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Sulfur-based denitrification treating regeneration water from ion exchange at high performance and low cost

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Abstract

Autotrophic denitrification with sulfur is an underexplored alternative to heterotrophic denitrification to remove nitrate from wastewater poor in organics. The application on ion exchange regeneration water (19.4-32.1 mS cm^{-1}) is novel. Three fixed bed reactors were tested at 15°C for >4 months, inoculated with activated sludge from sewage treatment. All were fast in start-up (<10 days) with high performance (94±2% removal efficiency). pH control with NaOH rendered higher nitrate removal rates than limestone addition to the bed (211±13 vs. 102±13 mg N L^{-1} d^{-1}), related to higher pH (6.64 vs. 6.24) and sulfur surface area. Bacterial communities were strongly enriched in *Sulfurimonas* (63-67%) and *Thiobacillus* (24-26%). In an economic comparison, sulfur-based denitrification (€5.3 kg^{-1} N) was 15% cheaper than methanol-based denitrification (€6.22 kg^{-1} N) and both treatments were opex dominated (85.9 vs. 86.5%). Overall, the technological and economic feasibility should boost further implementation of sulfurotrophic denitrification.

Keywords: IEX, biological nitrogen removal, capex, opex, neutralization

1. Introduction

Since the development of the Haber-Bosch process, mankind's disruption of the nitrogen cycle caused reactive nitrogen species to accumulate in the environment, one of the most serious sustainability threats at a global scale (Steffen et al., 2015). In Flanders for example, a nutrient flux hotspot, 20 kg N per capita per year is released into the environment, of which 28% to surface water causing eutrophication, toxic algal blooms, fish mortality and biodiversity loss (Coppens et al., 2016). Nitrate is a major reactive nitrogen species, present in treated municipal and industrial wastewaters, but also in some types of raw industrial wastewater. Biological denitrification is typically proposed as a cost-effective solution to remove nitrate, converting it to harmless nitrogen gas.

Some types of wastewater, ion exchange (IEX) regeneration water for example, contain nitrate but are devoid of organics or other suitable electron donors, rendering the need for external dosage. Several denitrification opportunities arise for such streams: heterotrophic denitrification *via* the addition of organic carbon, such as methanol, or autotrophic denitrification using sulfur or hydrogen gas as electron donor. Environmental and economic considerations would favor autotrophic denitrification, as sulfur and hydrogen gas are more sustainable and cheaper than methanol (Park & Yoo, 2009). Furthermore, autotrophic denitrification is characterized by lower sludge yields of 0.4-0.57 g VSS g^{-1} N (Oh et al., 2000; Park & Yoo, 2009) compared to heterotrophic denitrification (0.8-1.2 g VSS g^{-1} N) (Wiesmann, 1994), with concomitant lower sludge disposal cost. A detailed economic assessment is not available, but the advantages indicate potential cost savings, provided these are not outweighed by costs associated with pH correction and required reactor volume, directly linked to achievable nitrogen removal rates. Although sulfur-based denitrification has very few applications compared to heterotrophic denitrification, its technological readiness is slightly superior to hydrogen-based denitrification. Safety measures coupled to the use of hydrogen gas further substantiates the choice for sulfur-based denitrification.

The stoichiometry of sulfur-based denitrification is given by the equation below (1) (balanced for charge and elements) (Koenig & Liu, 2001).

$$1.11S + 1.06NO_3^- + 0.78H_2O + 0.3CO_2 + 0.086NH_4^+ \rightarrow 1.11SO_4^{2-} + 0.543N_2 + 0.06C_5H_7O_2N + 1.484H^+ + 0.238\ e^- \quad (1)$$

Elemental sulfur is oxidized to sulfate (2.39 g S g^{-1} N), for which the discharge limit for the receiving waterbody should also be taken into account. Insufficient buffering leads to a pH drop, alkalinity is required at a ratio of 5.93 g $CaCO_3$ g^{-1} N or 2.48 g $CaCO_3$ g^{-1} S to counter this drop in pH and to provide inorganic carbon for growth. When insufficient alkalinity is present in the wastewater, limestone can be mixed with the sulfur particles in a packed bed reactor. The denitrification rate is influenced by the available sulfur contact area and hence sulfur particle size, in which smaller sulfur particles bring about a higher surface area and concomitant higher nitrate removal rates (Koenig & Liu, 2001; Moon et al., 2006) The denitrification rate is also affected by pH and might be inhibited at values lower than 6.8 (Liu & Koenig, 2002). Optimal pH conditions range from 6.8 to 8.2 (Koenig & Liu, 2001) and the optimal temperature is about 35°C (Belmonte et al., 2016).

Few studies have evaluated elemental sulfur-based denitrification at lower temperatures

(<20°C), but all report a decrease in denitrification rate (Koenig & Liu, 2004; Sahinkaya et al., 2014). A full scale experiment for the treatment of groundwater reported nitrate removal rates of 120 mg N L^{-1} d^{-1} at temperatures <20°C (Schoonenberg et al., 1994). Furthermore, decreased denitrification rates are expected in saline wastewaters, as a 10% decrease of activity at 10 g NaCl L^{-1} has been reported (Campos et al., 2008). Although research on groundwater is available, research on industrial wastewater with high nitrate levels is limited to nitrified landfill leachate and diluted wastewater from steel industry (Lee et al., 2001; Nugroho et al., 2002).

In this study, the technological and economic feasibility of nitrate removal from ion exchange regeneration water was tested using sulfur-based denitrification, in an upstream fixed-bed reactor, with different external (NaOH) and internal (limestone) pH control mechanisms. The main challenges of this research related to the low temperature (15°C), relatively high salinity (18.5-32.3 mS cm^{-1}) and the variability of the influent characteristics. Additionally, activated sludge from sewage treatment was investigated as a suitable inoculum, because of its widespread availability, and the evolution of the bacterial community was investigated. Furthermore, it was investigated whether neutralization of the IEX regeneration water with limestone (instead of NaOH) would be feasible and improve the feasibility of the overall treatment train.

2. Materials and Methods

2.1. Influent characteristics and reactor setup

Six batches of IEX regeneration water were collected, originating from a full-scale plant demineralizing tap water. Five batches were industrially neutralized with NaOH while the sixth batch was collected prior to the neutralization (**Table 1**). The wastewater contained nearly no biodegradable organic carbon (3.3 ± 0.7 mg BOD_5 L^{-1}), phosphate (0.11 ± 0.04 mg P L^{-1}) and ammoniacal nitrogen (2.3 ± 1.0 mg TKN L^{-1}). It was rich in nitrate, high in conductivity and contained some alkalinity (**Table 1**). Phosphate and ammonium were added to allow microbial growth (1.5 mg P L^{-1} and 10 mg N L^{-1}).

