Sulfate reducing bacteria applied to domestic wastewater

van den Brand, Tessa; Snip, Laura; Palmen, Luc; Weij, Paul; Sipma, Jan; van Loosdrecht, Mark

DOI
10.2166/WPT.2018.068

Publication date
2018

Document Version
Final published version

Published in
Water Practice and Technology

© IWA Publishing 2018
542
Water Practice & Technology Vol 13 No 3
doi: 10.2166/wpt.2018.068

Sulfate reducing bacteria applied to domestic wastewater

Tessa van den Brand[a,*], Laura Snip[a], Luc Palmen[a], Paul Weij[b], Jan Sipma[c] and Mark van Loosdrecht[a,d]

[a] KWR Watercycle Research Institute, Groningenhaven 7, 3433PE Nieuwegein, The Netherlands

[b] Delfluent Services BV, Peuldreef 4, 2635 BX Den Hoorn, The Netherlands

[c] Paques BV, Tjalke de Boerstrjitte 24, 8561 EL Balk, The Netherlands

[d] TU Delft, van der Maasweg 9, 2629HZ Delft, The Netherlands

*Corresponding author. E-mail: tessa.van.den.brand@kwrwater.nl

Abstract

The application of sulfate reducing bacteria (SRB) to treat municipal wastewater is seldom considered. For instance, due to low sludge yield it can reduce the amount of excess sludge produced significantly. Several studies, mainly at laboratory-scale, revealed that SRB can proliferate in artificial wastewater systems at temperatures of 20°C and lower. So far, the application of SRB in a domestic wastewater treatment plant has been limited. Therefore, this study evaluates the proliferation of SRB at pilot-scale in a moderate climate. This study revealed that SRB were present and active in the pilot fed with domestic wastewater at 13°C, and outcompete methanogens. Stable, smooth and well-settled granule formation occurred, which is beneficial for full-scale application. In the Netherlands the sulfate concentration is usually low (<500 mg/L), therefore the application of SRB seems challenging as sulfate is limiting. Additional measurements indicated the presence of other sulfur sources, therefore higher sulfur levels were available, which makes it possible to remove more than 75% of the chemical oxygen demand (excluding sulfide) based on SRB activity. The beneficial application of SRB to domestic wastewater treatment might therefore be valid for more locations than initially expected.

Key words: domestic wastewater treatment, Europe, pilot, sulfate reducing bacteria, wastewater

INTRODUCTION

Wastewater treatment plants (WWTPs) make use of different microorganisms to treat the wastewater. Usually, these microorganisms are aerobic and have a high growth yield (0.5 g volatile suspended solids (VSS)/g chemical oxygen demand (COD)) which leads to large amounts of sludge (Henze *et al*. 2008; Metcalf and Eddy 1991). In conventional systems with aerobic COD removal, the excess daily sludge can be around 15–100 L/kg five-day biological oxygen demand (BOD_5) removed (Metcalf and Eddy 1991). The treatment and disposal of sludge are one of the major operational costs of WWTPs (Davis & Hall 1997; Spinosa & Vesilind 2001). Therefore, the use of anaerobic microorganisms with a lower growth yield than aerobic microorganisms is an interesting option to treat wastewater.

A group of anaerobic microorganisms that are potentially present in activated sludge are sulfate reducing microorganisms, which have a growth yield of 0.2 g VSS/g COD (Wang *et al*. 2009). These microorganisms can be members of the Bacteria or the Archaea, however it is common practice

Water Practice & Technology Vol 13 No 3
doi: 10.2166/wpt.2018.068

to refer to these microorganisms as sulfate reducing bacteria (SRB) as will be done in this article. During wastewater treatment, oxidation of organic compounds can occur by SRB.

The focus of many studies on SRB has been on the disadvantages of these microorganisms in WWTPs. Sulfate reduction can lead to the formation of sulfide, which is toxic and may result in corrosion. In addition, H_2S can be formed, which causes toxicity and odor problems. As the SRB compete for substrate with methanogens, the formation of methane can be reduced by the growth of SRB, decreasing the efficiency of biogas production.

Applying SRB to domestic wastewater can also have benefits (Lens *et al.* 1998; Lens & Kuenen 2001; Muyzer & Stams 2008; van den Brand *et al.* 2015c). Firstly, the production of sludge can be decreased when SRB are used due to a lower growth yield compared to heterotrophic biomass (Lens *et al.* 2002). This production can be even further decreased when coupled with autotrophic denitrification (Wang *et al.* 2009). Secondly, as sulfide is toxic to coliforms, its formation may lead to a reduction of coliforms (Abdeen *et al.* 2010). Thirdly, sulfide could also precipitate with heavy metals, leading to decreased concentrations of heavy metals in the effluent (Lewis 2010). Finally, the SRB are able to grow in granules, which leads to a more compact system compared to the conventional system, decreasing the footprint of the WWTP (Lens *et al.* 2002).

Due to a higher focus on the disadvantages of SRB compared to the benefits, limited research has been performed on treating domestic wastewater with SRB. Most of the studies regarding the application of SRB in domestic wastewater treatment have been performed in Hong Kong (Lau *et al.* 2006; Wang *et al.* 2009), as in Hong Kong the wastewater contains elevated sulfate levels, due to use of seawater for toilet flushing. This resulted in the development of the Sulfate reduction, Autotrophic denitrification and Nitrification Integrated process (SANI®, HKUST, Hong Kong). This process makes use of anaerobic and anoxic microorganisms to treat wastewater. A successful SANI® pilot plant containing SRB was operated for 225 days at an influent flow rate of 10 m^3/day and achieved 87% COD removal efficiency (Lu *et al.* 2011). In addition, the total observed sludge yield was 0.02 g VSS/g COD removed, also due to activity of autotrophic bacteria, resulting in a 90% reduction of sludge production.

