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# Sulfate-reducing anammox for sulfate and nitrogen containing wastewaters

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ABSTRACT

In a moving bed biofilm reactor (MBBR) at moderate temperature of 20°C sulfate reduction along with ammonium oxidation were established. In an upflow anaerobic sludge blanket reactor (UASBR), the same process took place at 36°C. Sulfate-reducing ammonium oxidation (SRAO) was performed using reject water as a substrate for micro-organisms and a source of $NH_4^+$, with $SO_4^{2-}$ being added as an electron acceptor. Bacterial strains belonging to the phylum *Planctomycetales* were detected from the biofilm of the MBBR; from the UASBR species representing phylum *Verrucomicrobia* were found. Average volumetric TN removal rates were 0.03 kg-N/$m^3$/d in the MBBR and 0.04 kg-N/$m^3$/d in the UASBR. $HCO_3^-$ concentrations exceeding 1000 mg/l had an inhibiting effect on the SRAO process. The stoichiometric ratio of $NH_4^+$ removal was significantly higher than that 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. Addition of hydrazine and hydroxylamine up to 12.5 mg/l had a positive effect on SRAO process performance, particularly in the UASBR. Hydrazine was naturally present in the reaction medium, indicating occurrence of the anammox process.

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

## 1. Introduction

Wastewaters produced from food industry (i.e. yeast production) may have high content of $NH_4^+$ and $SO_4^{2-}$ (>1,000 mg/l of each). When it is released into nature, ammonium can cause eutrophication of water bodies and deterioration of water quality, posing risk to fish stocks. High sulfate concentrations in wastewater can cause imbalance in the natural sulfur cycle of water bodies [1]. The anammox (anaerobic ammonium oxidation) process provides an alternative to the conventional nitrification–denitrification technology for nitrogen removal. The anammox reaction uses $NH_4^+$ as electron donor and $NO_2^-$ as electron acceptor, converting chemically the bonded nitrogen into $N_2$ gas.

Decreased concentrations of both nitrogen and sulfate led to a discovery of sulfate-reducing ammonium oxidation (SRAO), which was firstly assumed in a

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study discussing the treatment of vinasse wastewater in an anaerobic fluidized-bed reactor [1]. The following equations for the sulfate-dependent anammox process were proposed as follows:

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

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

$$N_4^+ + NO_2^- \rightarrow N_2 + 2H_2O \text{ (anammox-process)} \quad \Delta G_0 = -360 \text{ kJ/mol} \tag{3}$$

Also sulfide formation has been assumed to be possible in several studies [2–4].

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

Anammox reaction with this pathway of SRAO results in a summary equation [2–4]:

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

Several bacterial strains being involved in SRAO have been isolated. An autotrophic *Planctomycetes* bacterium named *Anammoxoglobus sulfate* was discovered [5], which oxidizes $NH_4^+$ into $NO_2^-$ using $SO_4^{2-}$ as an electron acceptor thus performing the reaction described by Eq. (2) as initially assumed in the study [1]. Possible involvement of sulfate-reducing bacteria in the nitrite-generating stage of SRAO has also been mentioned [3,6]. Ammonium is similar to methane in the molecular structure, thus in this stage sulfate reduction might be comparable to sulfate-utilizing methane oxidation. The possible half-reactions would be as follows [6]:

$$4NH_4^+ + 8H_2O \rightarrow 4NO_2^- + 32H^+ + 24e^- \tag{6}$$

$$3SO_4^{2-} + 24H^+ + 24e^- \rightarrow 3S^{2-} + 12H_2O \tag{7}$$

In addition, a non-neighboring organism of *planctomycete*, the *Bacillus benzoevorans* strain ASR, performing the entire two-staged SRAO reaction as given by Eq. (1), was found [7]. The reaction between $NH_4^+$ and $SO_4^{2-}$ was shown to be entirely biological by its nature with no abiotic chemical reactions taking place between these ions at 30°C and under normal pressure ([3] and [6]). Higher substrate concentrations and a low oxidation–reduction potential (ORP) were shown to be favorable for this bioprocess. Reports on SRAO show new technological solutions in the biological nitrogen removal from wastewaters, particularly in the case of waste streams containing both sulfate and nitrogenous compounds at high concentrations. High-content wastewaters with total Kjeldal nitrogen, 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 the process disruptions caused by sulfide inhibition and reducing nitrogen load in subsequent stages, including nitrogen removal. However, nitrogen removal coupled with sulfate reduction can take place with participation of bacteria representing various metabolic groups. Later, nitrate reduction to $N_2$ has also been shown to be stimulated by sulfate and methane [8].