The wastewater was treated in three parallel upstream packed bed reactors filled with sulfur particles (no replicates) (Supplementary information, section 1). For the variations tested in bed characteristics and pH control strategies, no replicate systems could be operated, yet the induced differences in performance were logical and consistent over time. The first reactor (L/S 0) contained only sulfur particles and pH was controlled by using 0.1 M HCl or NaOH solutions. Limestone was added to the second reactor (L/S 2.28) for pH control with a limestone/sulfur ratio of 2.28 on weight basis. This was the stoichiometric ratio considering the alkalinity present in the first batch of wastewater (51 ± 4 mg $CaCO_3$ L^{-1}) and the maximum expected nitrate concentration (100 mg N L^{-1}) in the IEX regeneration water, resulting in 0.22 g $CaCO_3$ g^{-1} S already present. For the third reactor (L/S 2.5), the available alkalinity was not taken into account and a limestone/sulfur ratio of 2.5 was used on weight basis, thus providing excess alkalinity. All reactors had a bed volume of 0.8L (including void volume) and total volume of 0.9L. Recirculation was imposed to fix the upflow velocity at 1 m h^{-1}, in accordance with the upflow velocity used in full-scale experiments (Schoonenberg et al., 1994). The volumetric nitrogen loading rate was changed by varying the influent flow rate, and thus the hydraulic retention time (HRT) (0.15-0.77d). All reactors were operated at 15°C. The sulfur particle size was 3-4mm, and limestone particle size was 5-7mm. All reactors were inoculated with 0.7 g VSS L^{-1} return activated sludge from a municipal wastewater treatment plant (Ghent, Belgium). Reactor operation consisted of several experimental phases as elaborated below (**Table 2**).

2.2. Reactor operation

2.2.1. Phase I: Target nitrogen **removal** *rate*

The earlier reported removal rate of 120 mg N L^{-1} d^{-1} in full-scale experiments on groundwater (<20°C) (Schoonenberg et al., 1994) was set as the target removal rate. The maximum expected nitrogen concentration was 100 mg N L^{-1}. Taking into account a removal efficiency of >90%, the loading rate was set at 130 mg N L^{-1} d^{-1} in order to safely meet a discharge limit of 15 mg N L^{-1}. In order to track the time needed to reach the target removal rate, daily samples of influent and effluent were taken for the determination of nitrogen concentrations, effluent biomass concentration, pH and EC.

2.2.2. Phase II: Maximum nitrogen removal rate

After achieving the target nitrogen removal rate, the loading rate was increased equally in all reactors to determine the maximum nitrogen removal rate. Loading rates were kept high to ensure sufficient substrate (nitrate) presence as not to limit the nitrogen removal rate. Again, reactor performance was monitored in time.

2.2.3. Phase III: Effect of pH, sulfur particle size and limestone/sulfur ratio

The L/S 0 reactor was used to determine the effect of the pH on the maximum denitrification rate. In phase I and II, the pH setpoint was 6.8. In phase III.a (day 52), the setpoint was changed to 6.2. After achieving a stable nitrogen removal rate (day 104, phase III.b), the pH was again increased to 6.8 to determine the reversibility of the pH-induced effect. In the L/S 2.28 reactor, the effect of sulfur surface area was tested by decreasing the sulfur particle size. Hereto, half of the sulfur and limestone bed were taken out of the reactor and crushed into smaller particles, which were subsequently blended with the remaining particles in the reactor. For the L/S 2.5 reactor, additional limestone was provided in two steps to see whether the effluent pH would become dischargeable (pH >6.5). In a first step (day 60), the limestone to sulfur ratio was increased to 2.7 and subsequently (day 98) to 2.8.

2.2.4. Phase IV: Neutralization with limestone and effect on denitrification

The used IEX regeneration water was acidic (pH = 1.94 ± 0.08). In industry, it is neutralized using NaOH (pH = 6.5-7.8). As potential add-on cost saver, wastewater neutralization with a limestone bed was examined, as pre-treatment to denitrification. In order to find a suitable HRT at which a neutral pH is obtained, non-neutralized wastewater was passed through the bed at five different HRTs (0.7, 1.25, 4.0, 8.0 and 20 hours). Subsequently, a 75mL limestone bed was added prior to the third bioreactor (L/S 2.5) at day 118, after which non-neutralized wastewater was fed to the combined train of limestone bed and denitrification reactor (Supplementary information, section 1). Actual HRT imposed to the neutralization bed was 1.15h. The nitrogen removal rate was monitored through time after changing to non-neutralized wastewater.

2.3. Bacterial community analysis

Samples were collected for bacterial community analysis from the inoculum, from each reactor at the end of phase II (day 56) and at the end of operation (day 137, 131 and 133 for L/S 0, 2.28 and 2.5 respectively). Samples were stored at -20°C prior to DNA extraction, which was performed as described previously (Vilchez-Vargas et al., 2013). The quality of the DNA extracts was validated with agarose gel electrophoresis. The DNA extracts were sent to LGC Genomics GmbH (Berlin, Germany) for Illumina sequencing on the Miseq platform. The sequencing data are deposited at the NCBI (National Center for Biotechnology Information) database under accession number PRJNA421155. Amplicon sequencing and data processing was performed as described in Supplementary material, section 2.

2.4. Chemical analyses

Nitrite, nitrate and sulfate were determined on a 761 Compact Ion Chromatograph (Metrohm, Switzerland). Ammonium nitrogen (*via* steam distillation) and total suspended solids (TSS) and volatile suspended solids (VSS) were analyzed according to Standard methods (APHA, 1992). The pH was measured with a Consort C5010 meter equipped with a Consort pH electrode (Consort, Belgium) and conductivity with a Consort C6010 meter with a Metrohm conductivity probe (Metrohm, Switzerland).

2.5. Statistical analyses

Statistical analysis was applied to determine whether certain nitrate removal rates or pH values were statistically significantly different. Prior to testing the null hypothesis, the data was screened and explored with boxplots. Normality was examined visually using normal QQ-plots and as a formal normality hypothesis test, a Shapiro Wilks test on the residuals was applied. The homogeneity of variances was checked with the Bartlett test.

