Low Temperature Partial Nitritation/Anammox in a Moving Bed Biofilm Reactor treating Low Strength Waste Water

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Supplementary Material:

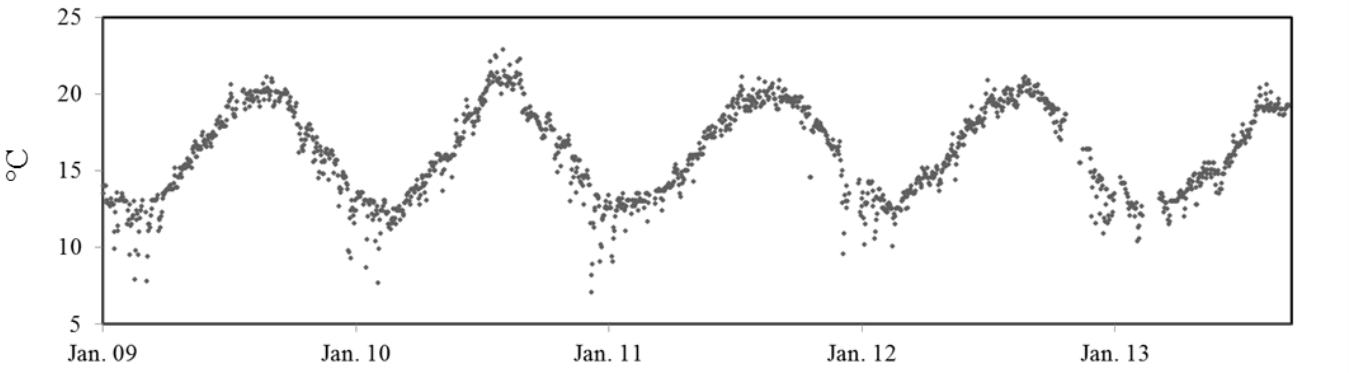

Figure SI 1: Five year temperature gradient in WWTP Neureut, Karlsruhe, Germany

Figure SI 2: Carrier material (Type K3, AnoxKaldnes, Sweden) used in this study. Scale bar 10 mm.

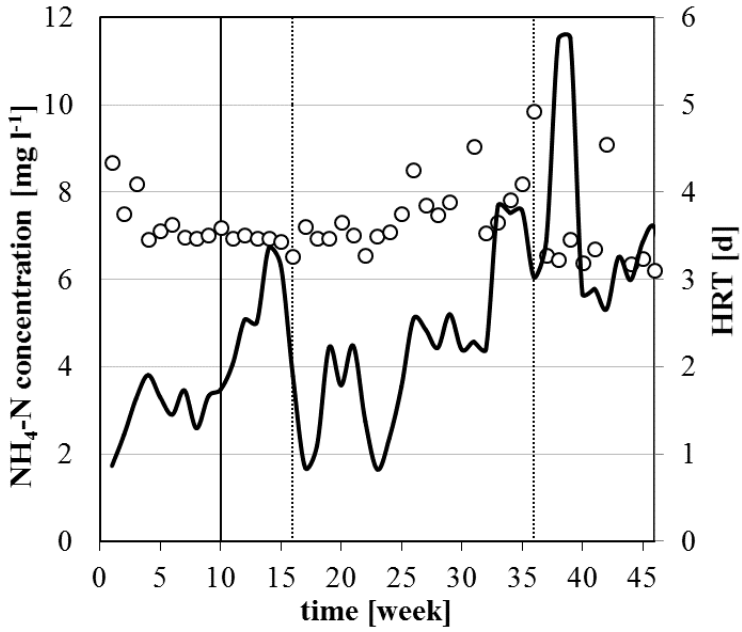

Figure SI 3: Weekly average of the effluent NH_4^+-N concentrations (empty circles) and HRT (solid line) in the MBBR during the period of reactor operation. In conformity with Figure 3 the change in influent concentration (dotted line) as well as start and end points of the temperature profile (dashed lines) are also indicated.