The conditions in the pilot plant operated in Hong Kong were favorable for the SRB as the sulfate concentrations in the influent were high (588 mg/L SO_4^{2-}) and the temperature was around 25°C (Lu *et al.* 2011). In moderate climates, such as in the Netherlands, the wastewater temperatures are lower (around 12°C) and the sulfate concentrations are significantly lower ($<$200 mg/L SO_4^{2-}) (van den Brand *et al.* 2015b). This could mean that the actual conditions are not suitable for SRB growth. Several laboratory studies have demonstrated the successful treatment of artificial sulfate rich domestic wastewater by SRB at temperatures of 20°C or lower (van den Brand *et al.* 2014a, 2014b, 2015a; Rubio-Rincón *et al.* 2017a, 2017b), but so far, SRB have not been tested for application in moderate climates with real wastewater at pilot-scale.

The aim of this research was to investigate how SRB perform in a pilot reactor with real domestic wastewater at low temperatures and limited sulfate levels. At WWTP Harnaschpolder (the Netherlands) the sulfate concentration fluctuates between 50–250 mg/L (personal communication), making it a suitable location for testing. At this WWTP, an upflow anaerobic sludge blanket (UASB) pilot plant operated to treat the presettled and raw wastewater during a period of 8 months.

MATERIALS AND METHODS

Pilot reactor

The pilot reactor was designed and built by Paques BV and was installed at WWTP Harnaschpolder, the Netherlands in order to work with real domestic wastewater. The reactor was operated

Water Practice & Technology Vol 13 No 3
doi: 10.2166/wpt.2018.068

continuously from September 2014 until June 2015. The volume of the reactor was 61 L (height 2.4 m, diameter 0.18 m). A schematic overview of the setup of the pilot reactor is presented in Figure 1.

Before the domestic wastewater reached the pilot reactor, it passed through a settling tank and an acidification tank. The settling tank contained a sieve in order to remove large substances such as toilet paper. In addition, it functioned as a primary clarifier. From the settling tank, the clarified wastewater was pumped into an acidification tank that controlled the pH at 7.6 by dosing either HCl or NaOH. The influent samples were taken from the acidification tank.

The pilot reactor was continuously fed from the acidification tank at a rate of 2.5 L/h. The recirculation flow in the pilot reactor was set at 10.8 L/h. In theory, (bio)gas could be formed in the reactor and therefore an outlet for off gas was created. A small container at the top of the reactor served as a sampling point for the effluent samples. The biomass samples were collected from the bottom of the pilot reactor. The temperature of the pilot reactor was not controlled, thus the temperature variation in the reactor originates from the temperature variations of the influent wastewater. The lowest temperature measured was 9.9°C and on average the wastewater temperature was 12.9°C.

The settling tank was manually filled and could not be filled at weekends. This resulted in a decrease of the influent flow rate of the pilot reactor during weekends from 2.5 L/h to 1 L/h. The weekly average influent flow was 1.96 L/h, which was used for further calculations. The hydraulic retention time was 24.4 hours during weekdays and 61.0 hours during the weekends, which on average during the week would be around 48 hours.

During startup, the pilot reactor was inoculated with activated sludge from WWTP Harnaschpolder and with anaerobic granular sludge from an anaerobic WWTP treating wastewater from several paper factories in Eerbeek (IndustrieWater Eerbeek, Eerbeek, the Netherlands) with equal volume. These two different sludge origins ensured a high COD consumption, which prevented toxic COD concentrations for SRB. The influent of the pilot reactor contained a relatively high VSS concentration (0.5 g/L), which ensured a continuous inoculum to the pilot, similar to the composition of WWTP Harnaschpolder.

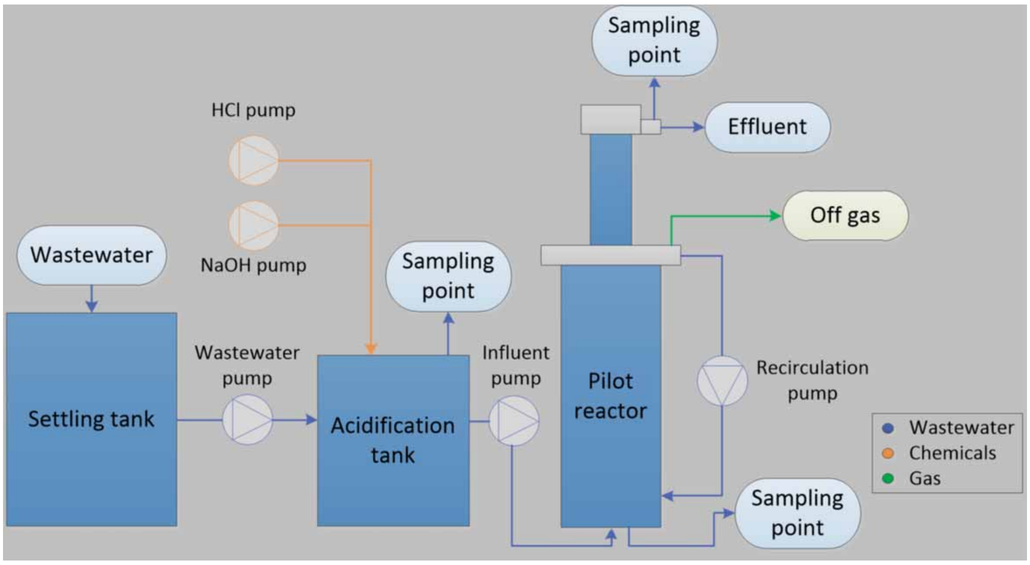

Figure 1 | Schematic overview of the pilot reactor set-up.

