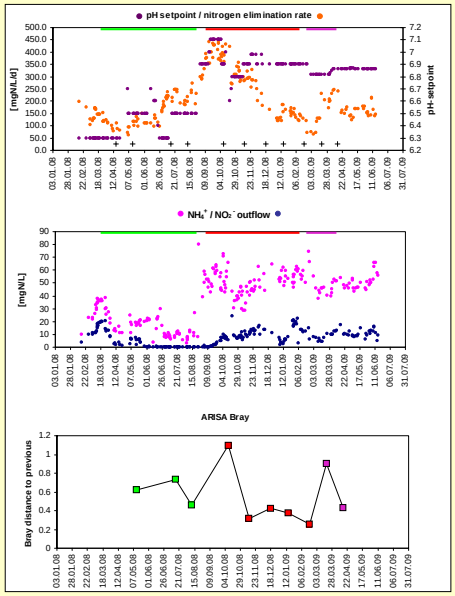

Figure 3: Top: pH-setpoint and Nitrogen elimination rate. Crosses mark sampling dates for microbial analysis. Middle: NH_4^+ and NO_2^- in the outflow. Bottom: moving window analysis of Bray distance based on ARISA data. Dissimilarity peaks as the system changes to a new stable state. Color codes indicate the grouping of microbial samples as shown in Fig. 2

Novel Anammox strain?

While ammonium oxidation was performed by *Nitrosomonas europaea* (not shown) or close relatives, at least two groups of probable anammox bacteria were detected (Fig. 4). Both types are phylogenetically distinct but most closely related to known anammox bacteria. The related *Brocadia* strain sp. 40 (■) was found in a lab scale anammox reactor in Uruguay. The SBR I type eventually became dominant in the reactor. Anammox and aerobic ammonium oxidizers both exhibited very low diversity during the 2008/2009 monitoring period

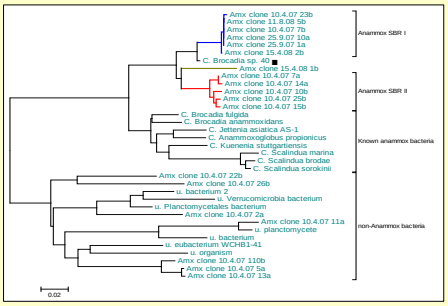

Figure 4: Minimum evolution tree of 16S rRNA gene sequences amplified with anammox bacteria specific primers

Conclusions

A lab-scale PNAA bioreactor for nitrogen elimination from urine was successfully operated over extended periods of time. Anammox bacteria, possibly belonging to a new strain were identified in the system. The diversity of the general (mostly heterotrophic) bacterial community increased considerably over the first months. Community structure changed dynamically and appeared to be subject to rapid regime shifts that were triggered by adjustments in operating parameters. The pH setpoint, the aeration regime, and the nitrogen concentration in the inflow were the most important variables shaping microbial community composition. The study shows that care needs to be taken in operating biological reactors as significant shifts in the microbial ecosystem can occur as a result of relatively minor changes in operating parameters.