The main objective of this study was to assess the feasibility of SRAO using real wastewater (e.g. reject water), characteristics of which fluctuate greatly over time. The adaptation abilities of bacterial consortia present in different inocula were compared. Evaluation of the inhibiting effect caused by high concentration of bicarbonate and nitrite on the flow-through SRAO culture was studied.

## 2. Materials and methods

### 2.1. Reactor configurations

Influent was supplied by time-controlled peristaltic pumps. The MBBR was mechanically stirred at 100–200 rpm (rotations per minute) and thermostated at 20°C (±0.5°C). The reactor had an active volume of 3.3 l, hydraulic retention time (HRT) was kept 1 d until day 239 and 2 d afterwards. The upflow anaerobic sludge blanket reactor (UASBR) was thermostated at 36°C (±0.5°C) and an upflow of the liquid phase was maintained by a continuously working peristaltic pump (Fig. 1). This reactor had an active volume of 0.75 l and HRT was kept 1 d until day 225 and 2 d afterwards. Temperatures were selected the same as in reactors where seeding material was taken from.

### 2.2. Influent

For comparison of effects of $NO_2^-$ versus $SO_4^{2-}$, as anammox electron acceptors, reject water was used as a source of $NH_4^+$. Reject water contained sufficient amounts of micro- and macro-elements [9]. Also the presence of Anammox bacteria in reject water was confirmed by PCR-DGGE (discussed below). Influent

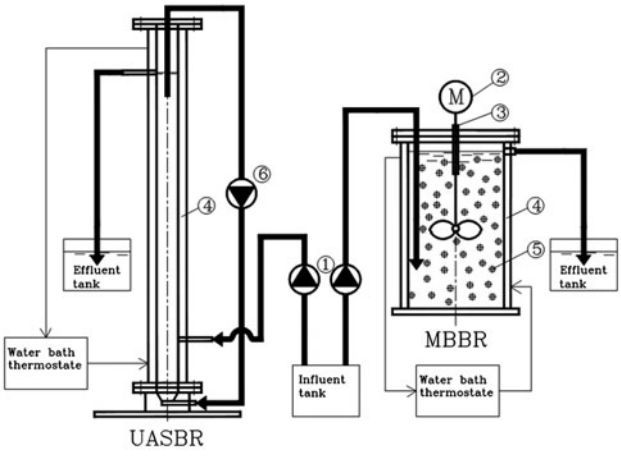


Fig. 1. Configuration of UASBR and MBBR systems used for research of the SRAO process. Numbers represent: (1) Influent pump, (2) Mechanical mixer, (3) Stirrer, (4) Water jacket, (5) Biofilm carriers, and (6) Recirculation pump.

was prepared by mixing reject water with tap water and $K_2SO_4$. Since day 241 (for the UASBR, day 227) $HCO_3^-$ concentration was held below 1,000 mg/l, considering literature data $HCO_3^-$ concentration on appropriate for the anammox process [10]. 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).

### 2.3. Seeding materials

The seeding material for UASBR came from anaerobic sludge from a yeast factory wastewater treating facility (Salutaguse, Estonia). The TN removal rate in the UASBR of this treatment facility was 4.8 kg-N/$m^3$/d. The MBBR was inoculated with 1,000 bioflow-9-type carriers (specific surface 800 m/$m^3$) with a well-established attached anammox biofilm (TN removal rate 0.5 kg-N/$m^3$/d, specific anammox activity of 0.73 gN/m/d) taken from a laboratory scale "conventional" anammox reactor treating reject water combined with $NaNO_2$. The micro-organisms detected in the seeding biomass for the MBBR included *uncultured Planctomycetales bacterium clone P4* (Fig. 2(a) and [11]). 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 in addition to bacteria from the phyla *Verrucomicrobia*, *Bacteroidetes*, *Firmicutes*, and *Proteobacteria* found in the treatment facility of Salutaguse yeast factory (Fig. 2(b)).

### 2.4. Analytical methods

The analyses of main nitrogen species: $NH_4^+$-N, $NO_2^-$-N, $NO_3^-$-N, $HCO_3^-$, $SO_4^{2-}$-S, total sulfide-S, and COD were performed according to APHA [12]. Hydrazine was determined on a Hach Lange DR2800 type spectrophotometer. 0.5% solution of sulfamic acid was used in order to eliminate interference from $NO_2^-$ and $NO_3^-$ as described in George et al. [13]. Hydroxylamine was measured by spectrophotometry at 705 nm according to the study [14]. Humic and fulvic substances (humic matter, HM) were analyzed by liquid chromatography [15]. The dissolved oxygen (DO) concentration, pH, and ORP were measured manually using the following equipment—Elke Sensor for DO, with Eutech ORP sensor for ORP, and pH with Jenway pH electrode.