Analytical methods

The influent and effluent composition of the pilot reactor was monitored regularly (3 times a week) by taking grab samples from the sampling points (Figure 1). From comparisons between grab samples

Water Practice & Technology Vol 13 No 3
doi: 10.2166/wpt.2018.068

and 24-hour-flow-proportional samples, it was concluded that grab samples are representative (results show in Appendix Table A.1).

To avoid sulfide losses during sampling, the samples (>15 mL) were collected in 1 drop of 1 M NaOH (Poinapen *et al.* 2009). The sulfide concentration was measured according to the methylene blue method (APHA 1995). Sulfate was measured spectrophotometrically with Hach Lange kit LCK 153 (Hach Lange, Düsseldorf, Germany). Prior to sulfate measurement, the sample was filtrated with an 0.45 μm glass fiber filter in order to remove the biomass. Total COD and the organic substrate concentration (COD_{VFA}) were measured with Hach Lange kit LCK 414 and 514. To measure the remaining organic substrates (COD_{VFA}), sulfide was first was removed via addition of $ZnSO_4$, after which the ZnS precipitates were filtered from the sample (Poinapen *et al.* 2009).

The influent and effluent samples were also analyzed for the following nutrient concentrations: ammonium, nitrate, nitrite and dissolved phosphate, using LCK304, 339, 341 and 348 respectively. The samples were filtered with an 0.45 μm glass fiber filter prior to analysis.

Samples were taken from the influent, effluent and the pilot reactor to examine the biomass. VSS and total suspended solids (TSS) measurements were performed as described in standard methods (APHA 1995). The samples were filtered with 2.7 μm glass microfiber filters (Sartorius, FT 3 1101 047, Goettingen, Germany) and incubated at 105°C (for TSS measurements) followed by 550°C (for VSS measurements). Furthermore, the growth of biomass in the reactor was also studied by measuring the fixed bed height in the reactor after stopping the recirculation pump and influent supply for 30 minutes to allow the biomass to settle.

Finally, the morphology of the sludge was analyzed regularly based on its settling velocity and floc or granule structure. Settling velocity was determined by a random selection of a floc and determination of the time required to settle 30 cm in a volumetric cylinder filled with tap water. The evolution of the sludge over time, in terms of shape and diameter, were analyzed microscopically.

Short-term batch activity tests

Short-term batch activity tests were performed using synthetic wastewater in order to maintain constant conditions throughout the different tests with sludge from 55 and 121 days of operation. The synthetic wastewater was composed according to the method mentioned in van den Brand *et al.* (2015d) and consisted of 2.68 mM $NaCH_3COO{\cdot}3H_2O$, 1.15 mM $NaC_3H_5O_2$ (300 mg COD_{VFA}/L in total), 0.09 mM K_2HPO_4, 0.04 mM KH_2PO_4, 2.89 mM NH_4Cl, 0.34 mM $MgCL_2{\cdot}6H_2O$, 0.39 mM $CaCl_2$ and 1 mL/L trace elements solution (Lau *et al.* 2006). A sulfate concentration of 500 mg/L was obtained by adding 7.3 g/L aquarium salt (Reef Crystals™). This ensured that there would be no sulfate limiting conditions during the activity tests. The SBR activity tests were carried out in threefold in 100 mL serum-bottles containing 50 mL of synthetic wastewater and 30 mL sludge from the pilot reactor. The bottles were closed with rubber stoppers and prior to each test flushed with N_2 gas to ensure anaerobic conditions. The temperature was controlled at 15°C. The activity of SRB was expressed as the rate of accumulation of sulfide in time per gram biomass.

RESULTS AND DISCUSSION

Mass balances

The reactor was operated for 8 months without significant operational problems. The average results of the sulfur and COD_{VFA} measurements from the influent and effluent samples are displayed in Table 1. The effluent concentrations of sulfate were below the detection limit (<15 mg/L). The formation of sulfide and removal of sulfate indicated that SRB were active in the pilot reactor. On

Water Practice & Technology Vol 13 No 3
doi: 10.2166/wpt.2018.068

Table 1 | Average concentrations of sulfate, sulfide and COD for the influent and effluent of the pilot reactor during the period of September 2014 until April 2015

Component	Influent	Effluent	Consumption or production
Sulfate (mg/L)	71 ± 41	Below detection limit (<15 mg/L)	71
Sulfide (mg/L)	11 ± 9	82 ± 75	71
COD total (mg/L)	512 ± 180	1,079 ± 313	567
CODVFA (mg/L)	208 ± 51	29 ± 12	179

average 71 mg of SO_4^{2-}/L was removed while 71 mg S^{2-}/L was formed (Table 1). The theoretical stoichiometry for SRB activity (neglecting biomass formation) shows that the removal of 100 mg COD requires 150 mg sulfate and produces 50 mg sulfide (Thauer *et al.* 1977). The formation of 71 mg S^{2-} would require 213 mg SO_4^{2-} mg, based on molar ratio, which was not available in the influent throughout operation of the pilot (Figure 2). Thus these results indicate a sulfur imbalance with a higher sulfur concentration measured in the effluent than in the influent.