In case that normality and homoscedasticity could be assumed, the null hypothesis was tested with one-way ANOVA. Pairwise differences or contrasts between values were tested with Tukey. In case of normality but heteroscedasticity, the null hypothesis could be tested with one-way ANOVA without the assumption of equal variances (also called Welch ANOVA). Pairwise differences or contrasts between values were tested with the Games-Howell Post-Hoc test (Post-Hoc test with Welch's correction). In case that normality could not be assumed, the null hypothesis was tested with a Kruskal Wallis rank sum test (nonparametric test) instead of one-way ANOVA. Pairwise Wilcoxon Rank Sum Tests with Holm correction for multiple testing were applied to determine the pairwise difference between values. All formal hypothesis tests were conducted on the 5% significance level ($p=0.05$), except for the homogeneity of variances (1%). All statistical analyses were executed in R version 3.3.1 (2016-06-21) on an x86_64-w64-mingw32/x64 (64-bit) platform running under Windows 8.1 Enterprisex64 (build 9600).

2.6. Economic comparison of sulfur- vs. methanol-based denitrification

To corroborate the hypothesized economic advantage of autotrophic denitrification over heterotrophic denitrification, a comprehensive economic comparison was conducted for the complete treatment of acidic IEX regeneration water, i.e. including neutralization and denitrification. Neutralization with NaOH dosage was compared to the use of a limestone bed. For denitrification, three scenarios were assessed: (i) sulfur-based denitrification with NaOH dosage for pH control (as applied in L/S 0), (ii) sulfur-based denitrification with limestone incorporated in the bed (as applied in L/S 2.28) with additional post-neutralization with NaOH to ensure a dischargeable effluent pH, and

(iii) methanol-based denitrification using HCl for pH control in a moving bed biofilm reactor (MBBR). In each scenario, a sedimentation tank was included, separating the effluent from the produced sludge which went to disposal. Capital expenditures (capex) for the reactor basin and sedimentation tank entailed construction, civil works, engineering, piping, pumps, pH sensor and transmitter and profit/risk. Operational expenditures (opex) comprised chemicals, pumping and mixing, sludge disposal, personnel, analyses and maintenance. As chemicals and sludge disposal considerably contributed to the denitrification cost, a sensitivity analysis was included evaluating the effect of individual and combined cost changes of 50%, up and down. Extensive details on data and assumptions linked to influent characteristics, process design and operation, depreciation and the sensitivity analysis can be found in supplementary material, section 3.

3. Results and discussion

3.1. Phase I: Target nitrogen removal rate

A fast start-up was observed, achieving the target removal rate after approximately 10 days (**Fig. 1**). Effluent nitrate concentrations were less than 5 mg N L^{-1} in all reactors and no nitrite accumulation occurred. Near stoichiometric sulfate production was observed from day 2 onward in L/S 2.28 and 2.5 and from day 14 in L/S 0, indicating autotrophic nature of the occurring denitrification (**Fig. 2, B**). The slightly slower start-up of L/S 0 was attributed to problems with the pH control in the first few days (Supplementary material, section 4). During phase I, the pH in L/S 2.28 and 2.5 decreased from pH 7.2 to 6.4, which was below the standard limit of discharge (pH>6.5).

3.2. Phase II: Maximum nitrogen removal rate

The highest maximum nitrate removal rate was obtained in L/S 0 (235 mg N L^{-1} d^{-1}), about a factor 1.8 higher than L/S 2.28 and L/S 2.5 (130 and 132 mg N L^{-1} d^{-1} respectively) (**Fig. 1**). This can partly be attributed to the higher sulfur content and thus surface area, since L/S 0 is filled only with sulfur particles (Liu & Koenig, 2002). Additionally, the lower pH in L/S 2.25 and 2.5 (6.24 ± 0.19 and 6.26 ± 0.21) compared to L/S 0 (6.64 ± 0.21) could have contributed. In L/S 0, a nitrate removal efficiency of 94 ± 2% was obtained, yielding dischargeable effluent quality (pH>6.5 and <15 mg N L^{-1}). There was no difference in maximum activity in L/S 2.28 and L/S 2.5, meaning a stoichiometric dosage of limestone sufficed. Removal rates were comparable to full scale experience for the treatment of groundwater (120 mg N L^{-1} d^{-1}) (Schoonenberg et al., 1994).

In L/S 2.28 and L/S 2.5, nitrite accumulation up to 2.6 mg L^{-1} was observed during phase II. The high loading rate in L/S 2.28 and L/S 2.5 (249 ± 29 and 270 ± 27 mg N L^{-1} d^{-1} respectively) resulted in low nitrate removal efficiencies of 42.3 ± 6.1 and 34.7 ± 6.7 respectively. The incomplete denitrification and concomitant accumulation of intermediates such as nitrite could be attributed to the slow dissolution of elemental sulfur, being the rate limiting step at high loading rates (Koenig & Liu, 2001). As L/S 0 contained more sulfur, the sulfur release rate was probably not limiting as no nitrite accumulation occurred. The average nitrogen removal rate in L/S 0 during phase II (211.2 ± 12.9 mg N L^{-1} $d^{-}1$) was significantly different (p<0.05) from the rates obtained in L/S 2.28 and 2.5 (101.9 ± 12.9 and 92.7 ± 14.6 mg N L^{-1} d^{-1} respectively).

3.3. Phase III: Effect of pH, sulfur particle size and limestone/sulfur ratio

In order to assess the contribution of the pH difference to the lower activity observed in L/S 2.28 compared to L/S 0, the pH of L/S 0 was varied during phase III. Firstly, it was lowered from 6.63 ± 0.21 in phase II to 6.38 ± 0.20 in phase III.a. Due to tube clogging between phase II and III in the recirculation of L/S 0, pH shortly dropped to 4.9, resulting in a severe drop in denitrifying activity on day 49. However, the nitrogen removal rate recovered fast and was stable at about 172.5 ± 16.8 mg N L^{-1} d^{-1}. A pH drop thus led to a significantly lower activity ($p<0.05$), preserving 87.0 ± 21.4 % of the activity (**Fig. 1, III.a**). Increasing the pH again to 6.67 ± 0.19 during phase III.b brought about a concomitant increase in activity (**Fig. 1, III.b**). After a lag phase, denitrifying activity rose up to 394.3 mg N L^{-1} d^{-1} on day 133. Reactor operation was stopped before reaching a stable rate. The drop in pH was significant ($p<0.05$), whereas the average value of the two higher pH values was not significantly different ($p>0.05$). The observed effect of the pH on denitrification activity is in accordance with literature, stating that a pH below 6.8 is sub-optimal and lowering the pH renders lower rates (Liu & Koenig, 2002). The sudden increase in activity during phase III.b could partially be attributed to the increase in pH closer to the optimum range, but also to an increase in biomass concentration since this amounted up to 4.07 ± 0.04 g VSS L^{-1} at the end of the reactor operation compared to the inoculated concentration of 0.7 g VSS L^{-1} at start-up. Reasons for the high removal rates could be linked to a relatively high sulfur specific area, suitable pH, high biomass enrichment and the fact that the effluent could still contain some nitrate, engaging a bigger portion of biofilm and stay further above the apparent affinity indices.