### 2.5. PCR-DGGE

Detailed information about microbial characterization is presented in the study [16]. Anammox microorganisms were determined via PCR with a wide-range primer set Eub27f/Eub1492r [17] in the first PCR round, and in the second PCR round by a Planctomycetes-specific primer Pla46f [18] coupled with an anammox-specific primer Amx368r [19]. *Verrucomicrobiales bacterium* clones were determined according to [17]. Nitrite oxidizing bacteria were determined with the Nitrospira-specific primer set NSR1113f/NSR1264r [20].

PCR with the primers Pla46f/Amx368r [21] was carried out with the following thermocycling parameters: 1 cycle for 5 min of initial denaturation at 95°C, 35 cycles at 94°C for 45 s, at 58°C for 1 min, and at 72°C for 1 min, and single final elongation at 72°C for 7 min.

DGGE was conducted using the eubacterial primer set GC-BacV3f/907r as described previously [24]. PCR with the primer set BacV3f/907r was conducted using the following thermocycling parameters: 1 cycle for 5 min of initial denaturation at 95°C, 30 cycles at 94°C for 30 s, at 52°C for 1 min, and at 72°C for 2 min, and single final elongation at 72°C for 10 min.

The gene sequences were amplified in a mastercycler personal thermocycler (Eppendorf, Germany). 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.

### 2.6. Sequencing

PCR for sequencing was performed with the BigDye® Terminator v3.1 Cycle Sequencing Kit (Life Technologies Corporation, USA). The sequences

