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ORIGINAL PAPER

Sulfate-reducing anaerobic ammonium oxidation as a potential treatment method for high nitrogen-content wastewater

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Abstract After sulfate-reducing ammonium oxidation (SRAO) was first assumed in 2001, several works have been published describing this process in laboratory-scale bioreactors or occurring in the nature. In this paper, the SRAO process was performed using reject water as a substrate for microorganisms and a source of NH_4^+, with SO_4^{2-} being added as an electron acceptor. At a moderate temperature of 20°C in a moving bed biofilm reactor (MBBR) sulfate reduction along with ammonium oxidation were established. In an upflow anaerobic sludge blanket reactor (UASBR) the SRAO process took place at 36°C. Average volumetric TN removal rates of 0.03 kg-N/m^3/day in the MBBR and 0.04 kg-N/m^3/day in the UASBR were achieved, with long-term moderate average removal efficiencies, respectively. *Uncultured bacteria clone P4* and *uncultured planctomycete clone Amx-PAn30* were detected from the biofilm of the MBBR, from sludge of the UASBR *uncultured Verrucomicrobiales bacterium clone De2102* and *Uncultured bacterium clone ATB-KS-1929* were found also. The stoichiometrical ratio of NH_4^+ removal was significantly higher than could be expected from the extent of SO_4^{2-} reduction. This phenomenon can primarily be attributed to complex interactions between nitrogen and sulfur compounds and organic matter present in the wastewater. The high NH_4^+ removal ratio can be attributed to sulfur-utilizing denitrification/denitritation providing the evidence that SRAO is occurring independently and is not a result of sulfate reduction and anammox. HCO_3^- concentrations exceeding 1,000 mg/l were found to have an inhibiting effect on the SRAO process. Small amounts of hydrazine were naturally present in the reaction medium, indicating occurrence of the anammox process. Injections of anammox intermediates, hydrazine and hydroxylamine, had a positive effect on SRAO process performance, particularly in the case of the UASBR.

Keywords Sulfate-reducing ammonium oxidation · Moving bed biofilm reactor · Upflow anaerobic sludge blanket reactor · Humic matter · Anammox intermediates

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Introduction

Sulfate-reducing ammonium oxidation (SRAO) process was first assumed by Fdz-Polanco et al. (2001) to explain "abnormal" losses of nitrogen and sulfate observed by the authors while researching the anaerobic treatment of yeast wastewater in a fluidized-bed reactor. Fdz-Polanco et al. (2001) have shown the

feasibility of methanogenesis and SRAO in a single reactor and proposed a summary equation describing the two-staged process:

$$SO_4^{2-} + 2\ NH_4^+ \rightarrow S_0 + N_2 + 4\ H_2O \quad \Delta G_0 = -46\ \text{kJ/mol}. \tag{1}$$

The proposed stages involve:

$$NH_4^+ + SO_4^{2-} \rightarrow S_0 + NO_2^- + 2\ H_2O \quad \Delta G_0 = +314\ \text{kJ/mol} \tag{2}$$

$$NH_4^+ + NO_2^- \rightarrow N_2 + 2\ H_2O\ (\text{anammox - process}) \quad \Delta G_0 = -360\ \text{kJ/mol}. \tag{3}$$

In the later studies conducted by the same authors, the pathway of SRAO, however, could not be reproduced again (Villaverde 2004). Possibility of SRAO with sulfide formation was noted by Strous et al. (2002), Lei et al. (2009) and Schrum et al. (2009)

$$4\ NH_4^+ + 3\ SO_4^{2-} \rightarrow 4\ NO_2^- + 3\ HS^- + 4\ H_2O + 5\ H^+ \quad \Delta G_0 = +338\ \text{kJ/mol}. \tag{4}$$

Coupled with the "conventional" anammox reaction, this pathway of SRAO results in a summary equation:

$$8\ NH_4^+ + 3\ SO_4^{2-} \rightarrow 4\ N_2 + 3\ HS^- + 12\ H_2O + 5\ H^+ \quad \Delta G_0 = -22\ \text{kJ/mol}. \tag{5}$$

The reaction (4) was assumed by Schrum et al. (2009) to occur in the subseafloor sediments both in tropical and temperate climatic zones, although the proceeding of SRAO in this particular pathway was not conclusively proven. The authors concluded that large areas of the ocean floor have conditions favorable for SRAO and it may be a significant sink for fixed nitrogen. Amend et al. (2003) calculated in situ the Gibbs energy of reaction (ΔG) of the lithotrophic SRAO reaction as one of many possible reactions involving sulfate and nitrogen species occurring in volcanic hydrothermal fluids. In addition to reactions (1) and (4), sulfur-reducing ammonium oxidation was computed to have negative ΔG value (normalized per electron transferred) under conditions present in hydrothermal fluids in island Vulcano (Italy):

$$2\ NH_4^+ + 3\ S_0 \rightarrow N_2 + 3\ H_2S + 2\ H^+. \tag{6}$$

In 2006, Zhao et al. reported achieving SRAO process in a laboratory-scale reactor using synthetic wastewater with a high glucose content at volumetric loading rates of NH_4^+-N, SO_4^{2-}-S and COD of 2.08 g-N/m^3/day, 2.38 g-S/m^3/day, and 104.17 gCOD/m^3/day, respectively, with a N/S ratio of 1:1.14 (Zhao et al. 2006). Liu et al. (2008) isolated and described a new autotrophic *Planctomycete* bacterium capable of oxidizing NH_4^+ into NO_2^- using SO_4^{2-} as an electron acceptor. Thus, the newly-discovered bacterium, named *Anammoxoglobus sulfate,* performs the first stage of SRAO, a reaction described by Eq. 2 as initially assumed by Fdz-Polanco et al. (2001). The discovery made by Liu et al. (2008) shows that anammox microorganisms have a more versatile metabolisms than previously assumed and that at least some of them may use electron acceptors alternative to NO_2^-. Zub et al. (2008) managed to maintain efficient sulfate removal in an industrial scale bioreactor treating baker's yeast wastewater, with sulfide generation being considerably less than could be assumed from sulfate reduction. The decrease in sulfide concentration was assumed to proceed at the expense of decay of trimethylglycine—NH_4^+ released from trimethylglycine reduced SO_4^{2-} with formation of elemental sulfur (Zub et al. 2008; Koplimaa et al. 2010). Lei et al. (2009) refer to a possible involvement of sulfate-reducing bacteria (SRB) in the nitrite-generating stage of SRAO. Ammonium is similar to methane in the molecular structure, thus in this stage sulfate reduction might be compared to sulfate-utilizing methane oxidation. The same idea was supported by Yang et al. (2009). The anaerobic oxidation of methane proceeds in two stages with half-reactions being as follows:

$$CH_4 + 3\ H_2O \rightarrow HCO_3^- + 9\ H^+ + 8\ e^- \tag{7}$$

$$SO_4^{2-} + 9\ H^+ + 8\ e^- \rightarrow HS^- + 4\ H_2O. \tag{8}$$

The possible half-reactions for SRAO, as suggested by Yang et al. (2009), would be as follows:

$$4\ NH_4^+ + 8\ H_2O \rightarrow 4\ NO_2^- + 32\ H^+ + 24\ e^- \tag{9}$$

$$3\ SO_4^{2-} + 24\ H^+ + 24\ e^- \rightarrow 3\ S^{2-} + 12\ H_2O. \tag{10}$$

A non-*planctomycete*, the *Bacillus benzoevorans* strain ASR, performing the overall two-staged SRAO reaction as given by Eq. 1, was discovered by Jing et al. (2010). SRAO is one of many possible metabolic pathways for this particular bacterium, with other pathways including utilization of various organic compounds. Hence, the SRAO process may be a more common metabolic pathway in nature and not restricted

to a few genera of bacteria. The reaction between NH_4^+ and SO_4^{2-} is entirely biological by its nature and no abiotic chemical reactions take place between these ions at 30°C and under normal pressure (Lei et al. 2009; Yang et al. 2009). Higher substrate concentrations and a low oxidation–reduction potential (ORP) were shown to be favourable for this biological reaction. Ammonium can also be oxidized into nitrate (Schrum et al. 2009), coupled with a subsequent heterotrophic denitrification utilizing organics (indicated in Eq. 12 as "CH_2O") as an electron donor:

$$NH_4^+ + SO_4^{2-} \rightarrow NO_3^- + HS^- + H_2O + H^+ \quad (11)$$

$$5''CH_2O'' + 4\ NO_3^- + 4H^+ \rightarrow 5CO_2 + 2N_2 + 7H_2O \quad (12)$$

$$4\ NH_4^+ + 4\ SO_4^{2-} + 5''CH_2O'' \rightarrow 5\ CO_2 + 2\ N_2 + 11\ H_2O + 4\ HS^-. \quad (13)$$

These discoveries show a potential for technological innovations in the biological nitrogen removal from wastewaters, particularly in the case of waste streams containing both sulfate and nitrogenous compounds at high concentrations. Autotrophic anaerobic nitrogen removal can be possible at the expense of sulfate for some wastewaters with high content nitrogen and low content of easily biodegradable organic matter, allowing thus to save treatment costs by reducing aeration needed for achieving nitritation. For wastewaters with high content of TKN, sulfate and organics, simultaneous removal of COD and nitrogen can be achieved in the anaerobic phase of treatment while avoiding accumulation of toxic sulfide, thereby preventing process disruptions caused by sulfide inhibition and reducing nitrogen load in subsequent stages including nitrogen removal.