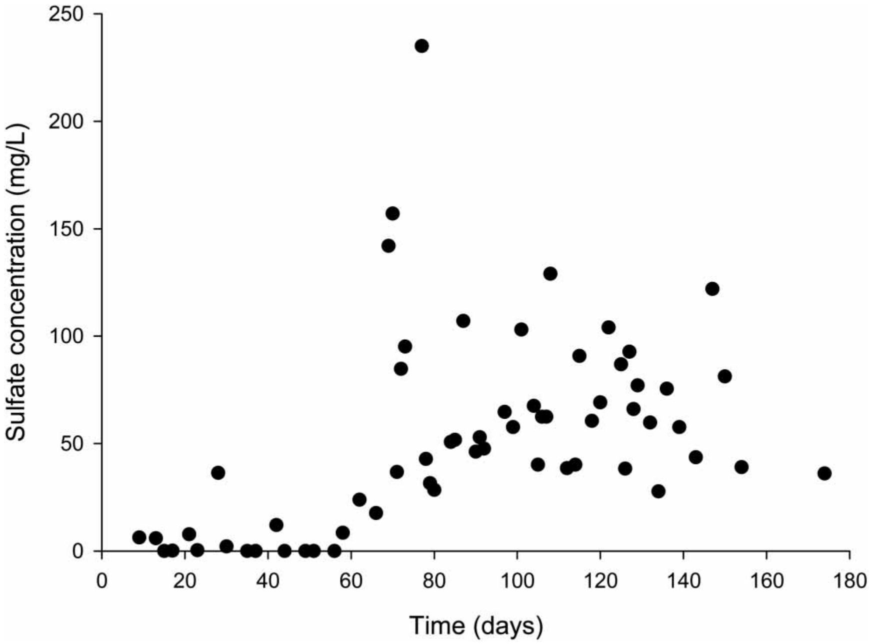

Figure 2 | Sulfate concentration (•) in the influent of the pilot reactor. Sulfate in effluent below detection limit (<15 mg/L).

Several possible explanations can be proposed for this imbalance in the sulfur (S) mass balance. Firstly, the measurements performed could neglect to take all S compounds present in the wastewater into account, for instance the presence of gypsum ($CaSO_4$). Additional tests showed that the filtration step prior measuring resulted in a significant decrease (>50%) in the measured sulfate concentration (data not shown), indicating that some of the sulfate was captured in particulates. However, the high biomass content in the effluent also required filtration of the sample. Furthermore, total S measurements indicated that more S was present than solely measured by sulfate (Table 2), which might have been present in the form of gypsum. The mentioned possible explanations jointly suggest that the actual sulfur content in the influent was higher than measured. As the sulfide analyses have shown to be reliable in this type of matrices (data not shown) further results and discussion are based on sulfide measurements.

Water Practice & Technology Vol 13 No 3
doi: 10.2166/wpt.2018.068

Table 2 | Comparison of sulfate and total S measurements of samples

Sample	Sulfate (mg S/L)	Total S (mg S/L)
Influent pilot	16.1 ± 3	31 ± 4
Effluent pilot	Below detection limit (<15 mg/L)	73.5 ± 1.5
Tap water at Harnaschpolder	17.1 ± 0.5	16.5 ± 0.5

SRB activity

The sulfate influent and sulfide influent and effluent concentrations are depicted in Figures 2 and 3, respectively. Considerable variations in concentrations in influent of the reactor were the result of the fluctuations of the influent composition of the WWTP (Butler *et al.* 1995). The effluent sulfide concentrations rose during the first 70 days of pilot operation, indicating that SRB activity was increasing. After 70 days, high concentrations of sulfide (~200 mg/L on average) in the effluent were observed, however these concentrations fluctuated considerably from 30 to 310 mg/L (Figure 3). Additional measurements of samples prior to filtration demonstrated that there was no sorbed sulfide present in the influent and therefore the sulfide measured in the effluent could be considered as the result of SRB activity.

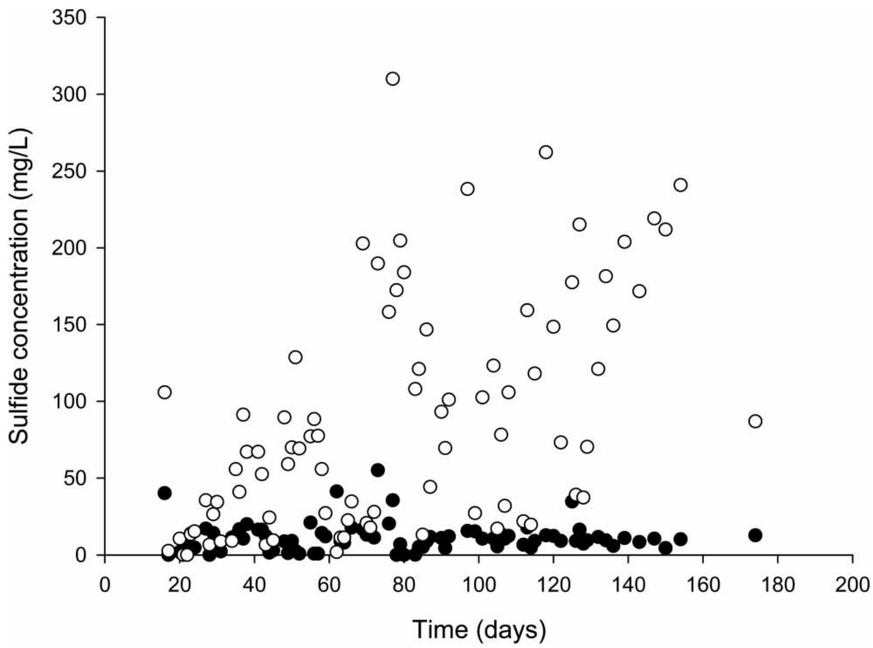

Figure 3 | Sulfide concentration in the influent (●) and the effluent (o) of the pilot reactor.