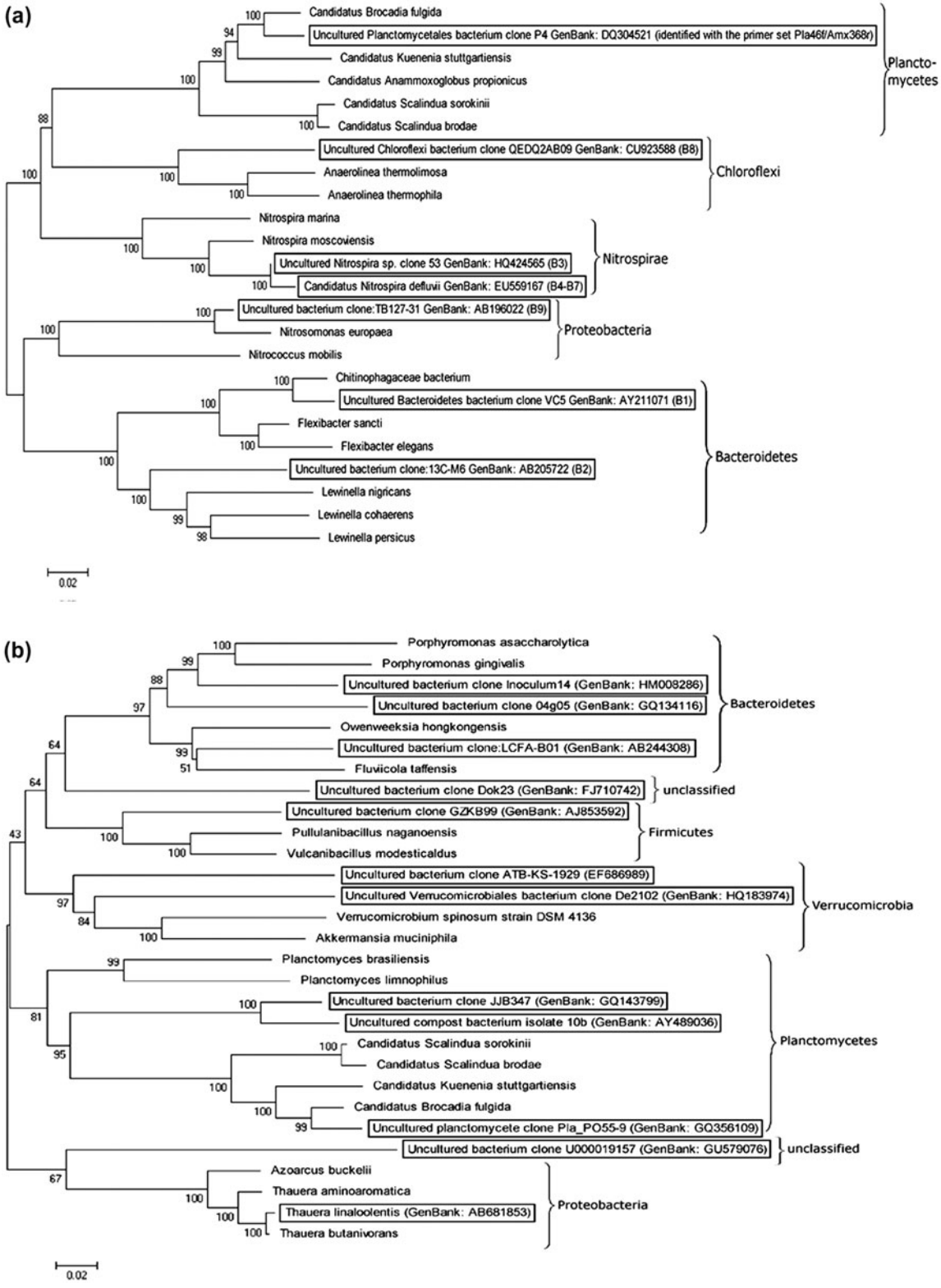


Fig. 2. 16S rDNA phylogenetic tree of some key micro-organisms detected in the inocula of the reactors. (a) MBBR and (b) UASBR.

acquired were compared with the available database sequences via a BLAST (basic local alignment search tool) search from the GenBank (http://www.ncbi.nlm.nih.gov/genbank/).

### 2.7. Pyrosequencing

The samples from the reactor were pyrosequenced at the Integrated Systems Biology Centre of Tallinn University of Technology. Universal 8F and 357R sequences were used for the PCR amplification of the V2–V3 hyper variable regions of 16S rRNA genes. The 357R primer included additionally a unique sequence tag to barcode each sample.

### 2.8. Phylogenetic analysis

Sequences obtained from PCR-DGGE analysis were compared with 16S rDNA sequences of related species. Phylogenetic tree showing these relationships was constructed with MEGA software version 5.0.

### 2.9. *Fluorescence* in situ *hybridization*

Fluorescent *in situ* hybridization (FISH) of harvested biomass of UASB was performed. Biomass was fixed in a 4% paraformaldehyde solution and the probe Amx820 with Cy3 label was used at 35% formamide to target the anammox genera "*Candidatus* Brocadia and Kuenenia". The samples were counterstained with the DNA stain 4′,6-diamidino-2-phenylindole (DAPI). Images were acquired on a Carl Zeiss Axioskop 2 Plus epifluorescence microscope (Jena, Germany) equipped with differential interference contrast.

## 3. Results and discussion

### 3.1. Start-up

For the MBBR, *inoculum* was taken from laboratory anammox MBBR system [11], which was fed with influent containing $NH_4^+$ and $NO_2^-$ (TN removal efficiency approximately 85%) similarily to the study [6], with a subsequent switching to the influent containing $NH_4^+$ and $SO_4^{2-}$. The average TN removal rate in this period was 0.02 kg-N/$m^3$/d. After electron acceptor was exchanged from nitrite to sulfate, TN removal efficiency for MBBR fell by day 80 to an average level of 19% (Fig. 3). However, similarly to Yang and Zhou [6], after start-up and acclimatization of this process for 50 d, the average effluent concentrations of ammonium-N and sulfate-S were 53 mg/l (influent 72 mg/l) and 58 mg/l (influent 75 mg/l), respectively. Sulfate removal efficiency of 30% was achieved [6]. Our experiments were similar despite they were done with real wastewater differently from these authors [6] who used synthetic wastewater. The average values for free ammonia (FA) corresponding to the $NH_4^+$-N content in the effluent were rather low—1.8 mg N/l. The average $NH_4^+$-N concentration was 69 mg/l for the influent