The impact of pH on the removal rate, however, did not fully explain the difference observed between L/S 0 and L/S 2.28 in phase II. A second contributing factor was the higher sulfur content and thus surface area in L/S 0, as it was filled only with sulfur particles whereas L/S 2.28 was packed with limestone as well. To investigate the impact on the removal rate, the particle size in L/S 2.28 was manually decreased by crushing the particles, thus increasing the sulfur surface area and potential sulfur release rate. The average sulfur particle diameter was decreased from 3.05 ± 0.98 to 1.67 ± 0.87 mm. This change resulted in an instant significant increase in denitrifying activity ($p<0.05$), with an average during phase III of 206.8 ± 30.7 mg N L^{-1} d^{-1}, about a factor 2 higher than the average rate during phase II (**Fig. 1, B II and III**). Particle size greatly affects the denitrification rate, with smaller particles bringing about higher rates due to a higher total surface area (Koenig & Liu, 2001; Moon et al., 2006). However, when sulfur particles become too small, washout might become an issue. Indeed, during phase III in L/S 2.28, small sulfur particles were occasionally observed in the effluent. A second concern when particle size becomes too small is clogging of and short circuiting of fluid flow in the bed, giving rise to technical issues and anaerobic zones. A thoughtful trade-off should be considered between denitrification rate and particle size to maximize rate but minimize potential problems. It is thus evident that both the pH and sulfur surface area played a part in the dissimilarity in maximum rate between L/S 0 and L/S 2.28 during phase II.

During phase II in L/S 2.28 and L/S 2.5, the effluent pH was below the discharge limit of 6.5. To alleviate this problem, the L/S ratio was increased to obtain better pH control and, by doing so, be able to discharge at acceptable pH levels. This approach was imposed on L/S 2.5, by increasing the L/S ratio to 2.7 on day 60 and subsequently to 2.8 on day 98. At an L/S ratio of 2.7, pH significantly increased ($p<0.05$) from 6.26 ± 0.21 during phase II to 6.60 ± 0.40 between day 60 and 98 (Supplementary material, section 4). The denitrification rate slightly yet insignificantly increased ($p<0.05$) to 97.7 ± 9.1 mg N L^{-1} d^{-1}. After increasing further to an L/S ratio of 2.8, the pH and nitrate removal rate significantly increased ($p<0.05$) to 6.95 ± 0.22 and 118.3 ± 18.1 mg N L^{-1} d^{-1} respectively. This increase was attributed to the pH, as it moved closer to the optimum range as mentioned before. Increasing the L/S ratio does decrease the total sulfur content in the reactor, which negatively impacts the denitrification rate. However, better controlling the pH and achieving a pH in the optimum range by using this approach, renders slightly higher denitrification rate, thus outweighing the disadvantage of the lower sulfur content.

3.4. Phase IV: Neutralization with limestone and effect on denitrification

As an alternative to the use of NaOH to neutralize the acidic IEX regeneration water, passage through a limestone bed was investigated. At low pH, limestone dissolved very rapidly but dissolution rates slowed down at pH values above 6 (Supplementary material, section 5). A HRT of 0.68h resulted in a pH of 6. Further elevating the HRT yielded less improvement, increasing to pH 6.48 at 19.84 hours. It was judged that 0.68h, i.e. 6-21 times less than the HRT applied in the denitrification reactors, could be a practically feasible retention time, ensuring that the savings in NaOH dosage would outweigh the cost of the required limestone bed volume. The alkalinity after passing a limestone bed at a HRT of 0.68h was 171 mg $CaCO_3$ L^{-1}, or about 1.7 g $CaCO_3$ g^{-1} N when assuming the maximum expected nitrate concentration in the IEX regeneration water (100 mg N L^{-1}). Besides increasing pH, the limestone bed also elevated the alkalinity and buffering capacity of the wastewater, bringing it to 29% of the stoichiometric need (5.9 g $CaCO_3$ g^{-1} N), whereas the first batch of neutralized IEX regeneration water only contained 8.6%. Using a limestone bed could thus, by providing additional alkalinity, decrease the NaOH consumption during denitrification in the sulfur packed bed reactor (L/S 0), and theoretically lower the required L/S ratio down to 1.8 for beds containing limestone.

To test the feasibility of combined limestone neutralization and denitrification, a limestone bed was inserted before the L/S 2.5 reactor with a HRT of 1.15h. Non-neutralized wastewater was fed to the reactor to investigate the effect on denitrification performance. The pH of the non-neutralized wastewater was 1.94 ± 0.08, which increased to 5.80 ± 0.24 after the limestone bed and eventually to 6.32 ± 0.15 in the effluent. The denitrification rate slightly decreased from 103.4 ± 15.1 to 78.1 ± 10.0 mg N L^{-1} d^{-1}, possibly due to the lower pH of the incoming wastewater in the sulfur bed (6.69 ± 0.39 vs. 5.80 ± 0.24 in phase III.b and IV respectively). Using some NaOH or a larger neutralization bed, might increase reactor performance. Overall, partially neutralizing the wastewater in a short-contact limestone bed allowed for good reactor performance.

3.5. Strong enrichment of sulfurotrophic denitrifiers

The community structure in the packed bed reactors was monitored over time through sequencing of the V3-V4 region of the bacterial 16S rRNA gene (**Fig. 3**). The observed fast start-up of each reactor would suggest that the inoculum already contained a considerable abundance of sulfur autotrophic bacteria. However, the abundance of putative sulfurotrophs was very low, e.g. related to the genera *Sulfurovum* (0.011%), *Sulfurospirillum* (0.027%), *Sulfuritalea* (0.752%), *Thiothrix* (0.023%), *Thiobacillus* (0.001%), *Sulfurimonas* (0.007%) and *Sulfuricurvum* (0.006%). After phase II (day 56), the L/S 0 community was most enriched, containing 76% *Sulfurimonas* and 13% *Thiobacillus* compared to 29 and 32% *Sulfurimonas* and 9 and 18% *Thiobacillus* in the communities of L/S 2.28 and 2.5, respectively. In L/S 0, the higher nitrate removal rate and hence cumulatively converted load during phase II likely caused faster enrichment. By the end of the operation, the bacterial community of all reactors was dominated by sulfurotrophs, with 67, 63 and 65 % *Sulfurimonas* and 24, 24 and 26% *Thiobacillus* in L/S 0, L/S 2.28 and L/S 2.5 respectively. *Sulfurimonas* was always the most dominant autotrophic denitrifier genus, although earlier reports usually highlight *Thiobacillus denitrificans* as dominant species in S-based denitrification (Koenig et al., 2005). *Sulfurimonas* species are widespread and are commonly isolated from sulfidic habitats and have been detected in denitrifying reactors (Han & Perner, 2015; Zhou et al., 2017).