The main objective of the current study was to assess the feasibility of SRAO in the case of real wastewater, characteristics of which may fluctuate greatly over time (reject water was chosen as an example). Evaluation of the inhibiting effect caused by nitrite (as energetically the most suitable electron acceptor for the anammox reaction) and a high concentration of bicarbonate on the flow-through SRAO culture were also among issues of high priority. Since the addition of anammox intermediates (hydrazine and hydroxylamine) has been reported as an option to overcome inhibition caused by nitrite on "conventional" anammox microorganisms, the effects of intermediates were also studied.

Materials and methods

Reactor configurations, seeding procedure

In current research a moving bed biofilm reactor (MBBR) system and a upflow anaerobic sludge blanket reactor (UASBR) system were used for the enrichment of anammox microorganisms. The 3.3 l effective volume MBBR working at 20°C (±0.5°C) and the 0.75 l UASBR working at 36°C (±0.5°C) were operated in parallel (Fig. 1). Temperature was kept by means of thermostated water jackets applying Assistant 3180 (Germany) type water bath thermostats. Both reactors were fed by time-controlled (time clock relay Talento 894, Grässlin, Germany), periodically switched-on peristaltic pumps (Seko P4, Italy). The hydraulic retention time was kept 1 day in both reactors until day 239 (in case of UASBR, day 225). MBBR was mechanically stirred at 100–200 rpm (rotations per minute). For the UASBR, a continuously working peristaltic pump was used in order to ensure circulation of the liquid phase.

Influent

Most studies of SRAO have been relied upon synthetic wastewaters or growth media (Zhao et al. 2006; Liu et al. 2008; Lei et al. 2009; Yang et al. 2009; Jing et al. 2010). In order to compare the effect of NO_2^-, the "classical" electron acceptor for the anammox reaction, with an "alternative" one, SO_4^{2-}, we used digested wastewater sludge supernatant (reject water) as a feeding medium and a source of NH_4^+ for bacteria. Reject water contained anammox microorganisms and sufficient amounts of microelements and other compounds needed for anammox process propagation (Zekker et al. 2011). Dilution of supernatant was prepared by mixing reject water with tap water once a week and stored at the room temperature. SO_4^{2-} was added as the solution of K_2SO_4 and mixed with the influent. In operation days 30–241 of the MBBR (days 16–227 of the UASBR), solution of $NaHCO_3$ was added to the feed in order to provide a better buffering capacity and ensure a sufficient amount of inorganic carbon for the autotrophic microorganisms. As excessive HCO_3^- concentration can possibly lead to a decrease in the anammox activity (Dexiang et al. 2008), the addition of HCO_3^- was ceased since day 241 (in case of UASBR, day 227) and 4 M HCl was

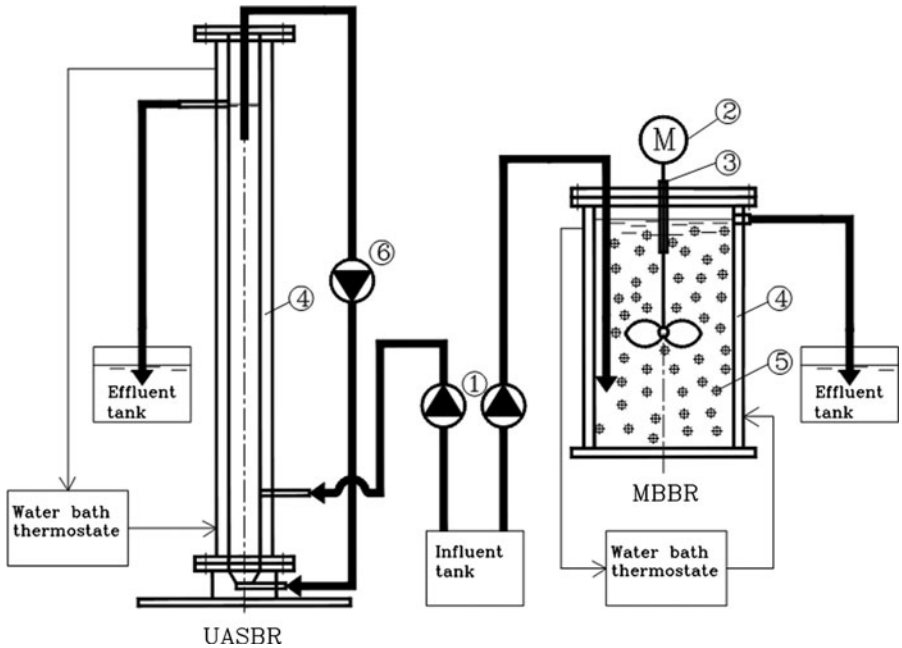

Fig. 1 Configuration of UASBR and MBBR systems used for research of the SRAO process 1: Influent pumps; 2: Stirrer; 3: Water seal; 4: Thermostated water jacket; 5: Biocarriers in the MBBR; 6: UASBR recirculation pump

used to reduce the influent HCO_3^- content since day 295 (in case of UASBR, day 281). The influent had the following ratios: COD: TN = 0.78:1 (range 0.39–1.10); COD: BOD_7 = 1.95:1 (range 1.82–2.03).

Seeding materials and inoculation procedure

The MBBR was inoculated by using 1000 ring-shaped Bioflow-9-type carriers (total specific surface 800 m^2/m^3, protected interior specific surface 500 m^2/m^3) with a well-established attached anammox biofilm. The carriers were taken from a laboratory-scale "conventional" anammox reactor treating a diluted supernatant of anaerobically digested municipal wastewater sludge combined with $NaNO_2$ to enable the anammox reaction to occur. The total surface of carriers placed into the MBBR was 0.8 m^2, with a specific anammox activity of 0.73 $gN/m^2/d$. The MBBR from which the carriers were taken from showed high simultaneous nitrite and ammonium removal rates (approximately 5 $kg\text{-}N/m^3/d$) and TN removal efficiencies around 85%.

The UASBR was seeded with anaerobic sludge obtained from the facility treating wastewater of the Salutaguse yeast factory. The TN removal rates in the UASB reactor of the Salutaguse yeast factory treatment facility where the sludge was taken from were 4.8 $kg\text{-}N/m^3/d$.

The microorganisms detected in the seeding biocarriers for the MBBR included: *uncultured Planctomycetales bacterium clone P4*, *uncultured anaerobic ammonium-oxidizing bacterium clone W1, Candidatus Nitrospira defluvii, uncultured Nitrospira sp. clone 53, Uncultured Nitrospira sp. clone S1-62, uncultured Bacteroidetes bacterium clone VC5 (genus Ferruginibacter), uncultured bacterium clone:13C-M6 (family "Saprospiraceae"), uncultured Chloroflexi bacterium clone QEDQ2AB09.*

Uncultured bacterium clone ATB-KS-1929 (order *Verrucomicrobiales*) was found in the seeding sludge for the UASBR. This seeding sludge originally contained also anammox organisms (unclassified *Planctomycetaceae* uncultured bacterium) in addition to unclassified *Porphyromonadaceae*, unclassified *Cryomorphaceae* uncultured bacterium, *Carnobacterium maltaromaticum*, *Carnobacterium gallinarum*, uncultured *Verrucomicrobia* bacterium), found in the treatment facility of Salutaguse yeast factory. Most of anammox microorganisms [*Brocadiales*—affiliated to the *Planctomycetes* (Van der Star et al. 2008; Jetten et al. 2009)] are strict anaerobic autotrophs and have extremely slow growth rate (doubling time approximately 11 days) (Strous et al. 1999). Differently from other similar biological wastewater treatment schemes applied for treatment of yeast wastewater, residual sludge from the anoxic stage was returned to the

beginning of the purification scheme (mixing tank). Thus retaining of anammox microorganisms in the system was facilitated with recirculation of sludge.The returned anoxic sludge retained also methanogenic activity (Zub et al. 2008). In later experiments also the presence of denitrifying sulfur-oxidizing *Sulfurimonas denitrificans* DSM 1251 and Sulfide-oxidizing bacterium N9-1 in this seeding sludge were detected.

Analytical methods

Samples of the influent and effluents from both the MBBR and the UASBR were collected at least once a week for prompt chemical analyses and physico-chemical measurements. The analyses of NH_4^+–N, NO_2^-–N, NO_3^-–N, HCO_3^-, SO_4^{2-}–S, total sulfide, and COD concentrations were performed using the standard methods according to APHA (American Public Health Association) (1985). Hydrazine was determined by spectrophotometrically using a Hach Lange DR2800 type spectrophotometer. Prior to the application of the Hach Lange HydraVer 2 reagent (containing *p*-dimethylaminobenzaldehyde), 0.5% solution of sulfamic acid was added to the sample as described by George et al. (2008) in order to eliminate interference from NO_x^- ($NO_2^- + NO_3^-$). A spectrophotometric method was applied for measurement of hydroxylamine as well, at the wavelenght of 705 nm as reported by Frear and Burrell (1955). Humic and fulvic substances were analyzed by liquid chromatography according to the method described by Ibrahim et al. (2008). Volatile fatty acids were measured by GC using a Shimadzu GC-2014 gas chromatograph equipped with a Phenomenex Zebron ZB-WAXplus GUARDIAN column. The DO concentration was measured by portable oxygen meter (Marvet Junior MJ2000, Estonia) and pH by a portable pH meter (Evikon, Estonia, or SensIon, Hach Lange). The oxidation–reduction potential (ORP) was measured by a Eutech redox electrode connected with a Jenway pH meter in a mV mode.

