REGIME SHIFT IN A NITRITATION-ANAMMOX SEQUENCING BATCH REACTOR

eawag aquatic research

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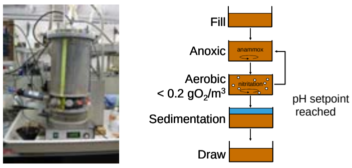
System stability and regime shifts

An experimental sequencing batch reactor for nitrogen removal from urine by partial nitritation and anammox was monitored for over a year. Over the course of the experiment the reactor experienced considerable dynamics in performance as well as changes in the microbial community. We hypothesize that these dynamics can be interpreted as ecological regime shifts. Regime shifts are defined as rapid reorganizations of ecosystems from one relatively stable state to another. While the concept is widely applied in ecology, it has not really been considered for microbial ecology. A better understanding of the causes and dynamics dynamics of regime shifts in microbial ecosystems is an important foundation to enable the stable operation of biological reactors.

Figure 1



Nitritation: $NH_3 + O_2 \rightarrow NO_2^- + H^+ + H_2O$
Anammox: $NH_3 + NO_2^- + H^+ \rightarrow N_2 + NO_3^- + H_2O$

PNAA (empirical):
$2.5\ NH_4^+ + 2.1\ O_2 \rightarrow 0.2\ NO_3^- + 1.15\ N_2 + 3.6\ H_2O + 2.8\ H^+$ (Jetten 2001)

Source separation of 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 the remaining wastewater. Urine separation is particularly interesting for decentralized wastewater 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).

Objectives

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.

•What drives microbial community composition?

•Can performance be linked to microbial community structure?

•Are communities stable during stable operation?

•How to improve performance and stability?

Methods

Chemical and physical parameters were measured continuously by probes or from samplings performed every 2-3 days, and analysis using standard methods. The microbial community in the reactor was sampled approximately monthly to monitor the long term development. Microbial populations were studied based on DNA extracted from sludge samples. Community structure was characterized by automated ribosomal intergenic spacer (ARISA) analysis.

Genetic markers (functional genes, 16S rRNA genes for anammox bacteria) for major processes of the nitrogen cycle were studied using quantitative real-time PCR.

Statistical analysis using R and the R and Biodiversity R packages.

Dynamics of population structure

The bacterial population showed gradual population changes during the initial monitoring period (also relative to 2007 samples). A strong shift occurred after the reactor reached peak performance. After this shift, the population entered a prolonged period of stability (Fig. 2, 3). We distinguished three phases in the microbial community development (green, red, purple indicators, Fig. 2 and throughout)

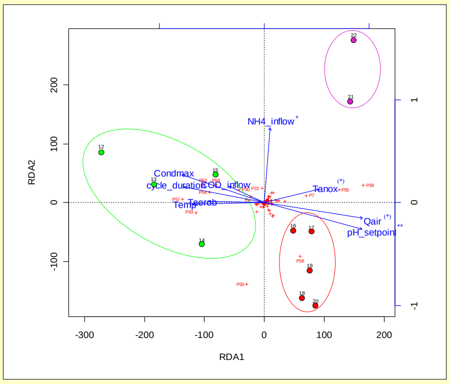


***Figure 2:** Biplot of Redundancy Analysis of 2008/2009 ARISA community data constrained by process control variables.*
Tanox: relative duration of anoxic phase. Qair: airflow.
*Significance by permutation test: ** p<0.01, *p<0.05, (*) p< 0.1. Colors and colored circles represent results from cluster analysis and correspond to phases of reactor performance (Fig. 4)*

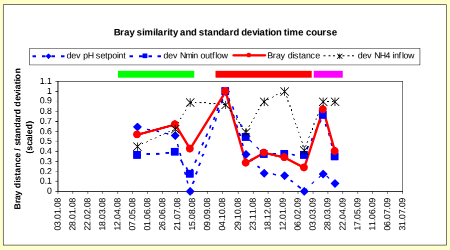


***Figure 3:** Analysis of Bray distance based on ARISA data and standard deviation between sampling events for selected parameters (scaled to 1). Dissimilarity and standard deviation peak as the system changes to a new stable state. Color bars indicate grouping of microbial samples as in Fig. 2.*