3.6. Economic feasibility of sulfur- versus methanol-based denitrification

An extensive economic evaluation of sulfur autotrophic denitrification for IEX regeneration water was performed. The economics of neutralizing the originally acidic wastewater (NaOH vs. limestone bed) and the subsequent biological treatment (sulfur-based vs. methanol-driven) was included (**Table 2, Table 3**).

For neutralization, the inclusion of a limestone bed enables to save 31% of the overall neutralization cost, and is therefore recommended for future implementation. Total neutralization costs were 0.40 and 0.28 EUR m^{-3}, for treatment with NaOH and Limestone+NaOH, respectively, as calculated from Table 3 (4.46 and 3.07 EUR kg^{-1} $N_{removed}$) with a removed nitrate concentration of 0.09 kg N m^{-3}. For both, opex is the largest cost class, representing 67.3 and 51.5% of the overall neutralization cost. The limestone bed increased capex only with 2%, but enabled 70.1% savings on the cost of the chemicals.

When deciding to go for autotrophic denitrification, total cost savings of around 15% could be expected when comparing to methanol-based denitrification. Most savings originated from sludge disposal, as autotrophs produce considerably less biomass. Further contribution could be found in energy and addition of chemicals. For energy, the rather intensive mixing requirement for the MBBR caused the cost difference. For chemicals, methanol addition amounted up to €1.06 kg^{-1} N whereas the cost for sulfur consumption was only €0.48 kg^{-1} N. The three treatment scenarios were opex driven systems, constituting 85.9, 77.4 and 86.5% of the total denitrification cost for the S/NaOH, S/L and Methanol/HCl scenario respectively. The additional alkalinity provided by the limestone bed results in extra operational cost savings of 1.7-3.8%.

The sensitivity analysis of the denitrification cost in the S/NaOH, S/L and Methanol/HCl scenario revealed that 50% variation in individual influential cost factors (chemicals and sludge disposal) changed the total denitrification costs by no more than 10.2%, while varying costs for multiple factors at the same time influenced the total denitrification cost up to 22% (Supplementary material, section 3). In the unlikely event that the price for methanol, HCl and sludge disposal would each decrease by 50%, the autotrophic treatment would be 3% more expensive than the heterotrophic treatment. In all other evaluated scenarios (increase and decrease of cost), autotrophic denitrification was between 1 and 26% cheaper than heterotrophic denitrification.

The combination of limestone neutralization with subsequent sulfur-based denitrification is proposed as a new treatment train for IEX regeneration water, resulting in a total cost of €8.37 kg^{-1} N, or €0.75 m^{-3}. Compared to the more conventional treatment of NaOH neutralization followed by methanol-based denitrification (€10.69 kg^{-1} $N_{removed}$ or €0.96 m^{-3}), total cost savings of 21.7% are expected. Total treatment cost in both scenarios are similarly divided among neutralization (±40%) and biological treatment (±60%).

3.7. From feasibility to implementation

For the first time, the combined technological and economic feasibility of sulfur-based denitrification for the treatment of IEX regeneration water was demonstrated. Next steps in the development of this technology could be pilot trials of the full treatment pipeline, including automation, realistic bed heights, bed refilling and long-term operation to evaluate the day-to-day robustness and the need for backwashing to prevent clogging and harvest biomass. To further validate the sustainability of the technology compared to heterotrophic denitrification, N_2O emissions and mitigation strategies should be investigated, as N_2O is a major environmental issue (Desloover et al., 2012). As sulfate is produced, the discharge limit must be taken into account which can depend on country and type of industry and receiving waterbody. In this specific case, dischargeable effluent quality was obtained as the produced sulfate (177-754 mg S L^{-1}) was below the imposed discharge limit (1000 mg S L^{-1}) (Fig. 2, A). Furthermore, the implementation of sulfur-based denitrification could be applied to similar carbon-lean, nitrate-rich effluents. Tertiary polishing in sewage treatment plants is an interesting example, particularly when stringent discharge limits are imposed and when organics are limited for sufficient denitrification or to compensate for insufficient suppression of nitrate formation in mainstream partial nitritation/anammox (Han et al., 2016).

4. Conclusions

Sulfur-based denitrification treating IEX regeneration water at 15°C was proven to be fast in start-up (<10 days) and robust in operation with high nitrate removal rates (up to 394 and 207 mg N L^{-1} d^{-1} in L/S 0 and L/S 2.28 respectively). A trade-off should be made between the desired denitrification rate (related to the pH and sulfur surface area), the pH control (external/internal) and prevention of technical issues (related to particle size). In an economic comparison, sulfur-based denitrification (€5.3 kg^{-1} N) was 15% cheaper than methanol-based denitrification (€6.22 kg^{-1} N) and both treatments were opex dominated (85.9 vs. 86.5%).

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Figure captions

Fig. 1: Nitrate loading and removal rate as a function of operation day for L/S 0 (A), L/S 2.28 (B) and L/S 2.5 (C). Roman numbers depict the phase of operation in each

reactor.

Fig. 2: Influent and effluent sulfate concentrations (A) and relative sulfate production (B) as a function of operation day in all three reactors. The horizontal line depicts the stoichiometric value for sulfate production (2.4 g SO_4^{2-} g^{-1} NO_3^--N).

Fig. 3: Rapid dominance of the sulfurotrophic organisms *Sulfurimonas* (dark green) and *Thiobacillus* (dark blue) among the 15 most abundant genera. Left bar: inoculum for all reactors. Other bars: for reactors L/S 0, 2.28 and 2.5, respectively, dominant community composition on operational day 56 and at the end of the test (day 137, 131 and 133, respectively)..

Tables

Table 1: Characteristics of the 6 different batches of ion exchange regeneration water treated throughout the experiment. (1-5: neutralized at industrial scale with NaOH; 6: not neutralized) (average ± standard deviation of daily measurements during feeding period). /: not measured.