Microstructural studies

Micrographs were taken by the scanning electron microscope (SEM) Zeiss D940 equipped with IdFix software and the SAMx 10 mm^2 SDD detector (an energy dispersive X-ray detector, based on the Silicon Drift technology). The biofilm for the SEM was prepared as described by Qiao et al. (2009). Finally, the samples were coated with gold for taking micrographs.

DNA extraction, nested PCR and denaturing gradient gel electrophoresis (DGGE)

Biomass DNA extraction was performed with the MoBio PowerSoil DNA extraction kit (MoBio Laboratories Inc.) according to the manufacturer's instructions. Fingerprinting of *Planctomycetes* and *Nitrospira* communities was conducted via PCR and DGGE. The first PCR round for amplification of *Planctomycetes* was performed with a wide-range primer set, Eub27f/Eub1492r (Lane 1991), and the second PCR round was performed with a *Planctomycetes*-specific primer Pla46f (Neef et al. 1998) with a GC-clamp (GC CGC CGC GCG GCG GGC GGG GCG GGG GC) coupled with an anammox-specific primer Amx368r (Sąnchez-Melsió et al. 2009). Nested PCR was carried out according to thermocycling parameters described by Sąnchez-Melsió et al. (2009). PCR was also performed to identify *Nitrospira* strains with the primer set NSR1113f/NSR1264r, which are specific for *Nitrospira* 16S rDNA, using a PCR program described by Dionisi et al. (2002). PCR products were purified with the JETquick Spin Column Kit (GENOMED GmbH) and then sequenced.

DGGE for detecting diversity of most abundant microorganisms was conducted using the eubacterial primer set GC-BacV3f/907r as described previously (Muyzer et al. 1993, 1996; Koskinen et al. 2006). The gene sequences were amplified in a Mastercycler Personal thermocycler (Eppendorf, Germany). The PCR reaction products were opposed to agarose gel electrophosesis with a 1% agarose (SeaKem® GTG® Agarose, FMC Bioproducts, Rockland, ME, USA) gel, which was stained with ethidium bromide and visualized under UV transillumination. DGGE was performed by using the INGENY PhorU System (INGENY, The Netherlands). PCR products were loaded on a 30–65% denaturing gel and run for 17 h at 90 V at a constant temperature of 60°C. The gels were stained with an ethidium bromide solution in 1× Tris-acetate-acetic acid-ethylenediaminetetraacetic acid (EDTA) (TAE) buffer to observe the bands by UV transillumination and subsequently the bands were excised for future reamplification and sequencing.

Sequencing and phylogenetic analysis

PCR for sequencing was performed with the BigDye® Terminator v3.1 Cycle Sequencing Kit (Life Technologies Corporation, USA). The sequences acquired were compared to the available database sequences via a BLAST (Basic Local Alignment Search Tool) search and the related sequences were obtained from the GenBank. In order to determine the phylogenetic position of the anammox 16S rRNA gene sequence acquired, it was compared with available database sequences via a BLAST search, obtaining the related sequences from the GenBank. Further analysis was carried out with MEGA software version 5.0 applying the neighbour-joining method.

Results and discussion

Start-up

Operation of MBBR system was organized similarly to Yang et al. (2009) starting up with influent containing NH_4^+ and NO_2^- (TN removal efficiency approximately 85%) with a subsequent switching to the influent containing NH_4^+ and SO_4^{2-}. A paradoxically high TN removal efficiency was recorded (93% decrease in NH_4^+–N, 88% in TN) one day after the MBBR was switched to influent containing SO_4^{2-} instead of NO_2^-, along with simultaneous decrease in SO_4^{2-} concentration (40%). The TN removal efficiency, however, rapidly decreased by day 50 (average TN removal efficiency between days 4–50 was 19%). The average TN loading rate in this period was 0.07 kg-N/m^3/day with corresponding TN removal rate 0.02 kg-N/m^3/day. The average NH_4^+–N concentrations in the start-up period were 69 mgN/l for the influent and 47 mgN/l for the effluent of the MBBR, respectively. In a similar manner to the study reported by Yang et al. (2009), a change of the electron acceptor led to a decrease in the efficiency of the anammox process.

The UASBR was launched 14 days after the MBBR had been started to feed with influent containing SO_4^{2-}. The same volumetric loading rates and hydraulic retention times (HRT) were kept for both reactors. HRT was kept 1 day until day 253 (day 239 in case of the UASBR), thereafter it was set to 2 days in order to facilitate a better process performance. Differently from the MBBR, the seeding sludge used to inoculate of the UASBR was not previously enriched with anammox organisms using a nitrite-containing feeding medium. However, in terms of TN removal, the UASBR showed quite similar results as the MBBR. Most probably, the mechanisms for N removal in these reactors were still different as revealed from the list of microorganisms identified by PCR-DGGE results. During the initial 36 days the average TN removal efficiency was 17%. The average TN loading rate in the UASBR for this period was 0.07 kg-N/m^3/day (calculated per surface of carrier material–0.30 g-N/m^2/day) with corresponding TN removal rate 0.01 kg-N/m^3/day (0.07 g-N/m^2/day). The average NH_4^+–N concentration in the start-up period for the effluent of the UASBR was 56 mgN/l (Fig. 2).

TN removal rates and efficiencies of the MBBR and the UASBR

Taking into consideration that higher concentrations of substrates (NH_4^+ and SO_4^{2-}) were thermodynamically favourable for the SRAO reaction as reported by Lei et al. (2009) and that higher consumption of NH_4^+ was experimentally observed than it could be predicted by the extent of SO_4^{2-} reduction based on the Eq. 1, the influent NH_4^+ content was approximately doubled while SO_4^{2-} content remained unchanged (Figs. 3, 4) in days 55–99 for the MBBR and 41–85 for the UASBR. For both reactors, increased influent NH_4^+ had no significant effect on the TN removal. During the mentioned period, the average TN loading rate was 0.15 kg-N/m^3/day (0.64 g-N/m^2/day), TN removal efficiency 18% and TN removal rate 0.03 kg-N/m^3/day (0.11 g-N/m^2/day). For the UASBR, the respective numbers were 0.15 kg-N/m^3/day, 11%, and 0.02 kg-N/m^3/day. The average NH_4^+–N concentrations for the influent, and effluents of MBBR and UASBR were 147 mgN/l, 120 mgN/l, and 135 mgN/l, respectively.

A decrease in loading rates occurred after day 100 (day 86 in the case of the UASBR) as the NH_4^+ concentration in reject water had decreased. Fluctuations in the loading rates happened due to variation of the supernatant composition over time. Loading rates were gradually increased until day 253 for the MBBR and day 239 for the UASBR. The output parameters of both reactors were, however, unstable during this

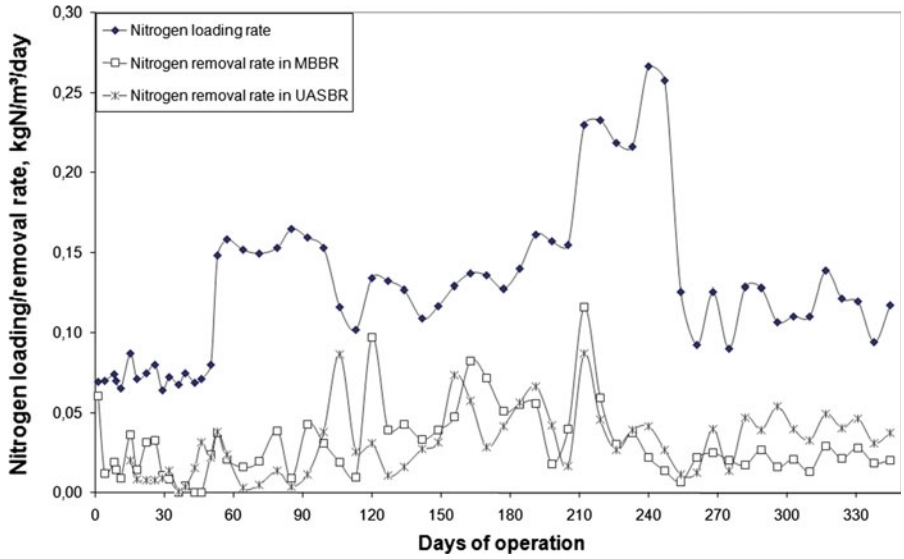

Fig. 2 Nitrogen loading rate (*diamonds*) and nitrogen removal rates for MBBR (*quadrates*) and UASBR (*stars*)

period and generally speaking, the nitrogen removal remained low. TN loading rate was increased from 0.10 kg-N/m^3/day (0.42 g-N/m^2/day) on day 113 for the MBBR (day 99 for the UASBR) to 0.27 kg-N/m^3/day (1.10 g-N/m^2/day) on day 240 for the MBBR (day 226 for the UASBR). The average TN loading rate for the entire period (days 100–253 for the MBBR and 86–239 for the UASBR) was 0.16 kg-N/m^3/day (0.67 g-N/m^2/day) with an average NH_4^+–N concentration in the influent being 152 mgN/l. TN removal efficiency fluctuated within a range of 5–72% (average 31%) for the MBBR and 10–75% (average 28%) for the UASBR, respectively. The corresponding average TN removal rates were 0.05 kg-N/m^3/day (0.19 g-N/m^2/day) for the MBBR and 0.04 kg-N/m^3/day for the UASBR, respectively. For the the MBBR, the effluent NH_4^+–N concentration averaged 109 mgN/l. For the UASBR, the same parameter was slightly higher, at a value of 118 mgN/l.