A decrease in temperature did not have any effect on sulfide formation, indicating that SRB activity was not influenced to such an extent that different effluent quality was achieved. This is in accordance with the laboratory study of van den Brand *et al.* 2014b, which reported that the effluent quality was not affected by a temperature decrease from 20 to 10°C; though the sulfide production rate decreased significantly (factor 1.9).

COD_{VFA} removal

The data shown in Figure 4 demonstrate that the COD_{VFA} was consumed from the start-up of the pilot reactor as the effluent concentration was below 50 mg/L from the beginning. As the SRB activity

Water Practice & Technology Vol 13 No 3
doi: 10.2166/wpt.2018.068

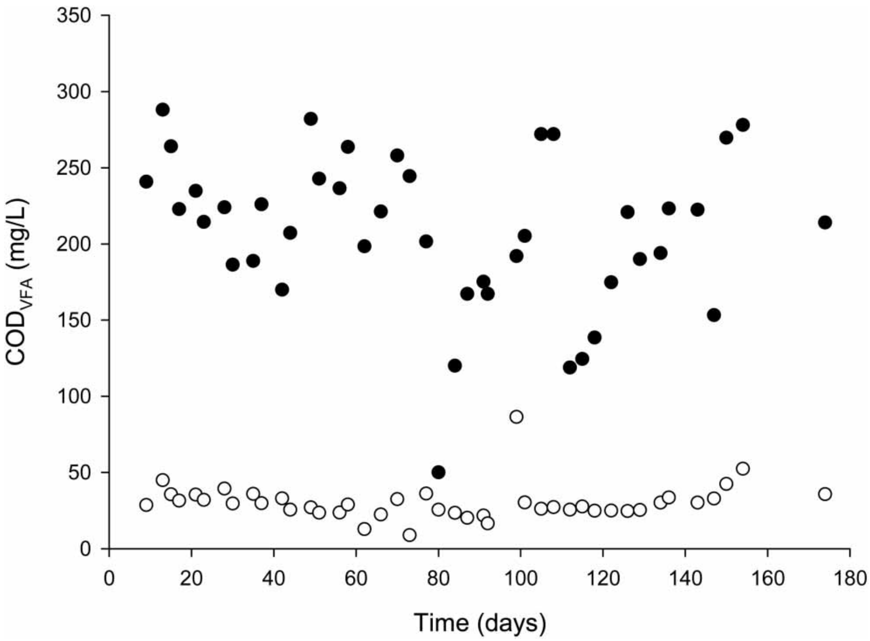

Figure 4 | COD_{VFA} concentration in the influent (●) and the effluent (o) of the pilot reactor during the period of September 2014 until April 2015.

started to increase after 70 days, it is likely that the COD_{VFA} was consumed by other microorganisms present in the inoculum in this start-up phase. Auto fluorescence examination revealed (data not shown) that methanogens were present in the inoculum and they could have been the cause of the COD_{VFA} consumption.

On average the VFA COD removal was 179 mg COD_{VFA}/L, while 71 mg S^{2-}/L was formed (Table 2). The formation of 71 mg S^{2-}/L would require 213 mg SO_4^{2-}/L, which would correspond to a consumption of 142 mg COD_{VFA}/L as stoichiometric conversion of SRB is 0.67 g COD/g SO_4^{2-} (Thauer *et al.* 1977; Oude Elferink *et al.* 1994). This would imply that 37 mg/L COD_{VFA} was consumed by other organisms than SRB and would result in a 79% COD_{VFA} removal caused by SRB activity based on sulfate reduction. However, as it is likely that other sulfur compounds besides sulfate were also used by the present SRB (see paragraph on mass balances), the actual COD removal resulting from SRB activity was different.

No gas production was detected, therefore minimal activity of methanogens is expected, but since methane can remain dissolved, methane formation cannot be excluded. This was confirmed by a decrease in autofluorescence signal from sludge from the pilot over time compared to its inoculum, indicating that the methanogens were outcompeted. As SRB usually outcompete methanogens in excessive amounts of sulfate (Harada *et al.* 1994; Mizuno *et al.* 1994; Omil *et al.* 1998), this indicates that another sulfur compound should be present to create a sulfate excess. Especially since additional nutrient analyses have indicated that marginal removal of ammonium, nitrate, nitrite or dissolved phosphate occurred within the pilot, suggesting that heterotrophs and autotrophs were not active in the pilot and could therefore not contribute to the removal of COD_{VFA}.