Batch (feeding period)	NO_3^- (mg N L^{-1})	NO_2^- (mg N L^{-1})	SO_4^{2-} (mg S L^{-1})	pH (-)	Electrical conductivity (mS cm^{-1})	Alkalinity (mg $CaCO_3$ L^{-1})
1 (d 1-12)	50.5 ± 4.0	0.25 ± 0.03	479 ± 24	7.07 ± 0.06	26.7 ± 0.8	53.0 ± 2.8
2 (d 13-31)	69.0 ± 1.7	1.66 ± 0.65	523 ± 9	6.91 ± 0.16	32.1 ± 2.2	68.8 ± 2.3
3 (d 32-66)	66.6 ± 2.7	2.65 ± 0.60	532 ± 14	6.58 ± 0.10	26.4 ± 5.5	66.0 ± 2.5
4 (d 67-89)	70.0 ± 2.93	2.93 ± 0.25	508 ± 24	7.09 ± 0.14	20.8 ± 1.6	78.5 ± 10.2
5 (d 90-135)	78.6 ± 7.9	7.89 ± 1.52	494 ± 44	7.62 ± 0.33	19.4 ± 3.2	107.4 ± 22.8
6 (d 118-133)	67.1 ± 5.1	0.58 ± 1.85	502 ± 47	1.94 ± 0.08	/	/

Table 2: Overview of the different phases applied to the three packed bed reactors and their duration.

	Phase I	**Phase II**	**Phase III**		**Phase IV**
			a	**b**	
L/S 0	Achieve target removal rate (day 1-12)	Maximum achievable removal rate (L/S 0: day 13-51) (L/S 2.28 & 2.5: 13-59)	Effect pH decrease on nitrate removal rate (day 52-103)	Effect pH increase on nitrate removal rate (day 104-133)	/
L/S 2.28			Effect sulfur surface area on nitrate removal rate (day 60-131)		/
L/S 2.5			Effect L/S ratio increase to 2.7 on effluent pH (day 60-97)	Effect L/S ratio increase to 2.8 on effluent pH (day 98-117)	Treatment of non-neutralized water using limestone bed (day 117-133)

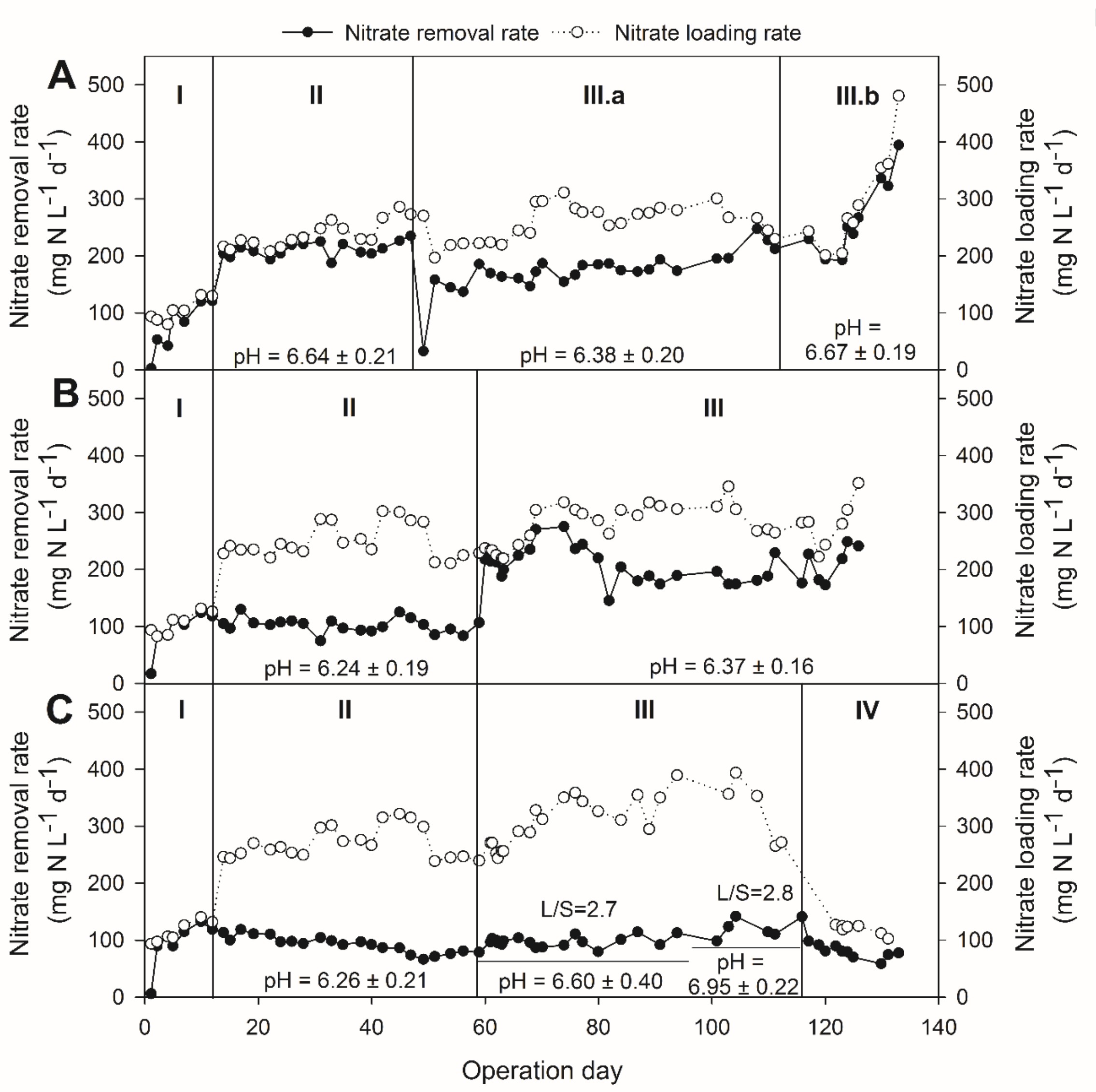
Table 3: Cost estimation for neutralization of regeneration water from ion exchange, including two scenarios. In order to enable comparison with the nitrogen removal costs (Table 4), costs are expressed per kg nitrogen removed. Details on data and assumptions linked to influent characteristics, process design and operation and depreciation can be found in supplementary material, section 3.