In order to improve the TN removal, HRT was doubled on day 254 (day 240 for the UASBR). The average loading rate from day 254 (day 240 for the UASBR) onwards was 0.11 kg-N/m^3/day (0.47 g-N/m^2/day) at an average influent NH_4^+–N concentration

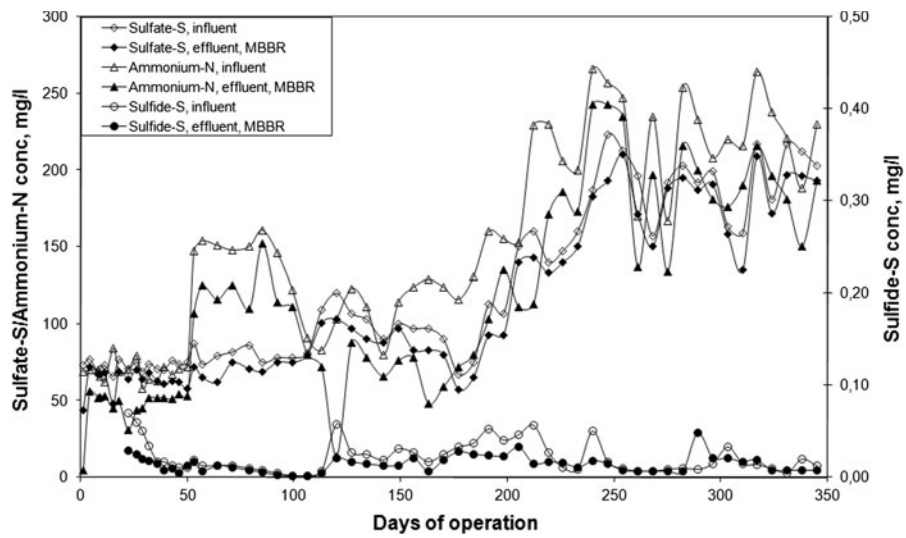

Fig. 3 Dynamics of NH_4^+-N and SO_4^{2-}-S in the MBBR. Influent NH_4^+-N (*empty triangles*); effluent NH_4^+-N (*filled triangles*); influent SO_4^{2-}-S (*empty diamonds*); effluent SO_4^{2-}-S (*filled diamonds*). Influent sulfide-S (*empty circles*); Effluent sulfide-S (*filled circles*)

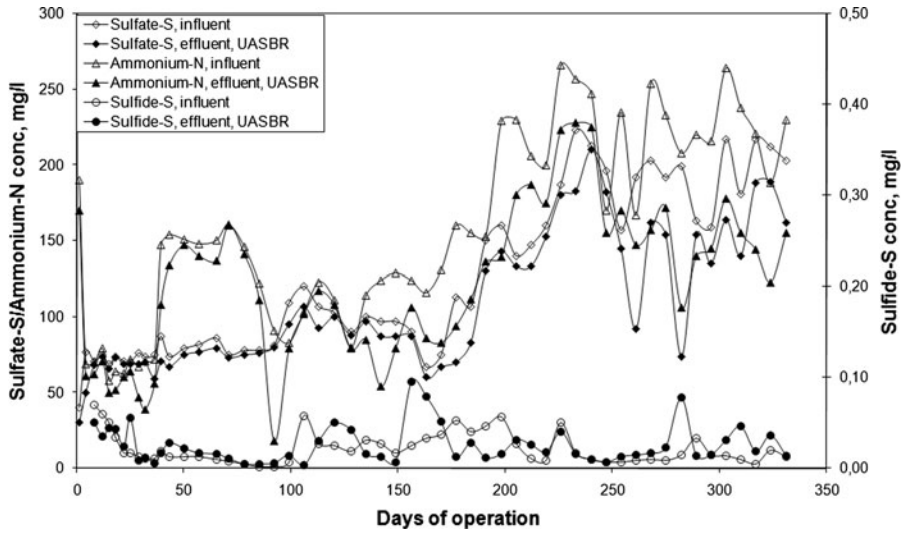

Fig. 4 Dynamics of NH_4^+-N and SO_4^{2-}-S in the UASBR. Influent NH_4^+-N (*empty triangles*); effluent NH_4^+-N (*filled triangles*); influent SO_4^{2-}-S (*empty diamonds*); effluent SO_4^{2-}-S (*filled diamonds*). Influent sulfide-S (*empty circles*); Effluent sulfide-S (*filled circles*)

of 221 mgN/l. These results were also affected by changes in the influent HCO_3^- and injections of the anammox intermediates into the reactors' media (discussed below). A combination of described measures led to a narrower range of fluctuations in the TN removal efficiencies and rates. In the MBBR, the average TN removal efficiency at an average NH_4^+–N concentration of 109 mgN/l (days 261–345) was 19%, within a range of 12–24%. Average TN removal rates in the MBBR for the same time interval were 0.02 kg-N/m^3/day and 0.09 g-N/m^2/day. For the UASBR, the average TN removal efficiency at an average NH_4^+–N concentration of 155 mgN/l (days 247–331) was 32%, within a range of 14–50%. Corresponding average TN removal rate in the UASBR was 0.04 kg-N/m^3/day.

During the entire experimental period, the average TN loading rate was 0.13 kg-N/m^3/day (0.52 g-N/m^2/day in the MBBR). The average TN removal efficiencies for the entire experimental study were 24% for the MBBR and 23% for the UASBR, respectively. The corresponding TN removal rates were 0.03 kg-N/m^3/day (0.13 g-N/m^2/day) for the MBBR and 0.03 kg-N/m^3/day for the UASBR. These results are significantly lower than those achieved in MBBRs fed with reject water using NO_2^- as the terminal electron acceptor for anammox reaction. In the latter reactors the average total nitrogen (TN) removal efficiency was 80–85% and the TN removal rate 0.50 kg-N/m^3/day (1.25 g-N/m^2/day) (Zekker et al. submitted).

The overall process stability of SRAO reactors, as manifested in the fluctuations of the effluent quality, was lower as well. For comparison, other researches of the SRAO process using synthetic wastewaters have achieved 40–45% NH_4^+ removal efficiencies (Zhao et al. 2006; Yang et al. 2009; Jing et al. 2010) while Fdz-Polanco et al. (2001) has reported 30–55% TKN removal studying treatment of vinasse-based wastewater.

The optimal N removal rate is dependent on the balance of SRAO and S-dependent denitrification (using S_0 and HS^-). In the case of SRAO, low ORP is favored. However, the ORP measured by us was high ($\sim +100$ mV). The presence of HS^- in non-inhibiting concentration or organic substance is needed that will guarantee the low ORP. If organics is present, the concentrations of nitrite and nitrate should be sufficiently low to avoid the prevalence of denitrifiers (substrate limitation). On the other hand, if their concentrations are high, the organics, Fe^{2+}, SO_4^{2-} concentrations should be sufficiently low, to obtain the suppression of denitrifiers (limitation of electron acceptors).

Effect of HCO_3^- on TN removal efficiency

High concentration of inorganic carbon may have an inhibiting effect on the anammox process. Dexiang et al. (2008) reported 1,500 mg/l HCO_3^- to be optimal

for anammox microorganisms, while at both lower and higher HCO_3^- concentrations the process performance deteriorated. Our studies of "conventional" anammox have indicated that an optimum concentration of HCO_3^- is likely less than 1000 mg/l (unpublished data). HCO_3^- concentrations of exceeding 1,000 mg/l showed no improving effect on the perfomance of the SRAO process. On the contrary, TN removal deteriorated both in the MBBR and UASBR after the concentration of HCO_3^- was increased; later, however, adaption of the anammox biomass to the elevated inorganic carbon concentration developed (Figs. 5, 6). Decreased HCO_3^- concentration in the influent resulting a decrease in the reactors' media following day 254 (in case of UASBR, day 240) is likely one of contributing factors to the more stable and effective performance of the SRAO process (Figs. 3, 4).

Factors affecting SO_4^{2-} reduction

SO_4^{2-} concentration was kept stable (around 75 mgS/l) until day 113 (day 99 for the UASBR), when it was increased alongside with NH_4^+ concentration until day 240 for the MBBR (day 226 for the UASBR). Onwards from these dates it was kept stable again, although fluctuations took place mainly due to processes occurring in the influent tank (Figs. 3, 4). More NH_4^+ was consumed throughout the experimental period than it can be concluded from the Eq. 1 referring to use of other electron acceptors than SO_4^{2-} coupled with NH_4^+ oxidation or reoxidation of reduced sulphur compounds into SO_4^{2-} (discussed below). Large fluctuations of SO_4^{2-} reduction were characteristic to the performance of both reactors (Figs. 3, 4, 7).