The SRB activity was investigated in more detail with short-term batch experiments at 15°C in which the sulfate concentration was not limiting (as seemed to be the case in the pilot reactor). After 55 days of operation, when a high productivity of sulfide by SRB in the pilot reactor was noticed (Figure 3), short-term batch activity tests were performed. The rate of accumulation of sulfide was 6.09 mg S/g VSS/h. Within 24 hours complete COD_{VFA} removal was achieved and a maximum sulfide concentration of 122 mg/L was produced. The production of 122 mg/L sulfide required 243 mg/L COD which indicates that 81% of the COD_{VFA} was consumed by SRB, a similar removal as obtained

Water Practice & Technology Vol 13 No 3
doi: 10.2166/wpt.2018.068

in the pilot. The percentage of COD_{VFA} removal by SRB was increased when the biomass from the pilot reactor set-up was tested 121 days after startup. The sulfide concentration reached 141 mg/L which requires 281 mg/L COD and indicates that 94% of the COD present was consumed by SRB. The higher sulfide concentration was also reached faster by sludge from day 121 compared to the biomass tested after 55 days of operation as clearly depicted in Figure 5. The rate of sulfide production was 26.3 mg S/gVSS/h for the sludge taken 121 days after start-up. The sulfide production rate of the sludge from the pilot (15°C) was in between the rates observed for 10 and 20°C in a laboratory study by van den Brand *et al.* 2014b. This indicates that the use of domestic wastewater results in similar SRB performance as when adapted to artificial wastewater feeding.

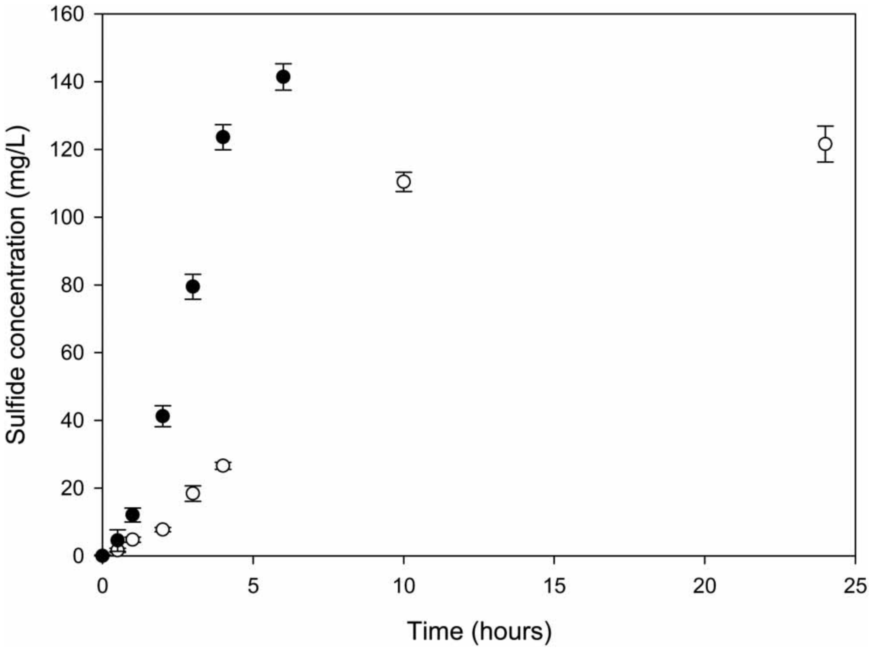

Figure 5 | Sulfide concentration from SRB activity tests with biomass of 55 days after start-up (o) and 121 days after start-up (•).

Biomass growth

The biomass growth was assessed by monitoring the VSS and TSS concentrations in the influent, sludge samples from the bottom of the pilot and effluent as well as by monitoring the bed height after settling. The results of the samples taken are shown in Table 3.

As shown in Figure 4, the COD_{VFA} concentration in the influent was higher than in the effluent (208 vs. 29 mg/L). In contrast, the total COD concentration in the influent was lower than in the effluent (1,079 vs. 512 mg/L, Table 1). This corresponds to the high VSS concentration (3.42 g/L) found in

Table 3 | Average concentrations of VSS and TSS in the influent, the pilot reactor and the effluent during the period of September 2014 until April 2015[a]

Component	Influent	Effluent	Pilot[a]
VSS (g/L)	0.40 ± 0.39	3.42 ± 2.30	2.55 ± 2.38
TSS (g/L)	0.48 ± 0.44	5.03 ± 3.48	3.32 ± 3.27
VSS/TSS (%)	16	32	23

[a]No well-mixed samples could be obtained from the reactor; the granules settle easy and as sampling occurred at the bottom from the reactor the VSS and TSS values are higher than expected.

Water Practice & Technology Vol 13 No 3
doi: 10.2166/wpt.2018.068

the effluent (Table 3). The average daily COD consumption with weekly influent flow rate of 1.96 L/h would be 8.4 g (179 mg/L * 1.96 L/h * 24 h/day). When assuming a standard conversion of 1.32 g COD/g biomass, the high production of VSS (4.55 g/L) would indicate that 283 g COD/day was flushed out on average (4.55 g VSS/L *1.96 L/h * 1.32 g COD/g VSS * 24 h/day). A washout of 283 g COD_{VFA}/day would lead to a complete flush-out of biomass from the reactor; which was obviously not the case as shown in Figure 6 which depicts a stabilized volume of 15 L biomass. Furthermore, the continuing COD_{VFA} consumption and sulfide formation suggested that biomass was still present and active in the pilot reactor. An explanation for the high VSS, TSS and total COD concentrations in the effluent (see Tables 1 and 3) could be the configuration of sampling point in the pilot reactor. As depicted in Figure 1, the sampling point was a small container at the top of the reactor which contained a high concentration of solids. It is likely that the presence of COD_{VFA} in the effluent along with the biomass led to biomass growth in the container, which affected the representativeness of the samples for the pilot reactor. Furthermore, this effect is amplified by the fact that settling of biomass can occur in this sample bucket.