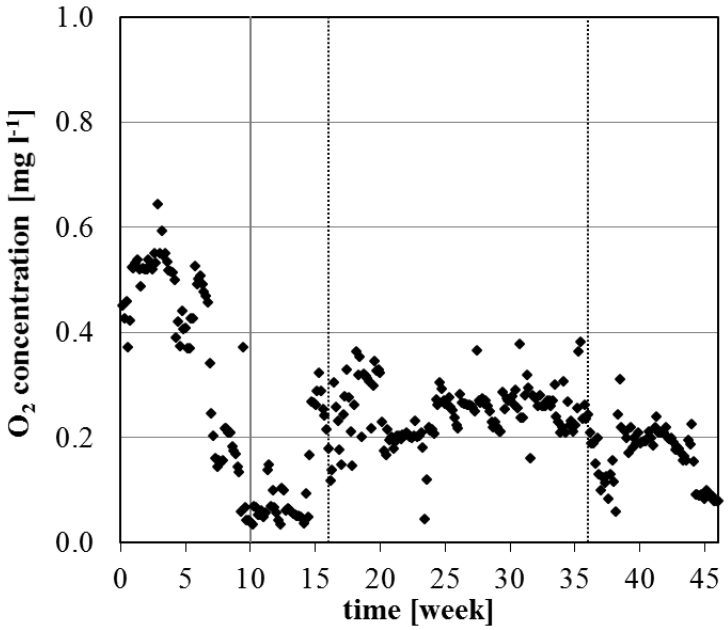

Figure SI 4: DO in the bulk phase of the MBBR during the period of reactor operation. In conformity with Figure 3 the change in influent concentration (solid line) as well as start and end points of the temperature profile (dashed lines) are also indicated.

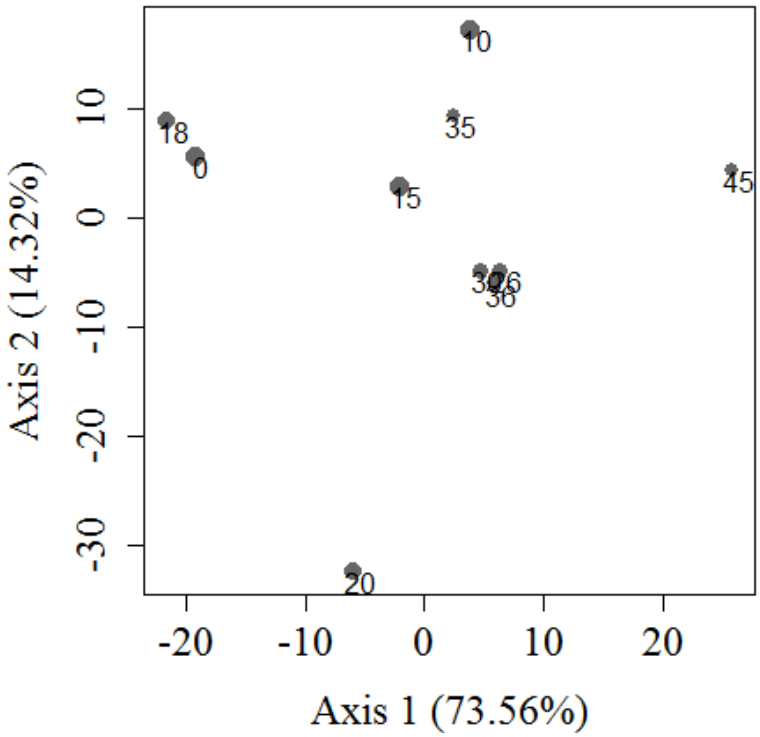

Figure SI 5: Redundancy analysis of samples (grey points) fitted with the operational week (numbers).