The SRAO process was heavily suppressed by increased NO_x^- concentrations. Peaks of NO_x^- in the influent (10 mg/l or more) resulted in major disturbances of the SRAO process manifesting itself a significant decrease of sulfate reduction (Fig. 7). Overall bioprocess stability was thereby greatly reduced as influent NO_2^- concentration was too low to enable sufficient nitrogen removal through the "conventional" anammox process while sulfate-reducing nitritation as given by Eq. 2 was inhibited. Higher NO_x^- concentration also promotes SO_4^{2-} resynthesis via sulfur-utilizing denitrification. Inhibition caused by influent high NO_x^- was, however, reversible, as indicated the partial recovery of SRAO in days 149–219 (days 135–205 for the UASBR). Onwards from day 219 (day 205 for the UASBR), a combined effect of high influent NO_2^- and HCO_3^- led to decreased process stability and efficiency again.

Low oxidation- reduction potential (average ORP value of −430 mV) is beneficial for SRAO (Fdz-Polanco et al. 2001). Calculated values of redox potential for the half reactions of reduction of N_2 to NH_4^+ $\left(\frac{1}{2}\,N_2 + 4\,H^+ + 3\,e^- \leftrightarrow NH_4^+\right)$ and SO_4^{2-} to S_0 $\left(SO_4^{2-} + 8\,H^+ + 6\,e^- \leftrightarrow S_0 + 4\,H_2O\right)$ at pH = 8 range between −330 and −360 mV. These values are close to the ORP typical of methanogenesis, indicating that the three processes, methanogenesis,

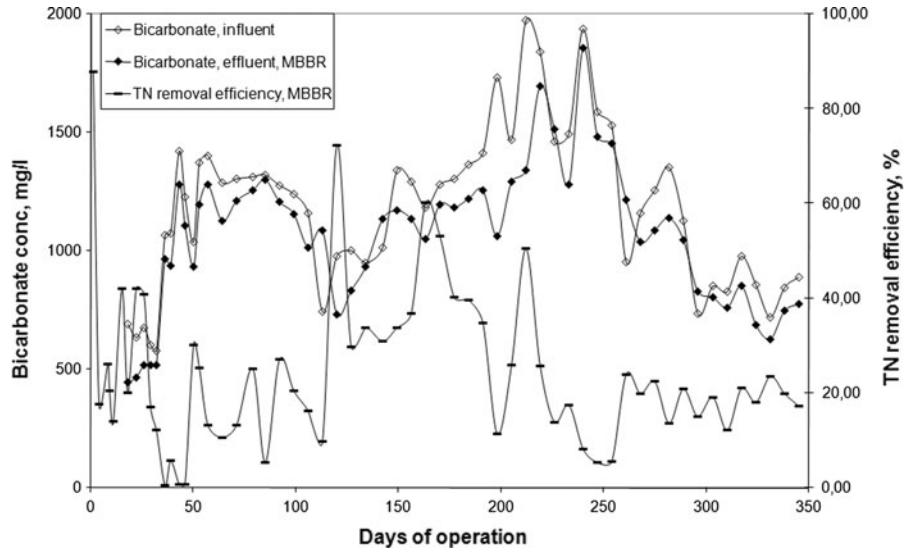

Fig. 5 Effect of influent and effluent inorganic carbon on TN removal efficiency in MBBR. Influent bicarbonate concentration (*black diamonds*); effluent bicarbonate concentration (*white diamonds*); TN removal efficiency (*stars*)

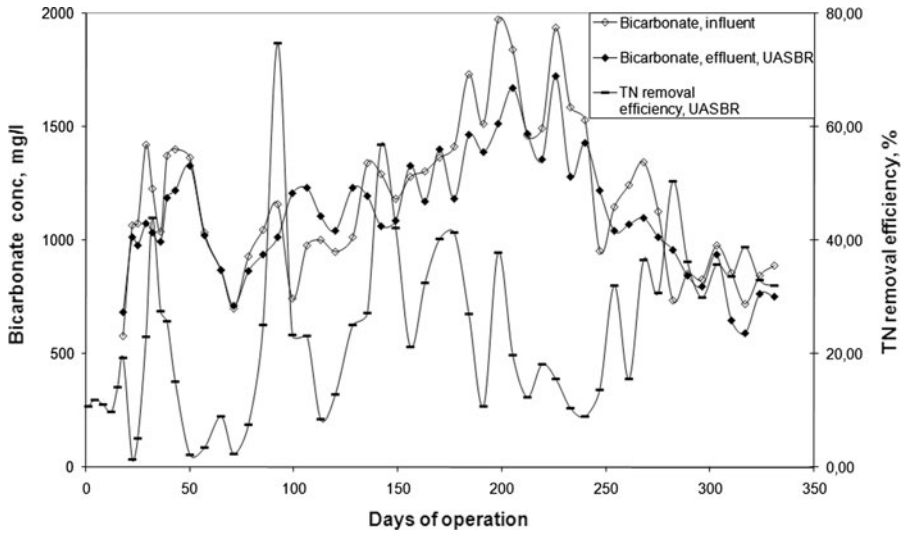

Fig. 6 Effect of influent and effluent inorganic carbon on TN removal efficiency in UASBR. Influent bicarbonate concentration (*black diamonds*); effluent bicarbonate concentration (*white diamonds*); TN removal efficiency (*stars*)

SO_4^{2-} reduction and NH_4^+ oxidation can theoretically coexist together in an anaerobic environment (Fdz-Polanco et al. 2001). The "conventional" anammox, however, is not sustainable in a medium where a high concentration of easily biodegradable organic matter is present (Chamchoi et al. 2008) as denitrifying bacteria outcompete the anammox organisms. Anammox process has still been successfully applied for treatment of landfill leachate (Liang et al. 2009) containing organic matter of more refractory nature.

High ORP values, generally more than +100 mV, are unfavourable for SRB. However, there are reports on other heterotrophic bacterial strains, except for SRB, reducing SO_4^{2-} as the terminal electron acceptor while utilizing organic substrates in a similar manner as SRB under anaerobic conditions (Liang et al. 2009). Four facultative anaerobic species, *Bacillus* sp., *Paenibacillus* sp., *Bacteroides* sp. and *Staphylococcus* sp. were assumed to possess the ability for SO_4^{2-} reduction, although no "hard" evidence was found to support this assumption. Oxidation of NH_4^+ coupled with SO_4^{2-} reduction mediated by *Bacillus* strains as proposed by Jing et al. (2010) may still be one of the possible pathways in addition to sulfate-reducing anammox (both stages as given by Eqs. 2, 3) carried out by *Planctomycetes* bacteria. However, in our experiments a minimal decrease in COD values (averagely 16% in the MBBR and 8% in the UASBR) in the effluent as compared with the influent emphasized minor SRB activity in both of the reactors. There are no reports on the possible ability of SRB to oxidize NH_4^+ anaerobically. Sulfate reduction with ammonium would normally need an electron donor, e.g. organic acid. Ammonium can thus be oxidized into nitrate (Schrum et al. 2009), coupled with a subsequent heterotrophic denitrification utilizing organics (indicated as "CH_2O") as an electron donor (Eq. 13). Denitrification in both MBBR and UASBR occurred via sulfur oxidation, as organics present in reject water was mainly of recalcitrant nature. Low-molecular organic acids such as acetic and propionic acids were not found in reject water, the concentrations of sulfide were very low (<100 μg/l).

Values of pH ranged within 7.58–8.75 in the MBBR and within 7.63–8.54 in the UASBR, with a small decrease taking place during the treatment process (as an average, 0.37 units for the MBBR and 0.29 units for the UASBR). Liu et al. (2008), however, reported an increase in pH in the course of autotrophic SRAO.

Factors affecting the stoichiometry of SRAO

The stoichiometric ratio of moles NH_4^+ consumed per moles SO_4^{2-} reduced was higher in both reactors than there could be expected from the extent of SO_4^{2-} reduction according to the Eq. 1. In most of the other studies concerning SRAO (Fdz-Polanco et al. 2001; Liu et al. 2008; Yang et al. 2009; Jing et al. 2010), the same stoichiometric ratio is in accordance with Eq. 1 or only slightly different, with the only notable exception being the experiments reported by Sabumon (2007, 2008, 2009). The latter showed disproportionally higher NH_4^+ removal, similarly to our results. In addition to SO_4^{2-}, other possible electron acceptors

