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Comparison of sulfate-reducing and conventional Anammox upflow anaerobic sludge blanket reactors

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Autotrophic NH_4^+ removal has been extensively researched, but few studies have investigated alternative electron acceptors (for example, SO_4^{2-}) in NH_4^+ oxidation. In this study, sulfate-reducing anaerobic ammonium oxidation (SRAO) and conventional Anammox were started up in upflow anaerobic sludge blanket reactors (UASBRs) at 36 (±0.5)°C and 20 (±0.5)°C respectively, using reject water as a source of NH_4^+. SO_4^{2-} or NO_2^-, respectively, were applied as electron acceptors. It was assumed that higher temperature could promote the SRAO, partly compensating its thermodynamic disadvantage comparing with the conventional Anammox to achieve comparable total nitrogen (TN) removal rate. Average volumetric NH_4^+-N removal rate in the sulfate-reducing UASBR1 was however 5–6 times less (0.03 kg-N/(m^3 day)) than in the UASBR2 performing conventional nitrite-dependent autotrophic nitrogen removal (0.17 kg-N/(m^3 day)). However, the stoichiometric ratio of NH_4^+ removal in UASBR1 was significantly higher than could be expected from the extent of SO_4^{2-} reduction, possibly due to interactions between the N- and S-compounds and organic matter of the reject water. Injections of N_2H_4 and NH_2OH accelerated the SRAO. Similar effect was observed in batch tests with anthraquinone-2,6-disulfonate (AQDS). For detection of key microorganisms PCR-DGGE was used. From both UASBRs, uncultured bacterium clone ATB-KS-1929 belonging to the order *Verrucomicrobiales*, Anammox bacteria (uncultured *Planctomycete* clone Pla_PO55-9) and aerobic ammonium-oxidizing bacteria (uncultured sludge bacterium clone ASB08 "*Nitrosomonas*") were detected. Nevertheless the SRAO process was shown to be less effective for the treatment of reject water, compared to the conventional Anammox.

[**Key words:** Sulfate-reducing ammonium oxidation; Upflow anaerobic sludge blanket reactor; Humic matter; Anammox intermediates; Autotrophic NH_4^+ removal]

Anammox-based technology for biological nitrogen removal has several advantages over conventional nitrification-denitrification since it needs less energy for aeration and no additional organic carbon is required due to autotrophic processes involved (1,2). Currently, the Anammox-related technology has the potential to achieve a neutral or even positive energy balance in complete wastewater treatment cycle (3–5) if, following to anaerobic treatment (biogas production), UASBRs are applied in the nitrogen removal stage (Driessen et al., 14th European Biosolids and Organic Resources Conference, 2010). Hence, this reactor type was selected in the study. The metabolic versatility of Anammox organisms, involving use of various substrates and electron acceptors has been shown (6). Although there is no genome information available on the ability of Anammox bacteria to consume SO_4^{2-} as electron acceptor, SO_4^{2-} reduction by them has been experimentally observed. A *Planctomycetes* bacterium named *Anammoxoglobus sulfate*, capable to oxidize NH_4^+ into NO_2^- using SO_4^{2-} as an electron acceptor, was isolated in 2008 from an enrichment culture (7). Sulfate-reducing ammonium oxidation (SRAO) process was first assumed by Fdz-Polanco et al. (8), who proposed a summary equation describing the two-staged process (Eq. 1), which has later been complemented with a possibility for sulfide formation noted previous literature (6,9,10) (Eq. 2):

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

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

In anaerobic, sulfide-saturated sediments of mesophilic springs carbohydrate fermentation and sulfur reduction are possible mechanisms employed by heterotrophic *Planctomycetes* for growth and survival (11). The alternative electron acceptors such as SO_4^{2-} may provide opportunities to reduce the need for aeration in the nitritation step preceding the Anammox process. This is especially important in the case of wastewaters with high content of both N- and S-compounds, such as the ones generated in oil refineries, fish canning, production of fertilizers (12), yeast factories etc. For wastewaters with high content of total nitrogen (TN), sulfate and organics, simultaneous removal of COD and nitrogen can thus be achieved in the anaerobic phase of treatment. Simultaneously the accumulation of toxic H_2S is avoided. If the SRAO process gives

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satisfactory results, dosage of sulfate (as Na_2SO_4, for example) or, in some cases even mixing the nitrogen-rich wastewater with sulfate-rich wastewater from food or fermentation industry instead of applying a pre-nitritation step would make the Anammox process even more energy-efficient.