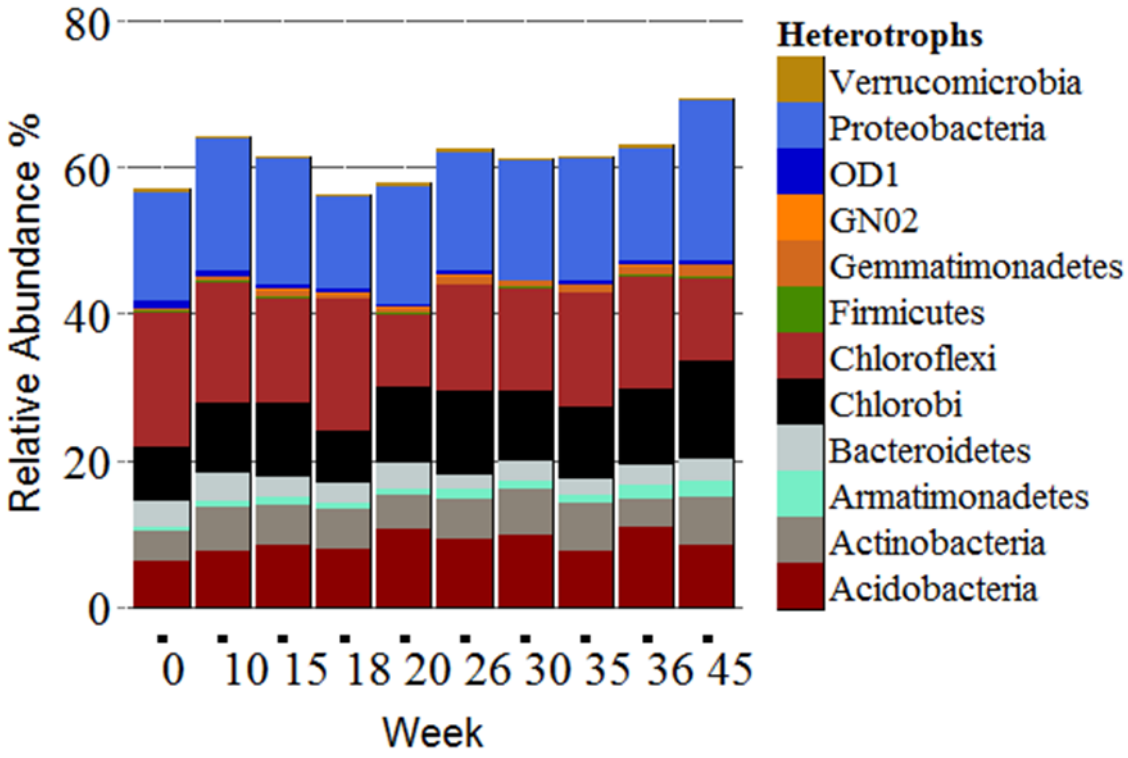

Figure SI 6: Relative read abundance (%) of partial 16S V4 rRNA gene sequences (at cut off, sum =100) of heterotrophic bacteria including denitrifying bacteria.

Table SI 1: Summary of Q_{10} coefficients for ex situ batch activity tests for anammox bacteria (AnAOB), ammonium oxidizing bacteria (AOB), and nitrite oxidizing bacteria (NOB)

	AnAOB			AOB			NOB		
Temperatur	30-20	20-10	30-10	30-20	20-10	30-10	30-20	20-10	30-10
°C									
20.0	2.1	3.7	2.7	2.0	2.3	2.1	1.7	2.2	1.9
17.5			2.6	1.8	3.7	2.6	1.4	1.4	1.4
15.5	2.2	3.2	2.6	1.3	2.7	1.9	2.4	2.2	2.3
14.0	1.9	4.7	3.0	2.3	2.0	2.1	2.2	2.3	2.3
13.0	1.8		3.3	1.6	2.7	2.1	1.8		3.2
12.0	1.9	3.4	2.6	2.2	5.4	3.4	2.4	1.3	1.7
10.0	2.4	3.0	2.6	1.6	2.8	2.1	2.7	2.0	2.0

1 Manometric batch experiments for direct comparison of unequal biomass types.

A test assay to measure conversion rates by means of the stochiometrically related consumption or production of gaseous molecules has been designed for application with suspended flocs, granulated bioaggregates or biofilms attached to carriers. Similar measurements are widely used for measurements of biochemical oxygen demand (BOD) with the Oxitop system (WTW, Germany). It has also been reported for measuring the nitrogen gas production caused by Anammox[3–5]. The assay itself was evaluated for its accuracy and reliability in expressing aerobic nitratation and nitratation. Further, basic parameters, such as vessel size, gas-liquid ratio, and shaking velocity have been optimized in respect to compare different biomass types.
Tested were biofilm carriers type K3 and BiofilmChip M (AnoxKaldnes) and suspended biomass.

