Microbial population dynamics during nitritation/anammox treatment of urine

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NoMix – Urine Source Separation

Source separation of urine is an alternative strategy in wastewater treatment (NoMix technology). The basic premise is to separate the nutrient elimination from urine from the treatment of organic matter by separating urine from the rest of wastewater. This allows for energy efficient wastewater treatment. The technology is suitable for decentralized treatment strategies, making it particularly interesting for developing countries or emerging economies. Various treatment strategies for source separated urine are currently under investigation at Eawag.

Sequencing Batch Reactor

In this project we studied a sequencing batch reactor (Fig.: left: Reactor, right: scheme of reactor operation) for nitrogen elimination from source separated urine by combined **nitritation and anammox**. The main goals are to

- **characterize the active processes**
- **characterize the stability of the system**
- **study potential sources of instability or inhibition**.

Understanding the composition and dynamics of the microbial population is a key to achieving these goals. We focused on the **heterotrophic, ammonium oxidizing, and anammox bacteria**.

Fill — Anoxic (anammox) — Aerobic < 0.2 gO_2/m^3 (nitritation) — Sedimentation — Draw
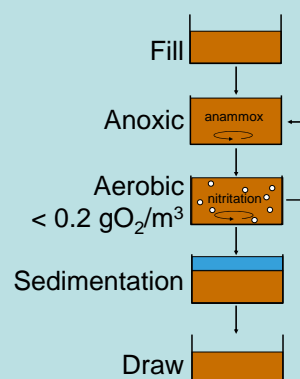
Methods

The reactor was sampled intensively during the start-up phase (switching from sludge supernatant to diluted urine), and then at reduced frequency to monitor the long term development. Microbial populations were studied based on DNA extracted from sludge samples followed by PCR amplification and DGGE analysis (general bacteria and anammox: 16S rRNA genes, ammonium oxidizers: *amoA* gene), sequencing of excised DGGE bands, and screening and sequencing of clone banks. Nitrogen dynamics were monitored regularly.

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.: Bacterial DGGE)

Startup phase | Long-term monitoring
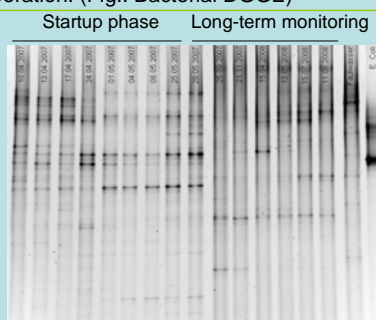
Ammonium oxidizers

According to DGGE, the inoculum contained three dominant *amoA* phylotypes. By the end of the startup phase the population began to change, interestingly showing a number of new phylotypes appearing. In the long term however, a single phylotype became and remained dominant. Dominant bands were sequenced and found to belong to two groups, both most similar to *Nitrosomonas europaea*. (Fig.: *amoA* DGGE)

Startup phase | Long-term monitoring
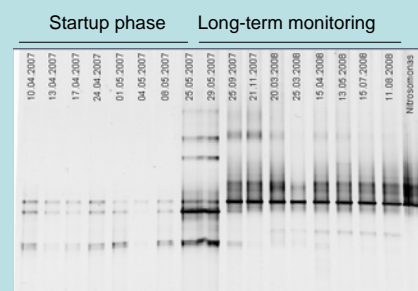
Anammox bacteria

At least two groups of probable anammox bacteria were detected. 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.
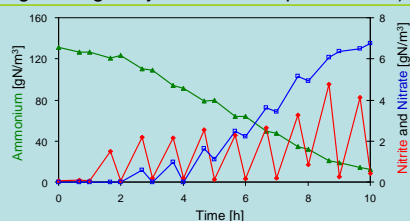
(Fig.: Minimum evolution tree of 16S rRNA gene sequences amplified with anammox bacteria specific primers)
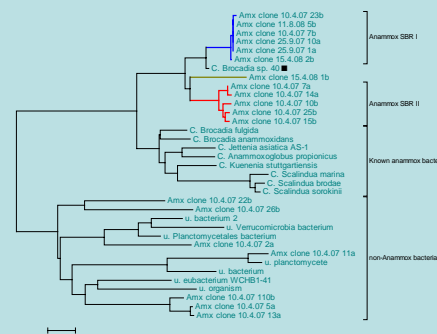
Performance

After initial problems with high nitrite accumulation, the reactor is now stable and eliminates nitrogen with ~ 90% efficiency.

(Fig.: Nitrogen dynamics on Sept. 15. 2008)

Conclusions and Outlook

The general (mostly heterotrophic) bacteria did not reach a stable population. This is interesting as heterotrophs appear to play an important role in detoxifying urine for the autotrophs, a prerequisite to successful nitritation / anammox. The ammonium oxidizer population became dominated by a single phylotype – future investigations will show if this is unfavorable in stress situations. Anammox appears to be mediated by new strains of anammox bacteria with unknown properties. The temporal development of the anammox population is still under investigation, but preliminary data indicates selection of a single phylotype (SBR I). Quantitative analyses of these and other key functional groups are planned.