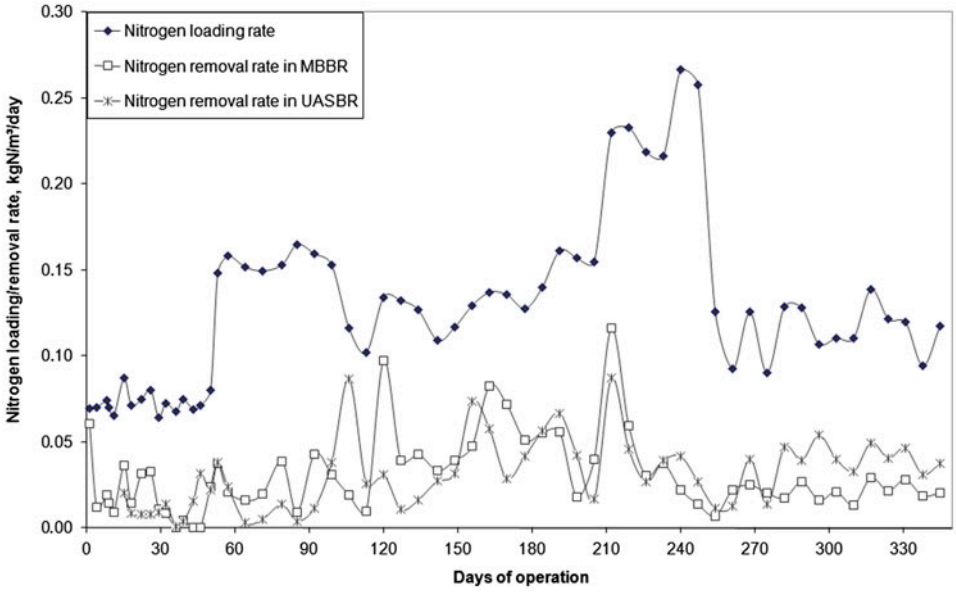


Fig. 3. Nitrogen loading rates (diamonds) and nitrogen removal rates for MBBR (quadrates) and UASBR (stars).

and 47 mg/l for the effluent of the MBBR, respectively. Inhibitory FA values for Anammox bacteria range from 13 to 90 mg N/l [22]. HRT was kept at 1 d until day 253 (day 239 in case of the UASBR), thereafter it was set to 2 d in attempt to facilitate higher TN and $SO_4^{2-}$ removal. Differently from the MBBR, the seeding sludge of the UASBR which had not been enriched with anammox organisms, the UASBR showed similar results to the MBBR: the average $NH_4^+$-N concentration in the effluent was low—56 mg N/l during the initial 36 d and the average TN removal efficiency was 17%. ORP values were below 100 mV in the influent and 100–140 mV in the effluents of both reactors, while the average pH value was 8.4 for the influent, dropping to 7.9 in the MBBR and 8.2 in the UASBR. In case of influent, ORP was affected both by storage of reject water (increase in ORP value due to oxidation/volatilization of sulfides etc.) and mixing reject water with tap water. Concentrations of $NO_2^-$ and $NO_3^-$ in the influent were mostly below 5 and 1 mg N/l, respectively; in the effluents, slight increase in $NO_3^-$ was observed while $NO_2^-$ remained in the same range. Sulfide concentrations both in the influent and the effluents were below 100 μg/l, remaining so during the entire experimental period.

### 3.2. TN removal rates and efficiencies of the MBBR and the UASBR

After reactor's start-up with lower influent loading, a better process performance was expected to overcome from the free energy barrier of SRAO (see Eq. (1)) by doubling influent $NH_4^+$ concentration ($SO_4^{2-}$ content left unchanged (Fig. (4)) considering lower sulfate removal) in days 55–99 for the MBBR and 41–85 for the UASBR. For both reactors, increased influent $NH_4^+$ concentration (average FA 7–10 mg N/l) had no significant effect on the TN removal efficiency. The TN removal efficiencies and removal rates were 18% and 0.03 kg-N/$m^3$/d for the MBBR and 11% and 0.02 kg-N/$m^3$/d for the UASBR. ORP values ranged 80–120 mV in the influent showing slight increase (up to 150 mV) in the effluents of both reactors. Average pH value was 8.7 for the influent, decreasing to 8.1 in the MBBR and 8.3 in the UASBR. In the effluent of MBBR, slight increase in both $NO_2^-$ an $NO_3^-$ was observed (<10 and <5 mg/l, respectively), while in UASBR, only $NO_3^-$ showed slight increase.

For the UASBR, loading rates were gradually increased until day 253 and 239. Unstable effluent parameters during this period were, however, recorded and the nitrogen removal remained low (Figs. 3 and 4). TN removal efficiency ranged 5–72% (average 31%) for the MBBR and 10–75% (average 28%) for the UASBR, respectively. Despite different temperatures applied (20 and 36°C), TN removal rates were similar—0.05 kg-N/$m^3$/d for the MBBR and 0.04 kg-N/$m^3$/d for the UASBR, respectively. Average FA values in the liquid phase were 7 and 8 mgN/L, for the MBBR and the UASBR, respectively. ORP values were similar to the previous period. Average pH values were 8.5 for the influent and 8.2 for both MBBR and UASBR. In both reactors, slight increase in $NO_3^-$, but not in $NO_2^-$ was observed.