1.1 Measurement principle

For aerobic activity measurements, the oxygen uptake rate (OUR) was measured indirectly by tracking the pressure decrease in the headspace. Via the stoichiometry of ammonium oxidation or nitrite oxidation and ideal gas law the specific activity of AOB and NOB was calculated, respectively. As long as oxygen diffusion rate from gas to liquid is higher than the OUR, the pressure decrease is directly proportional to the nitrogen conversion. During anaerobic activity measurements, the anammox process produces di-nitrogen gas, which leads to a pressure increase, which again is directly proportional to the nitrogen conversion and the specific anammox activity can be calculated via stoichiometry and ideal gas law:

$$r_{nitritation} = \frac{\Delta p \cdot V_{headspace}}{R \cdot T} \cdot \frac{1{,}5 \cdot 14}{V_{liquid} \cdot \Delta t \cdot 32} \qquad \text{Equation 1}$$

$$r_{nitratation} = \frac{\Delta p \cdot V_{headspace}}{R \cdot T} \cdot \frac{0{,}5 \cdot 14}{V_{liquid} \cdot \Delta t \cdot 32} \qquad \text{Equation 2}$$

$$r_{Anammox} = \frac{\Delta p \cdot V_{headspace}}{R \cdot T} \cdot \frac{1{,}02 \cdot 14}{V_{liquid} \cdot \Delta t \cdot 28} \qquad \text{Equation 3}$$

Where Δp is the pressure change in Pascal [N m^{-2}], r is the conversion rate in g $m^{-3}s^{-1}$, R is the gas constant of 8.3145 J mol^{-1} K^{-1}, T is the temperature in K, Δt is the time span in s, and V is the volume in m^3 (for liquid or headspace). The stoichiometric coefficients 1.5, 0.5 and 1.02 describe the ratio of consumed oxygen respective produced nitrogen to the nitrogen conversion and 14, 32, and 28 are the molar masses of nitrogen (in the substrate), oxygen and dinitrogen gas in g mol^{-1}.

1.2 Experimental setup

The experimental setup was based on the Oxitop Control system (WTW, Germany), which is an advancement from the BOD measurement devices from the same company. It allows individual measuring periods and readout of the raw pressure values in hPa. The device contains a piezoresistive strain gauge and is usually attached to screw cap bottles.

Conical 300 mL glass ware (Weck, Germany) has been chosen as reaction vessels, due to their wide opening which allows insertion of biofilm carriers. Custom made lids (PE) with two screw threads, one for the measuring device and one for a septum, have been used for gas and pressure tight sealing of the vessels. A quiver filled with a small amount of NaOH in the headspace of the vessel acted ad CO_2-trap to avoid pressure changes due to CO_2 uptake or release during the experiments. This setup was tested up to 2 bar pressure and showed no leakages.

The vessels have been further equipped with sensor spots for oxygen (presens, Germany) for fast and non-invasive testing of the initial DO concentrations. Filled with 200 mL liquid and equipped in the above described way, the vessels had a gaseous volume of 103 ± 1 ml.