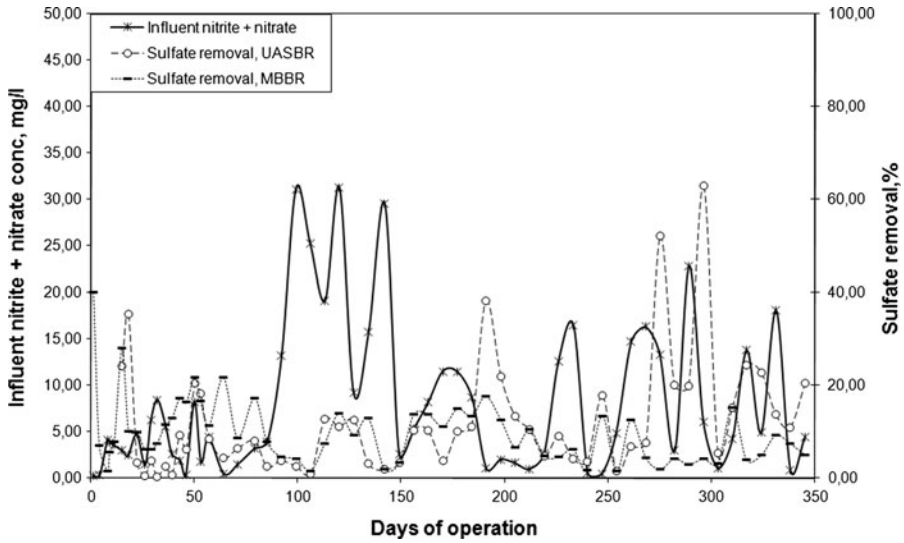

Fig. 7 Effect of influent nitrate + nitrite concentration on TN removal efficiency in MBBR and UASBR. Influent nitrate + nitrite concentration (*stars*); MBBR sulfate removal efficiency (*white circles*); UASBR sulfate removal efficiency (*filled bars*)

can be used in the biological oxidation of NH_4^+. Energetically the most favourable electron acceptor is NO_2^- (Eq. 3). Also dissolved O_2 (that ranged 0–0.2 mg/l) and NO_3^- present in the influent could be consumed (Sabumon 2007) in the process:

$$5\ NH_4^+ + 3\ NO_3^- \rightarrow 4\ N_2 + 9\ H_2O + 2H^+; \quad \Delta G_0 = -297\ \text{kJ/mol}. \tag{14}$$

Possible oxygen generation by photosynthetic oxygenic microorganisms can be neglected, as the room where the reactors were located lacked access to the sunlight and lights were only switched on for maintenance and sampling. The influence of Fe^{3+}, mentioned in the literature as one of the possible electron acceptors (Clément et al. 2005; Sawayama 2006; Strous et al. 2006) for anaerobic NH_4^+ oxidation (so-called Feammox process) was insignificant as total Fe was present in the influent at a concentration below 1 mg/l (confirmed by chemical analyses). Mn^{4+} is another theoretical electron acceptor for anammox (Strous et al. 2006; Javanaud et al. 2011) bacteria. Total Mn was estimated by calculations to be present in the influent at a negligible concentration and can therefore also be neglected. Even making an oversimplification, assuming that all dissolved O_2, NO_2^- and NO_3^- initially present in the influent were completely consumed for the oxidation of NH_4^+ and no SO_4^{2-} consumption occurred in the oxidation processes of organic matter, a disproportionally high NH_4^+ oxidation still persists. In fact, it has to be noted that the effluent contained more NO_3^- in most analyses than the influent. Although neither inert gas flushing nor addition of the reducing agent was used to maintain anaerobic conditions in the reactors, the access of O_2 from the air into the reaction vessel was still impeded as the mixer rod of the MBBR was equipped with water-seal and the discharge gates of the effluent tubes of both reactors were kept beneath water level in the effluent receiving vessels.

The above-mentioned high NH_4^+ removal ratio might be due to complex interactions between organics, nitrogen and sulfur compounds in the wastewater. Several mechanisms are possibly involved.

Involvement of reactive oxygen species

Under conditions of oxidative stress (for example, when exposed to oxygen in the influent tank), facultative anaerobes present in complex wastewaters containing considerable amounts of organics, nitrogen and sulfur compounds, can generate reactive oxygen species such as H_2O_2 and superoxide (O_2^-) in various biological pathways. H_2O_2 could be produced as a result of bacterial respiration in anoxic conditions involving organic carbon and/or any electron acceptor. Dismutation of O_2^- anion by superoxide dismutases yields H_2O_2. The catalase enzymes can readily break H_2O_2 into water and O_2, as a de-toxification mechanism (Sabumon 2007, 2008, 2009). This additional oxygen generated in the microenvironments could be efficiently utilized by ammonia oxidizing bacteria for nitritation in competition with other bacteria.

Humic matter

Humic matter (HM) may contribute to interactions between S and N compounds behaving as redox mediators both biologically and abiotically, amplifying the effect of small amounts of oxygen that can penetrate into an anaerobic reactor medium. HM can be present in the wastewater either in an oxidized (functional active groups—quinones) or reduced form (hydroquinones). HM boosts the oxidation of S^{2-} into elemental sulfur and reduction of NO_2^- and NO_3^- into dinitrogen gas even if present at small concentration (Aranda-Tamaura et al. 2007). Quinones can be chemically reduced by S^{2-} promoting its oxidation into S_0/polysulfide. Reduced HM and quinones can also serve as electron donors for the anaerobic microbial reduction of different electron acceptors such as NO_2^- or NO_3^-. Oxidized forms of HM can act as electron acceptors as shown for several bioprocesses such as anaerobic microbial oxidation of phenolic compounds (Cervantes et al. 2000). HM has been shown to accelerate the nitrification–denitrification processes. However, little is known about the direct influence of HM on the anammox process. Humic and fulvic acids were found to form most of the total organics present in the supernatant used in this study hence they can significantly affect the interactions between sulfur and nitrogen compounds. Our batch tests have shown that the addition of anthraquinone-2,6-disulfonate (as disodium salt) to the synthetic mineral growth medium increases the production of hydrazine by the anammox microorganisms more than 100% as compared with control test without anthraquinone-2,6-disulfonate addition. Nitrogen removal rate showed slight increase as well.

Sulfide-generating SRAO proceeding according to the Eqs. 4 and 6 occurring concomitantly with elemental sulfur generating SRAO may alter $\Delta NH_4^+/\Delta SO_4^{2-}$ stoichiometric ratio. Sulfide concentrations were, however, very low (<100 μg/l) (see Figs. 3, 4) in the effluents of both reactors, indicating rapid sulfide oxidation occurring in the reactor's media, although some of the sulfide was oxidized in the effluent tanks as well. In addition to the direct chemical reaction between sulfide and NO_3^-, HM-mediated electron transfer reactions lead to a drop in sulfide concentration.

There are no reports in the literature on SRAO processes if elemental sulfur can serve as an electron acceptor for ammonium oxidation as given by the Eq. 6. The reduction-reoxidation cycles of elemental sulfur can possibly be responsible for some of the ammonium oxidation, affecting the final $\Delta NH_4^+/\Delta SO_4^{2-}$ stoiciometric ratio.

Re-oxidation of elemental sulfur or sulfide into SO_4^{2-} can readily take place via sulfur-utilising denitrification/denitritation, resulting in a partial restoration of SO_4^{2-} and altering the final balance of NH_4^+ and SO_4^{2-} in the effluent, leading to an increase in the $\Delta NH_4^+/\Delta SO_4^{2-}$ ratio. A decrease in pH is one of the indicators of denitrification. Sulfur-utilizing denitrification/denitritation reactions include (Li et al. 2009):

$$S_0 + {}^{6}/_{5}NO_3^- + {}^{2}/_{5}H_2O \rightarrow SO_4^{2-} + {}^{3}/_{5}N_2 + {}^{4}/_{5}H^+ \quad \Delta G_0 = -548\ \text{kJ/mol} \tag{15}$$

$$HS^- + {}^{8}/_{5}NO_3^- + {}^{7}/_{5}H^+ \rightarrow SO_4^{2-} + {}^{4}/_{5}N_2 + {}^{4}/_{5}H_2O \quad \Delta G_0 = -739\ \text{kJ/mol} \tag{16}$$

$$S + 2\ NO_2^- \rightarrow SO_4^{2-} + N_2 \quad \Delta G_0 = -676\ \text{kJ/mol} \tag{17}$$

$$HS^- + {}^{8}/_{3}NO_2^- + {}^{7}/_{3}H^+ \rightarrow SO_4^{2-} + {}^{4}/_{3}N_2 + {}^{4}/_{3}H_2O \quad \Delta G_0 = -981\ \text{kJ/mol}. \tag{18}$$

Denitrification in both MBBR and UASBR occurred via sulfur oxidation, as organics present in reject water was mainly of recalcitrant nature. Presence of denitrifying sulfur-oxidizing *Sulfurimonas denitrificans* DSM 1251 and Sulfide-oxidizing bacterium N9-1 in the seeding sludge provides the evidence in favor of this denitrification mechanism. Low-molecular organic acids such as acetic and propionic acids were not found in reject water. If present, low-molecular organic acids, in addition of being a possible substrate for denitrifying bacteria, could be a possible alternative substrate for some of the anammox bacteria. The ability of certain anammox bacteria to consume acetic or propionic acids has been reported in several reports, like Güven et al. (2005), Kartal et al. (2007), Strous et al. (2006) and Van der Star et al. (2008). The hydrogenotrophic denitrification was ruled out in our systems by the values of ORP, which were positive, ranging 57–195 mV in the MBBR and 53–190 mV in the UASBR. Denitrification using organic substrates was low also as the average COD reduction was 16% in the MBBR and 8% in the UASBR, respectively.