A better controlled combination of factors led to fewer fluctuations in the TN removal efficiencies and rates after loadings of reactors were decreased after day 254 for the MBBR and day 240 for the UASBR. The results might be affected by changes in the influent $HCO_3^-$ (kept below 1,000 mg/L in the influent) and injections of the anammox intermediates into the reactors (discussed below). In the MBBR, the average TN removal efficiency at an average $NH_4^+$-N concentration of 109 mgN/l (FA = 5 mgN/l) was 20%. Average TN removal rate in the MBBR for the same time interval was 0.02 kg-N/$m^3$/d. For the UASBR, the average TN removal efficiency at an average $NH_4^+$-N concentration of 155 mgN/l (FA = 4 mgN/l) was 32%. Corresponding average TN removal rate in the UASBR was 0.04 kg-N/$m^3$/d. ORP values were 65–180 mV in the influent, 50–180 mV in the effluents of both UASBR and MBBR. Average pH values were 8.1 for the influent and 7.8 for both MBBR and UASBR. As during previous period, slight increase in $NO_3^-$, but not in $NO_2^-$ was observed in both reactors.

During the entire experimental period, the average TN removal efficiencies were 24% for the MBBR and 23% for the UASBR, respectively. Average TN removal rates were 0.03 kg-N/$m^3$/d for both reactors. These results are lower than those achieved in MBBRs fed with reject water and $NO_2^-$ as the electron acceptor [11]. For comparison, other researches of the SRAO using synthetic wastewaters have achieved 40–45% $NH_4^+$ removal efficiencies [6,7] while in the study [1] 30–55% TKN removal was reported for treatment of vinasse-based wastewater.

### 3.3. Stoichiometry of TN removal

According to Dexiang et al. [10], concentrations above 1,500 mg/l $HCO_3^-$ may limit Anammox process efficiency. Alternatively, higher $HCO_3^-$ levels can promote sulfur-based autotrophic denitrification and denitritation since $HCO_3^-$ acts both as inorganic carbon source and pH buffer, leading to partial outcompetition