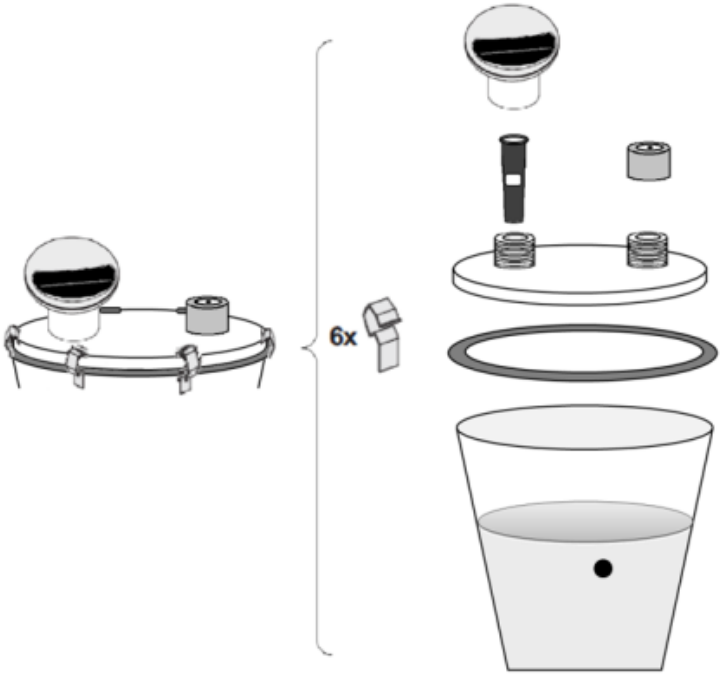

Figure SI 7: Reaction vessel with oxygen sensor spot and lid with screw treads for measuring device and quiver with NaOH as CO_2-trap (left) and septum (right), sealed with an O-ring and minimum six clamps

For evaluation of the measurement accuracy, some experiments were conducted in three-neck bottles (Schott) with 200 ml liquid and 140 ml headspace, which were used for similar anammox assays[4]. The two additional necks were equipped with an ion-selective electrode respective its reference

electrode (ISE, Endress + Hauser) to follow the nitrogen conversion via an additional measurement (sampling would have caused too much pressure disturbances during those manometric tests).
The experiments have been conducted in a shaking water bath with horizontal shaking (GFL, type 1086). A recirculating chiller (Huber Minichiller) was connected for experiments below room temperature.

1.3 Test preparation

Biofilm carriers have been prepared for the experiments as described in the manuscript: They were carefully rinsed with tap water to remove all residual substrate, suspended biomass was filtered with folded filters (pore size 8 µm). Next, the biomass was suspended in exact 200 mL medium (containing 500 mg l^{-1} $KHCO_3$, 27 mg l^{-1} KH_2PO_4, 300 mg l^{-1} $MgSO_4 \cdot 7H_2O$, 180 mg l^{-1} $CaCl_2 \cdot 2H_2O$ and trace elements (25 µg l^{-1} EDTA, 5 µg l^{-1} $FeSO_4$, 0.4 µg l^{-1} $ZnSO_4 \cdot 7H_2O$, 0.2 µg l^{-1} $CoCl \cdot 6H_2O$, 1 µg l^{-1} $MnCl_2 \cdot 4H_2O$, 0.25 µg l^{-1} $CuSO_4 \cdot 5H_2O$, 0.2 µg l^{-1} $NaMoO_4 \cdot 2H_2O$, 0.2 µg l^{-1} $NiCl_2 \cdot 6H_2O$, 0.2 µg l^{-1} $NaSeO_4 \cdot 10H_2O$ and 0.01 µg l^{-1} H_3BO_4). Sodium azide (24 µmol), a selective NOB inhibitor[6], was added only for testing the specific AOB activity as it has been reliably applied to suppress nitrite oxidation during previous batch experiments[7]. The closed vessels were then placed in the water bath for temperature adjustment.
For anammox measurements, the liquid in the vessel was sparged with dinitrogen gas until the DO was below the detection limit (1ppb) and then sealed. For nitritation or nitratation measurements the DO was checked and the liquid, if necessary, aerated till 80 % oxygen saturation. After complete temperature adjustment, the DO and temperature in the vessels was checked and the pressure measurement started.
After 10-40 min endogenous respiration, the substrate was injected through the septum. Either $(NH_4)_2SO_4$ and/or $NaNO_2$ were added as substrate. The substrate injection caused a pressure increase, which was sometimes accepted as clear indicator for the substrate addition. In other cases, the pressure was released directly afterwards by inserting a needle in the septum, connected to a dinitrogen gas filled gas bag in case of anaerobic tests or to ambient atmosphere in case of aerobic tests.