Dynamics of performance

By successive increases of the pH setpoint (and aeration, nitrogen elimination rate could be improved by ~400% (Fig. 4, top). The increase was not sustainable, however. Reduced cycle duration and a reduced duration of the anaerobic phase (Fig. 4, center) may have contributed to a breakdown of the anammox population (Fig. 5). Shortly after peak performance, nitrite accumulation was observed (Fig. 4, bottom).

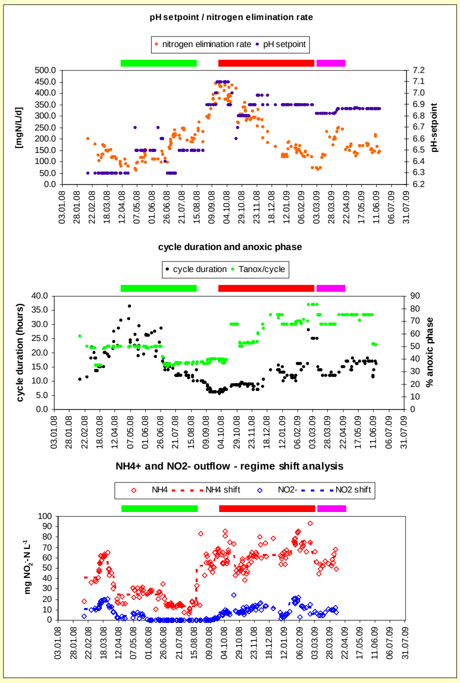


***Figure 4:** **Top**: Nitrogen elimination rate (performance) and pH setpoint. Peak performance was reached in september 2008. **Center:** cycle duration (affected by pH setpoint and rate of pH drop in the system) and relative duration of anoxic conditions. Short cycles increase the treated volume, but may have favored washing out of anammox bacteria **Bottom:** NH_4^+ and NO_2^- in the outflow. Regime shift analysis indicated phases of relative stability. The chemical parameters experienced regime shifts in accordance with those observed in the microbial community structure.*

Dynamics of functional groups

The initial increase in performance went along with an increasing anammox population, and a decrease in the abundance of *nosZ* (reduction of N_2O). Ammonium oxidizers changed little.

Anammox population did not recover until the end of the monitoring period.

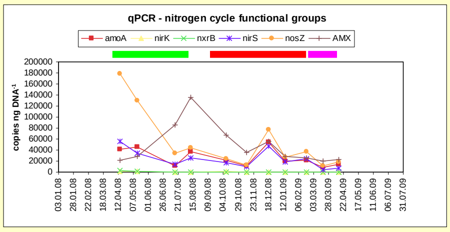


***Figure 5:** qPCR quantification of the abundance of key functional groups of nitrogen cycle processes in the reactor.*

Main Results

- Bacterial population structure (not diversity) changed in parallel to process performance trends
- pH setpoint, aeration (Qair, Tanox), and NH_4-inflow were identified as major drivers of community composition (Fig. 3)
- The dynamics in the reactor can be described as regime shifts – rapid changes of the ecosystem followed by phases of relative stability
- First regime shift caused by reduction of pH setpoint and changes in aeration that resulted in shorter cycles and less favorable conditions for anammox bacteria
- qPCR indicates that a breakdown of the anammox bacteria population was a main factor in the performance decrease of the reactor

Conclusions

Biological reactors act as complex ecological systems. Small changes in external parameters can trigger regime shifts that affect performance. These new states may be stable, affecting the ability to return the system to a more favorable state. Research into the ecological dynamics of wastewater treatment and other engineered systems is important to understand and predict system behavior.