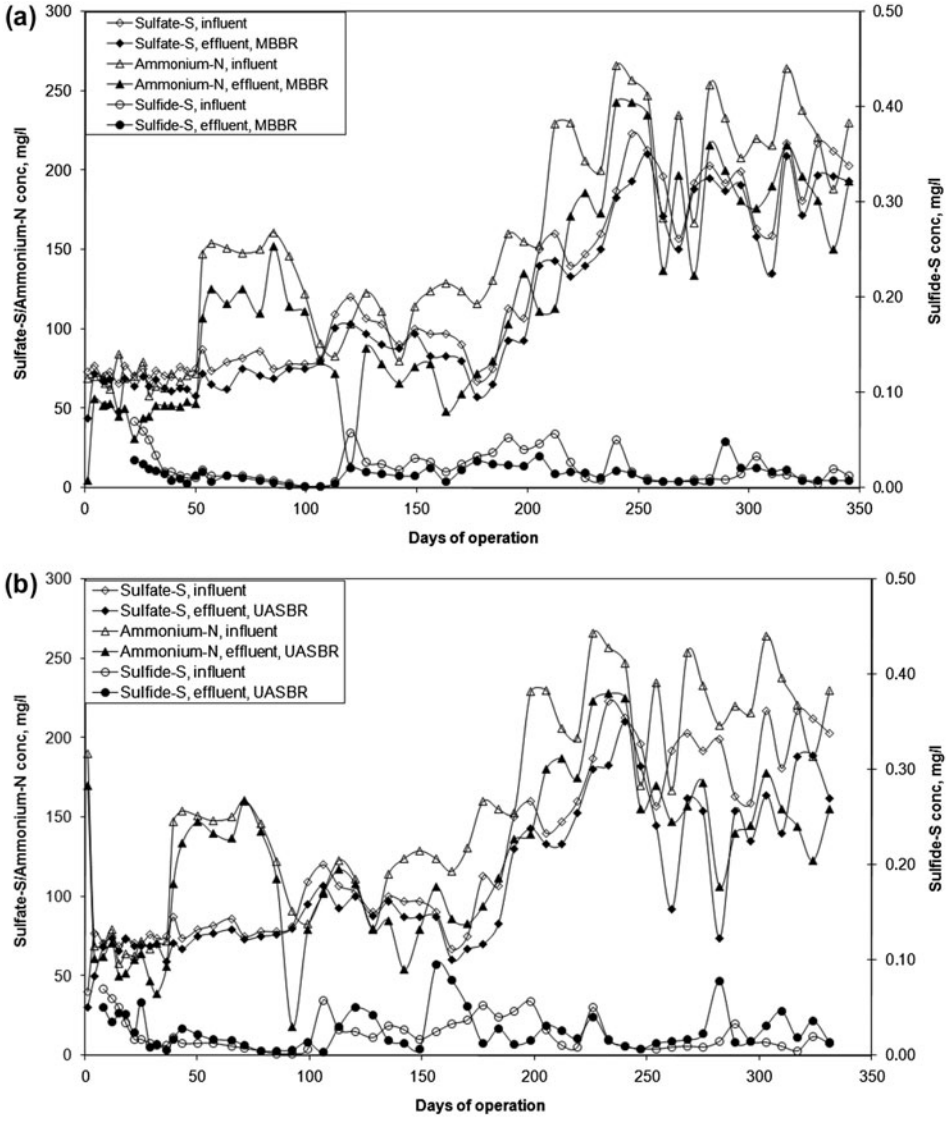


Fig. 4. Dynamics of $NH_4^+$-N and $SO_4^{2-}$-S in the (a) MBBR and (b) UASBR. Influent $NH_4^+$-N triangles, empty; effluent $NH_4^+$-N triangles, filled; influent $SO_4^{2-}$-S diamonds, empty; effluent $SO_4^{2-}$-S diamonds, filled. Influent sulfide-S: circles, empty; Effluent sulfide-S: circles,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

of Anammox bacteria by sulfur-utilizing autotrophic denitrifiers for available nitrite. $SO_4^{2-}$ 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). 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 sulfur compounds into $SO_4^{2-}$.

Most previous studies of SRAO [1,5–7] have reported a $\Delta NH_4^+/\Delta SO_4^{2-}$ stoichiometric ratio close to Eq. (1), with the only notable exception reported by Sabumon [23] that showed disproportionally higher $NH_4^+$ removal, similar to our results. Dissolved $O_2$ 0–0.2 mg/l in the influent was 0–0.2 mg/l, hence aerobic oxidation of $NH_4^+$ can be neglected. The observed $NH_4^+$ removal ratio might be due to complex interactions between organics, nitrogen, and sulfur compounds in the wastewater. Several mechanisms are possibly involved:

(1) Possibility of generation of reactive oxygen species such as $H_2O_2$ or $H^{\cdot}$ radicals, has been shown also in anaerobic medium. As a de-toxification mechanism, bacteria possessing

catalase enzymes can readily break $H_2O_2$ into water and $O_2$ that could be used by ammonium oxidizing bacteria for nitritation [23].

(2) HM, forming ⅔ of the total organics in reject water used in this study and present either in an oxidized (quinones) or reduced form (hydroquinones), can boost both biological and abiotic oxidation of $S^{2-}$ into $S_0$ and reduction of $NO_2^-$ and $NO_3^-$ into $N_2$ even if present at small concentration [23] This process eliminates toxic $H_2S$ and affects $NO_2^-$ concentration in two ways: HM-mediated $NO_2^-$ reduction competes with anammox process for available $NO_2^-$, on the other hand, additional $NO_2^-$ can be generated from reduction of $NO_3^-$ HM-mediated biological denitrification has been reported in the literature [24]. If HM mediate anammox process or can HM be an alternative electron acceptor for Anammox bacteria, need to be further studied.

(3) Re-oxidation of elemental sulfur or sulfide into $SO_4^{2-}$ can readily take place via sulfur-utilizing denitrification/denitritation, resulting in a partial restoration of $SO_4^{2-}$, which leads to an increase in the $\Delta NH_4^+/\Delta SO_4^{2-}$ ratio [25]. An evidence in favor of this pathway is provided by finding *Sulfurimonas denitrificans* DSM 1251 bacterium by pyrosequencing from sludge of UASBR as well as from seeding sludge.

(4) $NH_4^+$ can be physically adsorbed by sludge (biomass). This phenomenon has recently been demonstrated for aerobic granular sludge, indicating that the nitrification efficiency by aerobic granules would be overestimated if the adsorption contribution is neglected [26,27]. Physical adsorption or ion exchange of $NH_4^+$ have been shown to occur also in case of activated sludge or anammox granules, although at a smaller rate than in case of aerobic granular sludge [27]. Adsorption–desorption and ion exchange processes may also take place between other types of biomass and liquid phase, depending on the nature of extracellular polymers produced by the biomass.

Sulfide concentrations were lower than 100 μg/l (see Fig. 4) in the effluents of both reactors, indicating rapid sulfide oxidation. Low sulfide level prevented sulfide inhibition. Hydrazine was detected in the effluents of both reactors in the stationary operation phase (around 30 μg/L in both systems), indicating the anammox activity. Injections of hydrazine sulfate ($N_2H_4 \times H_2SO_4$) at low dosages (1 mg $N_2H_4$/l) into UASBR since day 268 showed TN removal efficiency increase over 30%. In case of MBBR, the mentioned dosage of hydrazine had virtually no effect.