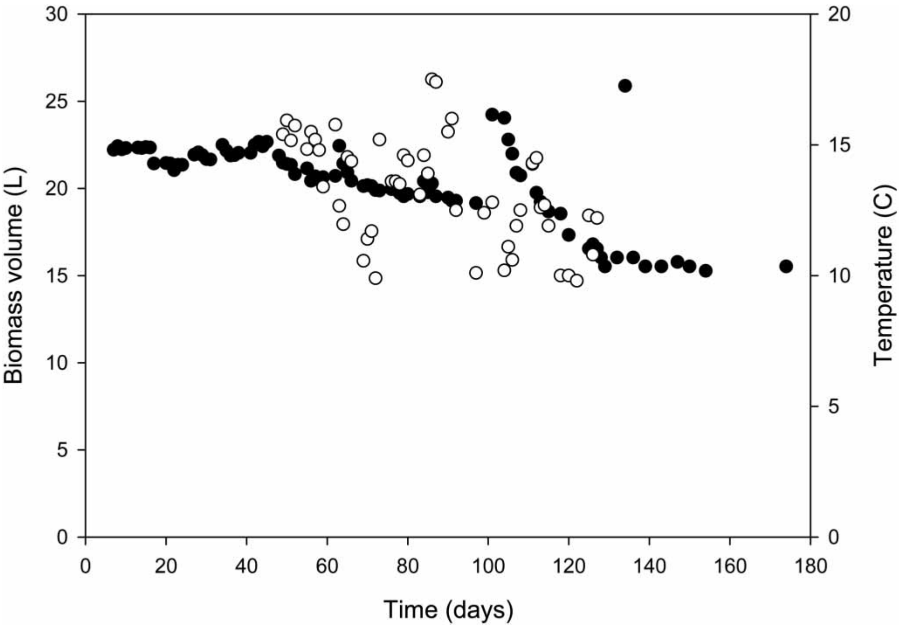

Figure 6 | The biomass volume (•) and temperature (o) within the pilot reactor.

The monitoring of the biomass volume in the pilot reactor showed a sudden increase after 100 days of operation (Figure 6), possibly caused by a change in the floc structure of the biomass, which is visible in the top of the bed. This change could not only affect the compactness of the biomass bed, it could also influence the assessment of the bed height in the reactor.

Morphology

SRB have been shown to granulate easily (Lens *et al.* 2002; van den Brand *et al.* 2015b. Application of granular sludge is an effective way to increase volumetric capacity and decrease reactor footprint (Nicolella *et al.* 2000), while remaining sufficiently biologically efficient. The fact that the sludge remained in a smooth and dense floc structure throughout operation of the pilot fed by domestic wastewater, as indicated in Figure 7, is therefore beneficial for lowering the environmental impact of a full-scale system. The fact that sludge sampling occurred at the bottom of the pilot will have influenced the type of granules selected for this experiment. The change in floc structure observed after

Water Practice & Technology Vol 13 No 3
doi: 10.2166/wpt.2018.068

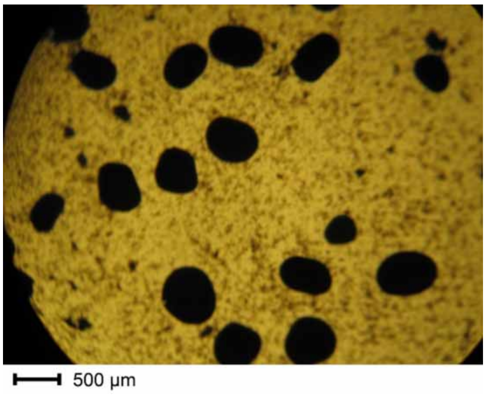

Figure 7 | Floc structure of sludge in the pilot.

100 days is only for the top part of the bed, and therefore did not affect the selected granules, which could only be taken from the bottom of the reactor.

The settling velocity of the sludge decreased slightly (20%), which could be explained by the slight decrease in temperature of tap water in which the experiment was performed (Figure 8). Nonetheless, there was no deterioration of floc structure or settleability during the entire operation. A lower temperature therefore did not affect the floc structure or settleability in a negative way. The settling velocity (~100 m/hour), is much higher than the upflow velocity in the pilot (~0.5 m/hour), which illustrates that the sludge was not washed out easily.