Role of hydrazine and hydroxylamine

Hydrazine was detected in the effluents of both reactors in the stationary operation phase (averagely 31 μg/l in the MBBR and 26 μg/l in the UASBR), indicating the anammox activity. Injections of hydrazine sulfate ($N_2H_4 \times H_2SO_4$) since day 282 (day 268 for the UASBR) at low dosages (1 mg N_2H_4 per litre of the active volume of a reactor) resulted in a better process performance in the UASBR as the TN removal efficiency increased above 30%. In case of MBBR, the mentioned dosage of hydrazine had virtually no effect. Hydrazine injected into the reactors was nearly entirely consumed by the next day. Earlier, the influence of hydrazine on the "conventional" anammox process has been researched (Bettazzi et al. 2010), but there are no reports in the literature on the effects of hydrazine on the SRAO process. As reported by Bettazzi et al. (2010), the maximum NO_2^- removal rate increased about 40% after 50% of the anammox activity was lost due to peak nitrite concentrations used in the traditional anammox experiment by using hydrazine concentrations of 2 mg N/l. In our case peak bicarbonate concentrations were involved as an inhibiting factor in flow-through conditions in both reactors, in addition to inhibitory effects caused by NO_x^-. Recovery of the UASBR from inhibition caused by several inhibitory factors shows the benefits of addition of this Anammox intermediate, which circumstance has perspectives in practical applications.

Since day 316 (day 301 for the UASBR), a mixture of hydrazine and hydroxylamine was injected at a dosage of 12.5 mg/l of each. The dosage selection was based on batch tests (data not shown). Hydroxylamine was added in the form of hydroxylamine hydrochloride ($NH_2OH \times HCl$). By 24 h, both substances injected were mostly consumed in both reactors with residual hydroxylamine concentrations in effluents ranging 0.17–0.37 mg/l. Residual hydrazine concentrations were 0.9–4.1 mg/l in the effluent of the MBBR and 0.72–0.83 mg/l in the effluent of the UASBR.

PCR-DGGE results

In MBBR *uncultured Planctomycetales bacterium clone P4* and two other species close to *Brocadia caroliniensis* were present while in UASBR only one species close to it was found (*Uncultured planctomycete clone* Pla_PO55-9 16S, GenBank: GQ356109.1, Fig. 8). Other bacteria identified in UASBR were a more distant *planctomycete Uncultured bacterium clone* Dok23 16S, GenBank: FJ710742, *Uncultured bacterium clone* U000019157, GenBank: GU579076.1 and two species belonging to the phylum *Verrucomicrobia* (Uncultured *Verrucomicrobiales bacterium clone* De2102, GenBank: HQ183974 and *Uncultured bacterium clone* ATB-KS-1929, GenBank: EF686989). The mechanisms for N removal in MBBR and UASBR might be different as microorganisms mostly from phylum *Planctomycete*s were typical to MBBR and bacteria from the phylum *Verrucomicrobia* to the UASBR.

SEM observations

The biofilm had a porous and uneven structure, no filamentous bacteria were detected (Fig. 9). Mineral particles were also found in the biofilm (Fig. 10).

Conclusions

The SRAO process is feasible for the treatment of a supernatant from sludge digestion, although significantly less efficient and less stable than the "conventional" anammox process. Average TN removal efficiencies of 24 and 23% were achieved in the MBBR and UASBR, respectively. *Uncultured bacteria clone P4* and *uncultured planctomycete clone Amx-PAn30* were detected from the biofilm of the MBBR, from sludge of the UASBR *uncultured Verrucomicrobiales bacterium clone De2102* and *uncultured bacterium clone ATB-KS-1929* were found. The SRAO process took place as one reaction of the multiple complex interactions between *N*-compounds, *S*-compounds and organics (primarily humic matter), resulting in a significantly higher removal ratio of NH_4^+ than it can be concluded on the basis of the extent of the SO_4^{2-} reduction. The high NH_4^+ removal ratio can be attributed to re-oxidation of elemental sulfur or sulfide into SO_4^{2-} taking place via sulfur-utilizing denitrification/denitritation. Presence of denitrifying sulfur-oxidizing *Sulfurimonas denitrificans* DSM 1251 and Sulfide-oxidizing bacterium N9-1 in the seeding sludge provided the evidence that SRAO was occurring independently and was not a result of two separate processes—sulfate reduction

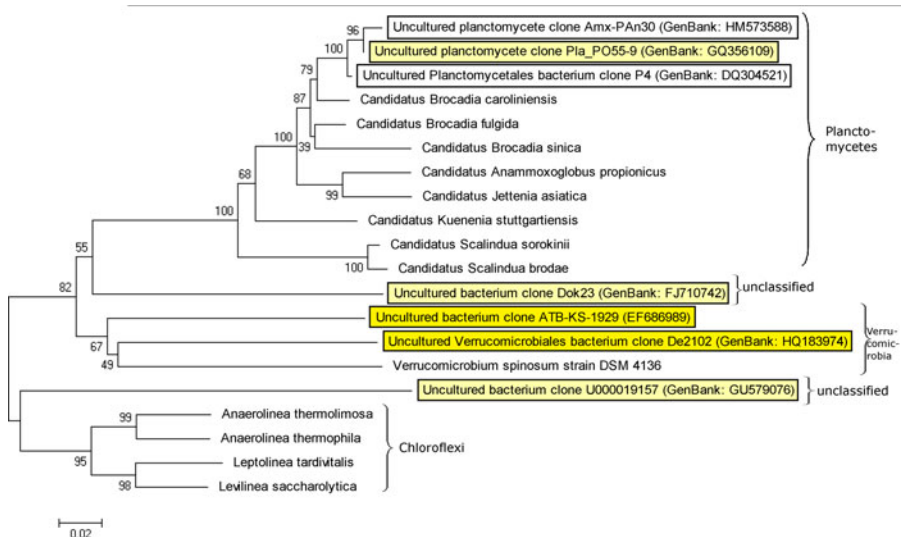

Fig. 8 Phylogenetic neighbour-joining tree, reflecting the relationships between identified sequences. *Numbers* at the nodes are percentages of bootstrap values. Branch lengths correspond to sequence differences as indicated by the *scale bar*

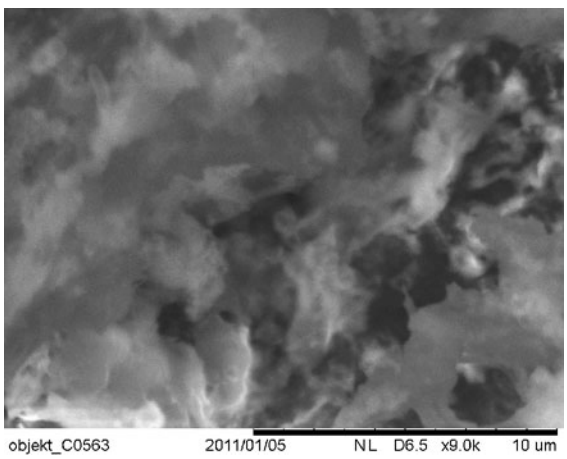

Fig. 9 Scanning microscopy image of biofilm of MBBR. Magnification 5000×, bar 10 μm

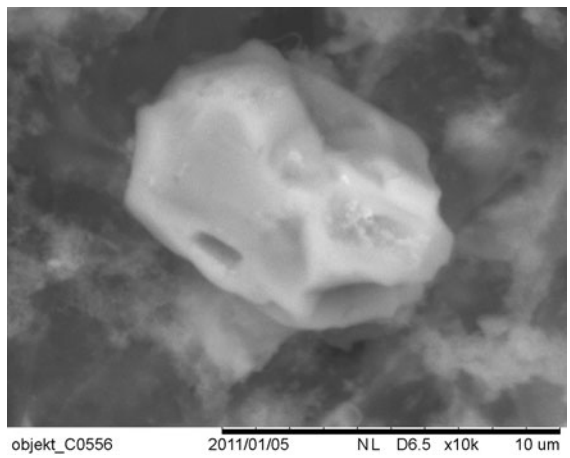

Fig. 10 Scanning microscopy image of precipitate in biofilm of MBBR. Magnification 10000×, bar 10 μm

and anammox. Small amounts of hydrazine were naturally present in the reaction medium, indicating occurrence of the anammox process. Influent NO_x^- concentrations exceeding 10 mg/l disrupted the SRAO process. High HCO_3^- exceeding 1,000 mg/l might contribute to the overall deterioration of the bioprocess performance as well. Injections of hydrazine had an ameliorating effect on the performance of the UASBR, no significant effect was observed in the case of the MBBR.