1.4 Accuracy

Test assays for manometric respirometry provided reproducible and accurate results. The maximum pressure change was always consistent with the amount of converted substrate, which was confirmed by IC measurements of initial and final samples. The general accuracy of these batch experiments was proven in other studies for anammox[4,5]. Therefore, the accuracy evaluation was focused on nitritation and nitratation. The indirect indicator for nitrogen conversion, the oxygen depletion was

measured indirectly. Therefore both the oxygen depletion rates as well as the nitrogen conversion rates have been verified by comparison of initial and final samples as well as via online measurements. Figure SI 8 shows a profile of direct and indirect measurement of NH_4^+ depletion with online DO and pH tracking. NH_4^+ depletion was measured directly via tracking the NH_4^+ concentration with an ion-selective electrode and indirectly calculated out of the pressure decrease.

1.5 Optimal test conditions

Optimal test conditions were determined out of different vessel sizes, filling degrees of liquid, number of carriers respective biomass concentration and shaking velocities.

300 ml glass vessels with 200 ml reaction volume were chosen for the activity measurements as this size provided a height to diameter ratio that allowed good mixing of the reaction volume and filling degree left a sufficient headspace. The horizontal shaking created waves that break when touching the lid, which maximized mixing in the liquid and the gas phase. Mixing was insufficient in higher vessels.

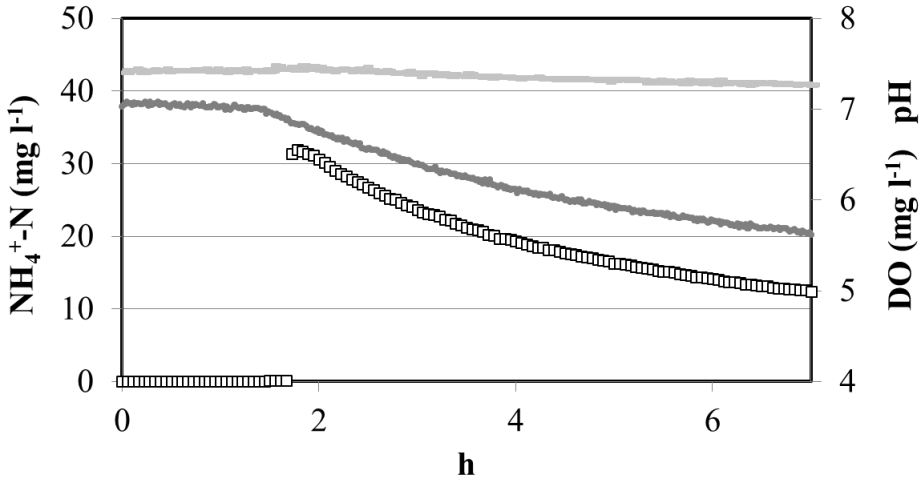

Figure SI 8: Example for profiles during manometric respirometry (recorded in a 340 ml Schott bottle with 3 bottlenecks, allowing online measurements). Shown are pH (light grey line) and DO (dark grey line), recorded with optical sensor spots (presens), NH_4^+-N concentration (filled squares), measured with an Ion-Selective Electrode (ISE, Endress+Hauser) and NH_4^+-N concentration (empty squares), calculated out of the measured pressure difference (Oxitop Control N, WTW) via the ideal gas equation and the stoichiometry of nitritation (at 30°C and 100 ml head space, $\Delta p = 1$ hPa equals $\Delta c = 3.7 \cdot 10^{-3}$ mmol NH_4^+ or $\Delta\beta = 0.05$ mg NH_4^+-N).

The best mixing was observed at 130 rpm: Slower shaking limits the mixing and faster shaking lead occasionally to liquid spilling into the quiver containing the sodium hydroxide.

Experiments with different biomass concentrations (0.2-2 g l^{-1} TSS) showed a direct correlation between conversion rates and biomass concentration. High amounts of biofilm carriers did affect the

mixing, especially in case of BiofilmChip M carriers, a very flat carrier. We observed good mixing with up to 5 carriers (max 3 carriers in case of BiofilmChip M).

Literature

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