### 3.4. Detected bacteria

In the MBBR *uncultured Planctomycetales bacterium clone P4*, originating from reject water and two other species close to it were present [16]. In UASBR only one species from the phylum *Planctomycetes* was found, originating from the inoculum (*Uncultured planctomycete clone* Pla_PO55-9 16S, GenBank: GQ356109.1). The two species from the phylum *Verrucomicrobia* (Uncultured *Verrucomicrobiales bacterium clone* De2102, GenBank: HQ183974 and *Uncultured bacterium clone* ATB-KS-1929, GenBank: EF686989) also originated from the *inoculum*.

The FISH studies indicated that Anammox bacteria were more abundant in the MBBR than in the UASBR

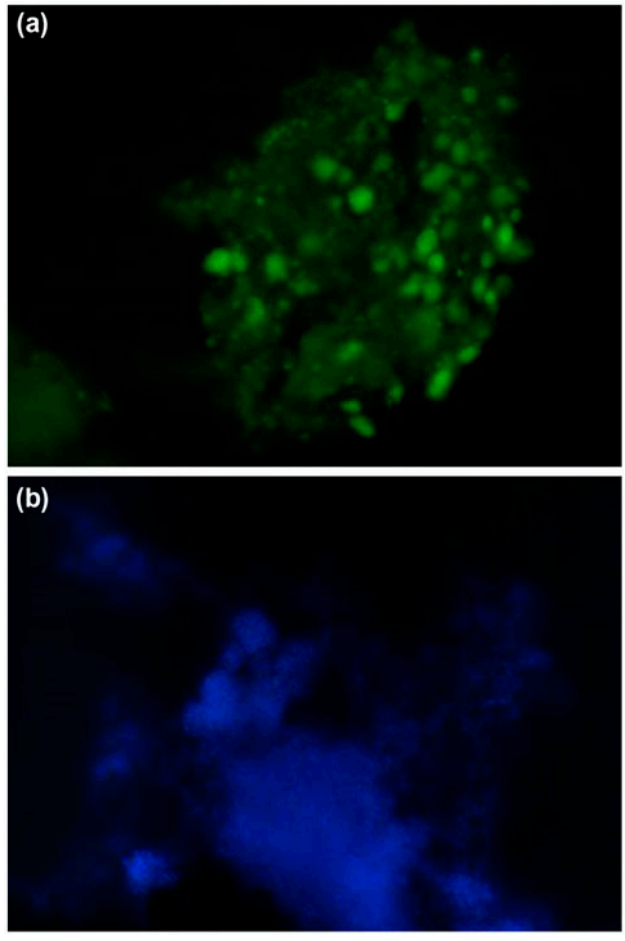


Fig. 5. Micrographs of the anammox biofilm, with a FISH staining displaying Anammox bacteria by Cy3-labeled Amx820 in MBBR (a) and by DAPI staining (b) of UASBR system after a 300 d of operation. The scale bar in the right corner is 50 μm.

biomass, which correlated with the higher substrate utilization rates present in the MBBR when compared with UASBR and showed higher Anammox-to-denitrification ratio in the MBBR compared to the UASBR (Fig. 5(a) and (b)).

## 4. Conclusions

The treatment of a supernatant from anaerobic sludge digestion by the SRAO process is feasible, however, due to low efficiency and low stability, unsuitable for most practical applications. There is still some perspective while occurring simultaneously with degradation of organics during the anaerobic treatment of some types of wastewaters, like vinasse or yeast wastewater. Maximum TN removal rates achieved at moderate temperature in MBBR and at higher temperature UASBR were similarly 0.03 kg-N/$m^3$/d and sulfate removal rates of around 0.01 kg-S/$m^3$/d. Species belonging to the phylum *Planctomycetales* were detected from the biofilm of the MBBR; from sludge of the UASBR species belonging mostly to the phylum *Verrucomicrobia* were found, indicating that the mechanisms for N removal in MBBR and UASBR were different. The SRAO process took place as one reaction of the multiple complex interactions between N-compounds, S-compounds, and organics (primarily HM), both biological and physicochemical in nature, resulting in a significantly higher removal ratio of $NH_4^+$ than SRAO stoichiometry predicts. According to UASBR performance and the composition of microbial community it can be assumed that the phylum *Verrucomicrobia* can also be involved in sulfate-dependent ammonium oxidation. Presence of hydrazine in the medium indicated the occurrence of the anammox process. Injections of intermediates had a positive effect only on the performance of the UASBR.

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