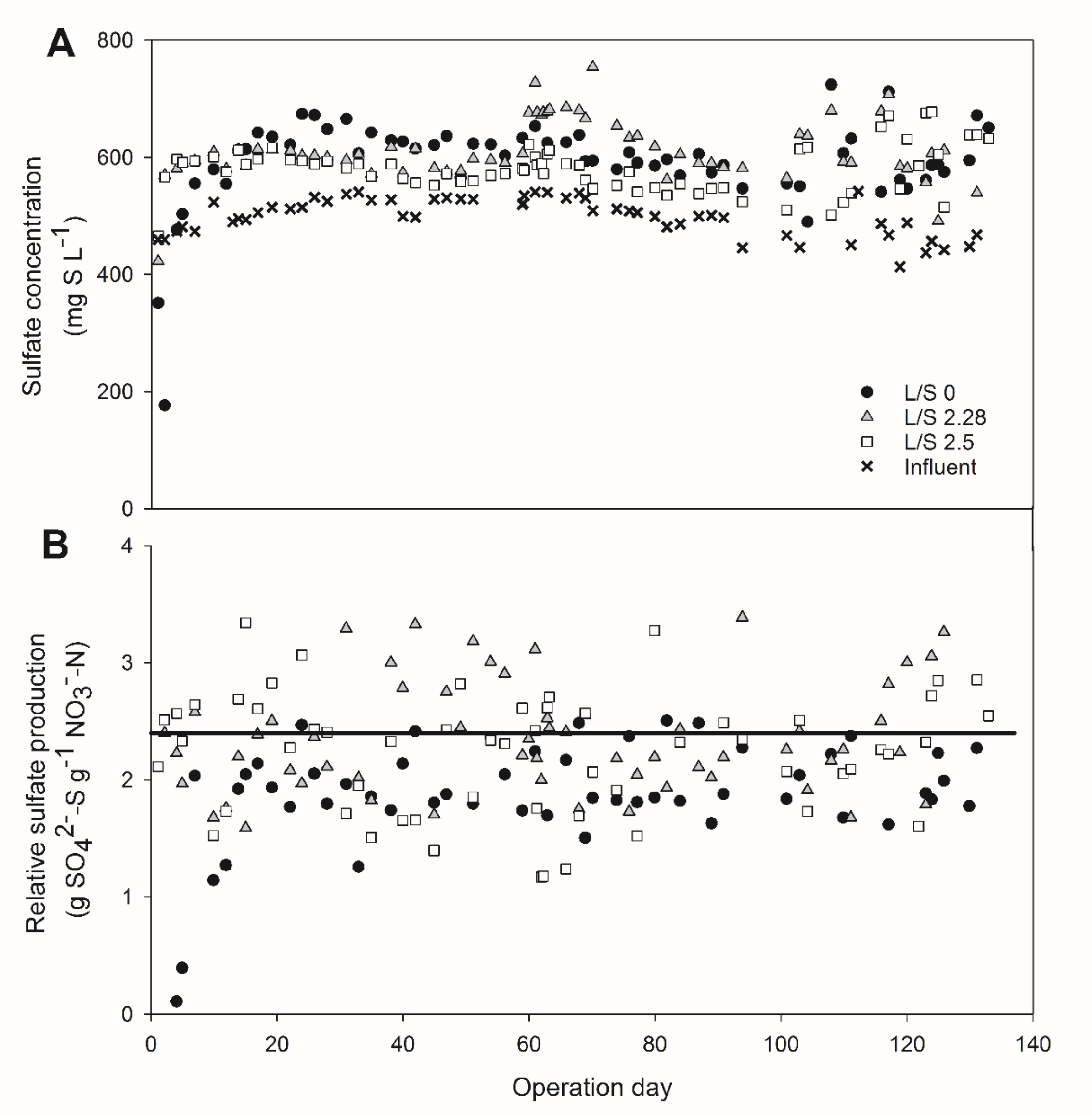
			NaOH dosage			**Limestone bed + add-on NaOH dosage**	
		€	**€ kg^{-1} $N_{removed}$**	**%**	**€**	**€ kg^{-1} $N_{removed}$**	**%**
Capex							
	Neutralization tank	682 413	1.46	32.7	697 616	1.49	48.5
Opex							
	Chemicals		2.04	45.6		0.61	19.7
	Pumping and mixing		0.45	10.0		0.45	14.5
	Maintenance		0.52	11.6		0.53	17.2
	Sum opex		**3.01**	**67.3**		**1.59**	**51.5**
Capex + opex			**4.47**	**100**		**3.08**	**100**

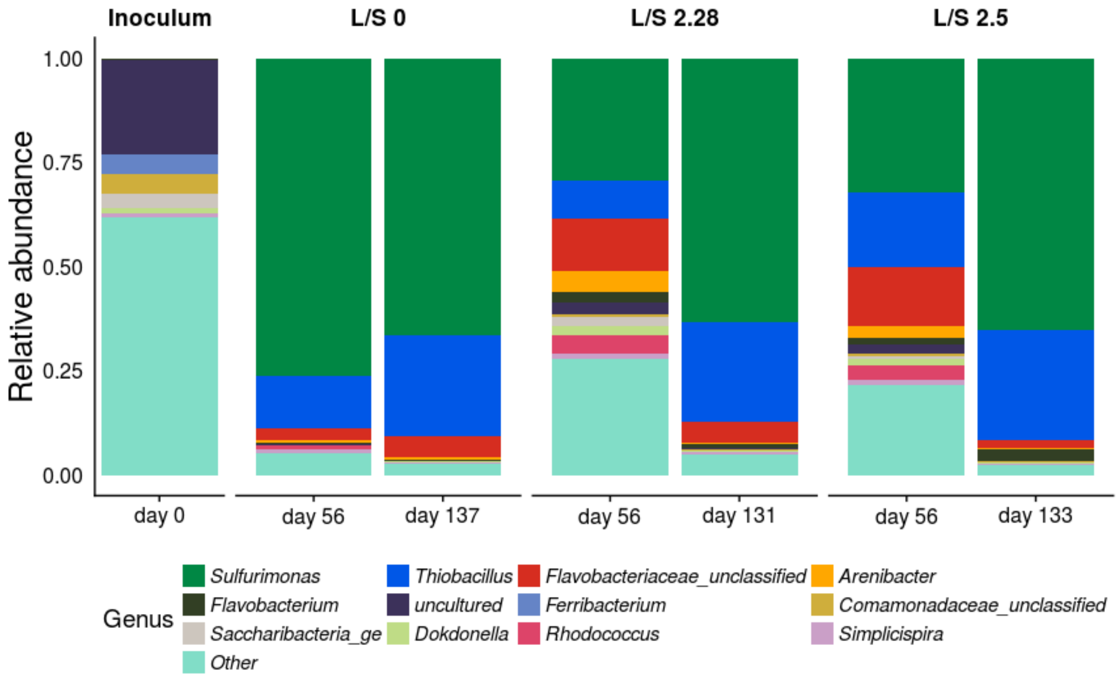
Table 4: Capital and operational costs estimation (capex and opex) for denitrification on neutralized regeneration water from ion exchange, including two autotrophic and one heterotrophic scenario, with the column heading indicating the compounds used as electron donor and for pH correction. Details on data and assumptions linked to influent characteristics, process design and operation and depreciation can be found in supplementary material, section 3.