The aims of this study were to achieve quick start-up of the Anammox UASBRs and to compare the SRAO process with the conventional Anammox process (inoculated with the same seed) using a real wastewater. Since addition of low-molecular quinoid analogs of humic matter has been reported as an option to increase TN removal efficiency of denitrifiers (13), the effects of AQDS were also researched.

MATERIALS AND METHODS

Reactor configurations, seeding procedure In this research 0.75 L thermostated UASBR1 (for SRAO) at 36 (±0.5)°C and 1.5 L volume UASBR2 (for conventional Anammox) at 20 (±0.5)°C were operated in parallel (Fig. 1). The influent was fed by periodically switched-on peristaltic pumps (SEKO, Italy). The hydraulic retention time (HRT) kept was one to two days.

Influent The effect of NO_2^- vs. SO_4^{2-} as Anammox electron acceptors was studied by feeding the reactors with reject water from anaerobic digestion of municipal wastewater sludge obtained from Tallinn municipal wastewater treatment plant. The latter was diluted with tap water. For the UASBR1, SO_4^{2-} was added to the influent as the solution of K_2SO_4 keeping an approximate molar NH_4^+/SO_4^{2-} ratio of 2. For feeding of the UASBR2 and batch tests, nitrite for the Anammox reaction was provided by adding $NaNO_2$ to ensure the influent molar NO_2^-/NH_4^+ ratio close to 1.32 as the optimum for Anammox reaction. Reject water contained Anammox microorganisms – *Planctomycetales* bacterium clone P4 (GenBank ID: DQ304521) and sufficient amounts of micro- and macronutrients for Anammox propagation (14). The influent of reactors had the following average ratio of carbon and nitrogen compounds: COD/TN = 0.78:1 (range 0.39:1–1.10:1). The biodegradability of influent expressed as COD/BOD_7 was 1.95:1 (range 1.82:1–2.03:1).

Seeding of UASBRs Both UASBRs were seeded with anaerobic sludge containing Anammox bacteria, obtained from the facility treating wastewater of the Salutaguse Yeast Factory (Salutaguse, Estonia) rich in both SO_4^{2-} and NH_4^+. After the inoculation, the volatile suspended solids (VSS) of UASBR1 and UASBR2 were 1.87 g/L and 1.10 g/L, respectively. The average TN removal rate in the UASB reactor of the Salutaguse yeast factory wastewater treatment facility was around 4.80 kg-N/(m^3 day).

Batch assays To study the effect of a model compound of a quinone analog for humic matter (HM) – anthraquinone-2,6-disulfonate disodium salt (AQDS) – on TN and SO_4^{2-} removal rate by Anammox bacteria, batch assays were conducted at 20 (±0.5°C). The same temperature was selected for batch tests with NO_2^- and with SO_4^{2-} as electron acceptors in order of better comparability of batch tests at least with UASBR2. Stable temperature was maintained using a water bath thermostat (Assistent® 3180, Glaswarenfabrik Karl Hecht GmbH, Germany). Batch tests were performed using sludges from UASBR1 and UASBR2, maintaining a VSS concentration of 1.8–2.0 g/L. $(NH_4)_2SO_4$-water solution was used as a synthetic Anammox medium in case of UASBR1 and NH_4Cl–$NaNO_2$-water solution in case of UASBR2. Acidic solution (3 mL) and 3 mL of alkaline solution of micronutrients were added into the substrate of batch tests along with the 40 mL of macronutrients solution as described in previous literature (12,15).