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References

Amend JP, Rogers KL, Shock EL, Gurrieri S, Inguaggiato S (2003) Energetics of chemolithoautotrophy in the hydrothermal system of Vulcano Island, southern Italy. Geobiology 1:37–58

APHA (American Public Health Association) (1985) Standard methods for the examination of water and wastewater, 16th edn. APHA, Washington DC

Aranda-Tamaura C, Estrada-Alvarado MI, Texier A-C, Cuervo F, Gómez J, Cervantes FJ (2007) Effects of different quinoid redox mediators on the removal of sulfide and nitrate via denitrification. Chemosphere 69:1722–1727

Bettazzi E, Caffaz S, Vannini C, Lubello C (2010) Nitrite inhibition and intermediates effects on anammox bacteria: a batch-scale experimental study. Process Biochem 45:573–580

Cervantes FJ, van der Velde S, Lettinga G, Field JA (2000) Quinones as terminal electron acceptors for anaerobic microbial oxidation of phenolic compounds. Biodegradation 11(5):313–321

Chamchoi N, Nitisoravut S, Schmidt JE (2008) Inactivation of ANAMMOX communities under concurrent operation of anaerobic ammonium oxidation (ANAMMOX) and denitrification. Bioresour Technol 99:3331–3336

Clément J-C, Shrestha J, Ehrenfeld JG, Jaffé PR (2005) Ammonium oxidation coupled to dissimilatory reduction of iron under anaerobic conditions in wetland soils. Soil Biol Biochem 37:2323–2328

Dexiang L, Xiaoming L, Qi Y, Guangming Z, Liang G, Xiu Y (2008) Effect of inorganic carbon on anaerobic ammonium oxidation enriched in sequencing batch reactor. J Environ Sci 20:940–944

Dionisi HM, Layton AC, Harms G, Gregory IR, Robinson KG, Sayler GS (2002) Quantification of *Nitrosomonas oligotropha*-like ammonia-oxidizing bacteria and *Nitrospira* spp. from full-scale wastewater treatment plants by competitive PCR. Appl Environ Microbiol 68:245–253

Fdz-Polanco F, Fdz-Polanco M, Fernández N, Urueña MA, Garciá PA, Villaverde S (2001) Combining the biological nitrogen and sulfur cycles in anaerobic conditions. Water Sci Technol 44(8):77–84

Frear DS, Burrell RC (1955) Spectrophotometric method for determining hydroxylamine reductase activity in higher plants. Anal Chem 27:1664

George M, Nagaraja KS, Balasubramanian N (2008) Spectrophotometric determination of hydrazine. Talanta 75(1):27–31

Güven D, Dapena A, Kartal B, Schmid MC, Maas B, van de Pas-Schoonen K, Sozen S, Mendez R, Op den Camp HJM, Jetten MSM, Strous M, Schmidt I (2005) Propionate oxidation by and methanol inhibition of anaerobic ammonium-oxidizing bacteria. Appl Environ Microbiol 71(2):1066–1071

Ibrahim MBM, Moursy AS, Bedair AH, Radwan EK (2008) Comparison of DAX-8 and DEAE for isolation of humic substances from surface water. J Environ Sci Tech 1:90–96

Javanaud C, Michotey V, Guasco S, Garcia N, Anschutz P, Canton M, Bonin P (2011) Anaerobic ammonium oxidation mediated by Mn-oxides: from sediment to strain level. Res Microbiol. doi:10.1016/j.resmic.2011.01.011

Jetten MSM, van Niftrik L, Strous M, Kartal B, Keltjens JT, Op den Camp HJM (2009) Biochemistry and molecular biology of anammox bacteria. Crit Rev Biochem Mol Biol 44:65–84

Jing C, JianXiang J, Ping Z (2010) Isolation and identification of bacteria responsible for simultaneous anaerobic ammonium and sulfate removal. Sci China Chem 53(3):645–650

Kartal B, Rattray J, van Niftrik LA, van de Vossenberg J, Schmid MC, Webb RI, Schouten S, Fuerst JA, Sinninghe Damsté J, Jetten MSM, Strous M (2007) *Candidatus* ''*Anammoxoglobus propionicus*'' a new propionate oxidizing species of anaerobic ammonium oxidizing bacteria. Syst Appl Microbiol 30:39–49

Koplimaa M, Menert A, Blonskaja V, Kurissoo T, Zub S, Saareleht M, Vaarmets E, Menert T (2010) Liquid and gas chromatographic studies of the anaerobic degradation of baker's yeast wastewater. Procedia Chem 2(S1):120–129

Koskinen PE, Kaksonen AH, Puhakka JA (2006) The relationship between instability of H_2 production and compositions of bacterial communities within a dark fermentation fluidized-bed bioreactor. Biotechnol Bioeng 97:742–758

Lane DJ (1991) 16/23S rRNA sequencing. Nucleic acid techniques in bacterial systematics. John Wiley and Sons, Chichester, pp 177–204

Lei Z, Ping Z, YuHui H, RenCun J (2009) Performance of sulfate-dependent anaerobic ammonium oxidation. Sci China Ser B Chem 52(1):86–92

Li W, Zhao Q-L, Liu H (2009) Sulfide removal by simultaneous autotrophic and heterotrophic desulfurization–denitrification process. J Hazard Mater 162(2–3):848–853

Liang Z, Liu J-X, Li J (2009) Decomposition and mineralization of aquatic humic substances (AHS) in treating landfill leachate using the Anammox process. Chemosphere 74:1315–1320

Liu S, Yang F, Gong Z, Meng F, Chen H, Xue Y, Furukawa K (2008) Application of anaerobic ammonium-oxidizing consortium to achieve completely autotrophic ammonium and sulfate removal. Bioresour Technol 99:6817–6825

Muyzer G, De Waal EC, Uitterlinden AG (1993) Profiling of complex microbial population by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16rRNA. Appl Environ Microbiol 59:695–700

Muyzer G, Hottenträger S, Teske A, Waver C (1996) Denaturing gradient gel electrophoresis of PCR amplified 16S rDNA—a new molecular approach to analyze the genetic diversity of mixed microbial communities. In: Akermanns AD, van Elsas JD, de Brujin FJ (eds) Molecular microbial ecology manual. Kluwer Academic Publishers, Dordrecht

Neef A, Amann RI, Schlesner H, Schleifer KH (1998) Monitoring a widespread bacterial group: in situ detection of planctomycetes with 16S rRNA-targeted probes. Microbiology UK 144:3257–3266

Qiao S, Kawakubo Y, Cheng Y, Nishiyama T, Fujii T, Furukawa K (2009) Identification of bacteria coexisting with anammox bacteria in an upflow column type reactor. Biodegradation 20:117–124

Sabumon PC (2007) Anaerobic ammonia removal in presence of organic matter: a novel route. J Hazard Mater 149:49–59

Sabumon PC (2008) Development of a novel process for anoxic ammonia removal with sulfidogenesis. Process Biochem 43:984–991

Sabumon PC (2009) Effect of potential electron acceptors on anoxic ammonia oxidation in the presence of organic carbon. J Hazard Mater 172:280–288

Sąnchez-Melsió A, Cįliz J, Balaguer MD, Colprim J, Vila X (2009) Development of batch-culture enrichment coupled to molecular detection for screening of natural and man-made environments in search of anammox bacteria for N-removal bioreactors systems. Chemosphere 75:169–179

Sawayama S (2006) Possibility of anoxic ferric ammonium oxidation. J Biosci Bioeng 101(1):70–72

Schrum HN, Spivack AJ, Kastner M, D'Hondt S (2009) Sulfate-reducing ammonium oxidation: a thermodynamically feasible metabolic pathway in subseafloor sediment. Geology 37(10):939–942

Strous M, Kuenen JG, Jetten MSM (1999) Key physiology of anaerobic ammonium oxidation. Appl Environ Microbiol 65:3248–3250

Strous M, Kuenen JG, Fuerst JA, Wagner M, Jetten MSM (2002) The anammox case—a new experimental manifesto for microbiological eco-physiology. Antonie van Leeuwenhoek 81:693–702

Strous M, Pelletier E, Mangenot S, Rattei T, Lehner A, Taylor MW, Horn M, Daims H, Bartol-Mavel D, Wincker P, Barbe V, Fonknechten N, Vallenet D, Segurens B, Schenowitz-Truong C, Médigue C, Collingro A, Snel B, Dutilh BE, Op den Camp HJM, van der Drift C, Cirpus I, van de Pas-Schoonen KT, Harhangi HR, van Niftrik L, Schmid M, Keltjens J, van de Vossenberg J, Kartal B, Meier H, Frishman D, Huynen MA, Mewes H-W, Weissenbach J, Jetten MSM, Wagner M, Le Paslier D (2006) Deciphering the evolution and metabolism of an anammox bacterium from a community genome. Nature 440:790–794

Van der Star WRL, van de Graaf MJ, Kartal B, Picioreanu C, Jetten MSM, van Loosdrecht MCM (2008) Response of anaerobic ammonium-oxidizing bacteria to hydroxylamine. Appl Environ Microbiol 74(14):4417–4426

Villaverde S (2004) Recent developments on biological nutrient removal processes for wastewater treatment. Rev Environ Sci Biotechnol 3:171–183

Yang Z, Zhou S, Sun Y (2009) Start-up of simultaneous removal of ammonium and sulfate from an anaerobic ammonium oxidation (anammox) process in an anaerobic up-flow bioreactor. J Hazard Mater 169:113–118

Zekker I, Rikmann E, Tenno T, Lemmiksoo V, Menert A, Loorits L, Kahu K, Tomingas M, Tenno T. Anammox enrichment from reject water on blank biofilm carriers and carriers containing nitrifying biomass—operation of two moving bed biofilm reactors (MBBR) (submitted to Biodegradation)

Zekker I, Rikmann E, Tenno T, Menert A, Lemmiksoo V, Saluste A, Tenno T (2011) Modification of nitrifying biofilm into nitritating one by combination of increased free ammonia concentrations, lowered HRT and dissolved oxygen concentration. J Environ Sci 23(7):1113–1121

Zhao Q-l, Li W, You S-J (2006) Simultaneous removal of ammonium-nitrogen and sulfate from wastewaters with an anaerobic attached-growth bioreactor. Water Sci Technol 54(8):27–35

Zub S, Kurissoo T, Menert A, Blonskaja V (2008) Combined biological treatment of high-sulfate wastewater from yeast production. Water Environ J 22(4):274–286