		Sulfur/NaOH			**Sulfur/Limestone**			**Methanol/HCl**		
		€	**€ kg^{-1} N$_{removed}$**	**%**	**€**	**€ kg^{-1} N$_{removed}$**	**%**	**€**	**€ kg^{-1} N$_{removed}$**	**%**
Capex										
	Reactor basin	248 006	0.53	10.0	433 422	0.93	17.6	284 757	0.61	9.8
	Sedimentation tank	56 350	0.12	2.3	51 750	0.11	2.1	56 350	0.12	1.9
	Profit/risk	45 653	0.10	1.8	72 776	0.16	3.0	51 166	0.11	1.8
	Sum capex	**350 010**	**0.75**	**14.1**	**557 948**	**1.19**	**22.6**	**392 273**	**0.84**	**13.5**
Opex										
	Chemicals		1.64	30.1		0.99	18.7		1.56	25.1
	Pumping and mixing		0.01	0.2		0.03	0.6		0.24	3.9
	Sludge disposal		0.61	11.4		0.61	11.5		1.25	20.1
	Personnel		1.78	33.5		1.78	33.7		1.78	28.6
	Analyses		0.25	4.8		0.25	4.8		0.25	4.1
	Maintenance		0.27	5.0		0.42	8.0		0.30	4.8
	Sum opex		**4.55**	**85.9**		**4.08**	**77.4**		**5.38**	**86.5**
Capex + opex			**5.30**	**100**		**5.27**	**100**		**6.22**	**100**

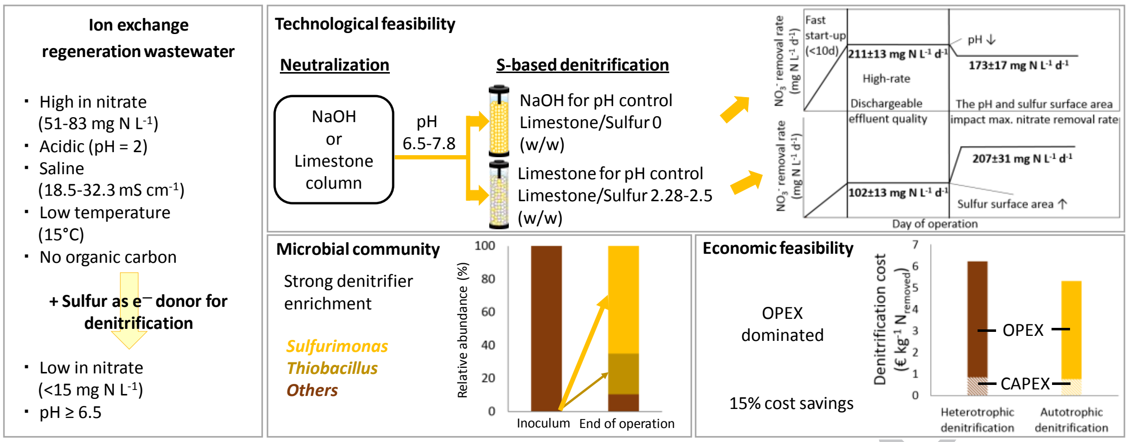
Figures


Nitrate removal rate
Nitrate loading rate
A
B
C
I
II
III.a
III.b
III
IV
pH = 6.64 ± 0.21
pH = 6.38 ± 0.20
pH = 6.67 ± 0.19
pH = 6.24 ± 0.19
pH = 6.37 ± 0.16
pH = 6.26 ± 0.21
pH = 6.60 ± 0.40
pH = 6.95 ± 0.22
L/S=2.7
L/S=2.8
Nitrate removal rate (mg N L^{-1} d^{-1})
Nitrate loading rate (mg N L^{-1} d^{-1})
0
100
200
300
400
500
0
20
40
60
80
100
120
140
Operation day


A
Sulfate concentration (mg S L^{-1})
0
200
400
600
800
● L/S 0
△ L/S 2.28
□ L/S 2.5
× Influent
B
Relative sulfate production (g SO$_4^{2-}$-S g^{-1} NO$_3^-$-N)
0
1
2
3
4
0
20
40
60
80
100
120
140
Operation day


Inoculum
L/S 0
L/S 2.28
L/S 2.5
Relative abundance
1.00
0.75
0.50
0.25
0.00
day 0
day 56
day 137
day 56
day 131
day 56
day 133
Genus
Sulfurimonas
Thiobacillus
Flavobacteriaceae_unclassified
Arenibacter
Flavobacterium
uncultured
Ferribacterium
Comamonadaceae_unclassified
Saccharibacteria_ge
Dokdonella
Rhodococcus
Simplicispira
Other

- Fast in start-up (<10 days) with high nitrate removal rates up to 394 mg N L^{-1} d^{-1}.
- Robust in operation, yielding dischargeable effluent quality.
- The pH and sulfur surface area impact the maximum nitrate removal rate.
- High abundance of *Sulfurimonas* (63-67%) and *Thiobacillus* (24-26%).
- Opex dominated, saving 15% on total cost compared to heterotrophic denitrification.


Ion exchange regeneration wastewater
High in nitrate (51-83 mg N L-1)
Acidic (pH = 2)
Saline (18.5-32.3 mS cm-1)
Low temperature (15°C)
No organic carbon
+ Sulfur as e⁻ donor for denitrification
Low in nitrate (<15 mg N L-1)
pH ≥ 6.5
Technological feasibility
Neutralization
NaOH or Limestone column
pH 6.5-7.8
S-based denitrification
NaOH for pH control Limestone/Sulfur 0 (w/w)
Limestone for pH control Limestone/Sulfur 2.28-2.5 (w/w)
NO3- removal rate (mg N L-1 d-1)
Fast start-up (<10d)
211±13 mg N L-1 d-1
High-rate
Dischargeable effluent quality
pH ↓
173±17 mg N L-1 d-1
The pH and sulfur surface area impact max. nitrate removal rate
102±13 mg N L-1 d-1
207±31 mg N L-1 d-1
Sulfur surface area ↑
Day of operation
Microbial community
Strong denitrifier enrichment
Sulfurimonas
Thiobacillus
Others
Relative abundance (%)
100
80
60
40
20
0
Inoculum
End of operation
Economic feasibility
OPEX dominated
15% cost savings
Denitrification cost (€ kg-1 N removed)
7
6
5
4
3
2
1
0
OPEX
CAPEX
Heterotrophic denitrification
Autotrophic denitrification