The procedure of the batch tests and analytical methods has been described in detail in previous literature (14). Data and statistical analyses were performed by the MS Excel 2010 Analysis ToolPak. Homogeneity of group variances and the difference between group means were checked using the F-test and the two-way *t*-test, respectively. The level of significance was set at $\alpha < 0.05$.

PCR-DGGE, sequencing and phylogenetic analysis The PCR-DGGE was performed as described in previous literature (12,15). 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 from the GenBank (http://www.ncbi.nlm.nih.gov/genbank/). The samples from the treatment facility of Salutaguse Yeast factory were pyrosequenced at the Integrated Systems Biology Centre of Tallinn University of Technology. Universal 8F and 357R primers 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 (Plaza et al., Proceedings of the IWA-WEF Spec. Conf. Nutrient Recovery and Management, Miami, FL, 2011). 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.

RESULTS AND DISCUSSION

Description of operation of the reactors Operation of the reactors was divided into periods based on the characteristics of effluent quality, HRT, loading rates (and in the final, IV period, dosage of intermediates as discussed below). The main parameters of operation of the UASBR1 (SRAO) and the UASBR2 (Anammox) are given in Table 1. The UASBRs were started up with HRTs of one day. Selection of the HRT was based on comparison with other studies (9,16). Surprisingly, the seeding sludge showed a more rapid adaptation in the UASBR2 than in the UASBR1, and in the latter the TN removal rates were significantly (p-value <0.05) lower than in the UASBR2 (see Table 1, Figs. 2 and 3).

Considering disproportionally high ratio of $\Delta NH_4^+ - N_{consumed}/\Delta SO_4^{2-} - S_{consumed}$, comparing with the ratio emanating from Eq. 1 or 2, the NH_4^+ content in the influent for the UASBR1 was doubled between days 41–100 while the SO_4^{2-} content was retained unchanged (Table 1, Fig. 2a). For the UASBR1, the increased concentration of NH_4^+ in the influent had no obvious effect on promoting the TN removal, although higher substrate concentrations have been reported to facilitate the SRAO reaction in earlier studies (9). The data presented in Table 1 show that at comparable ammonium loading rates, nearly complete deammonification was achieved in UASBR2 while in the UASBR1 it remained less than 1/3.

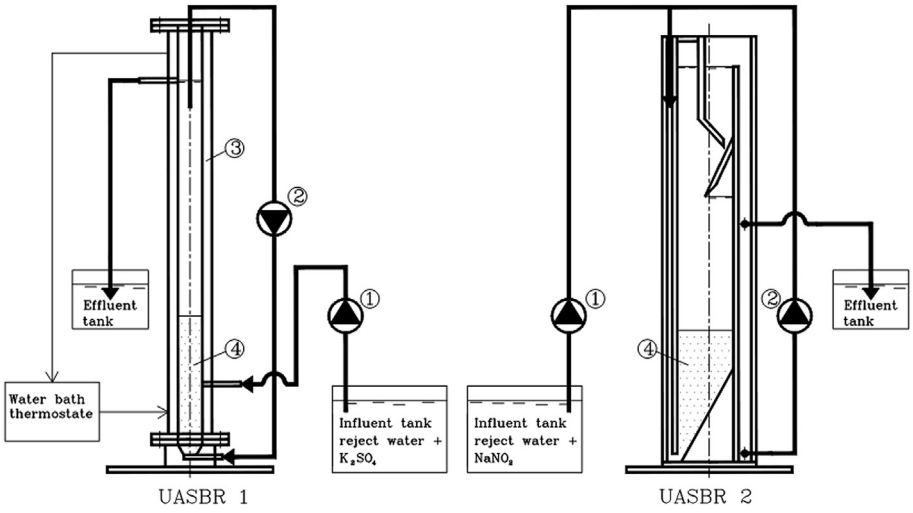

FIG. 1. Scheme of the UASBR1 (performing SRAO) and the UASBR2 (performing conventional Anammox process).