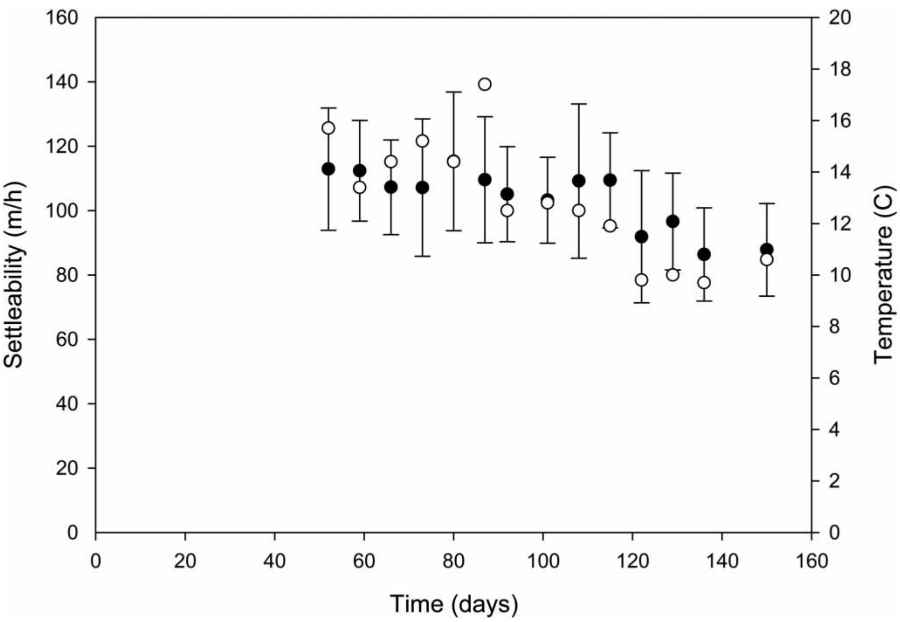

Figure 8 | Settleability of sludge (●) and temperature (○) in the pilot over time.

Outlook

This study has shown the potential of SRB activity in domestic wastewater, and its ability to remove COD_{VFA}. In order to move from science to practice, several research directions should be covered in follow-up studies. For instance, the ability of SRB to perform in a broader perspective, such as at even lower temperatures (<5 °C) in, for example, Scandinavia (Sallanko & Pekkala 2008) as well as up-scaling to full-scale operation.

Water Practice & Technology Vol 13 No 3
doi: 10.2166/wpt.2018.068

In order to understand more about the underlying processes occurring in the pilot reactor, it is important to gain more clarity about the origin of sulfur in the wastewater that is converted by SRB. This is of importance to the COD_{VFA} consumption, which can vary with the sulfur source available. A possibility to further investigate the COD_{VFA} consumption during growth of SRB is by aerating a sample in which sulfur is completely converted into sulfate. Furthermore, SRB can also be influenced by nutrients and conditions specific for certain process steps within WWTP, such as the formation of nitrate and nitrite (van den Brand *et al.* 2015d; Rubio-Rincón *et al.* 2017a). SRB activity can also affect other crucial microbial processes, such as phosphorus removal (Rubio-Rincón *et al.* 2017b) or the effectiveness of the digester. This additional research will contribute to design a complete and optimal wastewater treatment technology including SRB, or to the activity of SRB in conventional aerated wastewater treatment and will give direction to up-scaling activities.

Before applying SRB to domestic WWTPs, it is advisable to examine the growth yield of SRB in more detail, to determine the effectiveness of the decrease in sludge production. The benefits from using microorganisms with a low growth yield to treat wastewater could be maximized if SRBs are combined with autotrophic denitrification, as is the case in the SANI process (Wang *et al.* 2009). Other promising technologies to achieve nitrogen removal are the Anammox® (Strous & Jetten 2004) or Sharon processes (Hellinga *et al.* 1998), of which combinations with SRB could be explored as well. In addition, other intended benefits of SRB to treat (domestic) wastewater, such as coliform reduction and the potential to remove heavy metals, should be established and quantified.

CONCLUSIONS

Based on the operation of the unaerated pilot reactor fed with domestic wastewater at an average temperature of 13°C for 8 months continuously at WWTP Harnaschpolder, the following conclusions can be drawn:

- The pilot study clearly confirms that SRB were active in domestic wastewater at temperatures below 15°C. COD_{VFA} removal occurred dominantly by SRB activity.
- SRB can outcompete methanogens at low temperatures in domestic wastewater treatment at these specific conditions.
- SRB are capable of thriving in wastewater under conditions that are less favorable compared to the conditions of the pilot reactor in Hong Kong (30°C and excessive amounts of sulfate), expanding the expected range in which SRB can be applied.
- The sludge in the pilot study formed a stable and smooth granule, resulting in sufficient sludge settling characteristics.
- In order to achieve maximum benefit from the advantages of applying SRB in domestic wastewater treatment, additional research regarding the specific processes of coliform reduction, removal of heavy metals and micropollutants are required.
- A limiting sulfate concentration was expected, however additional measurements revealed the presence of an unknown sulfur source, therefore SRB could remain active. Therefore the beneficial application of SRB in domestic WWTP could be reconsidered for all locations were insufficient sulfate levels were expected.

ACKNOWLEDGEMENTS

This research has been co-financed by a grant from the Dutch Ministry of Economic Affairs within the funding program 'Top consortia for Knowledge and Innovation (TKI)' in cooperation with KWR, Delfluent Services BV and Paques BV.

Water Practice & Technology Vol 13 No 3
doi: 10.2166/wpt.2018.068

REFERENCES

Abdeen, S., Di, W., Hui, L., Chen, G.-H. & van Loosdrecht, M. C. M. 2010 Fecal coliform removal in a sulfate reducing autotrophic denitrification and nitrification integrated (SANI) process for saline sewage treatment. *Water Science & Technology* **62**(11), 2564–2570.

APHA 1995 *Standard Methods for the Examination of Water and Wastewater*, 19th edn. American Public Health Association, Washington DC, ISBN:0-87553-223-3.

Butler, D., Friedler, E. & Gatt, K. 1995 Characterizing the quantity and quality of domestic wastewater inflows. *Water Science & Technology* **31**(7), 13–24.

Davis, R. D. & Hall, J. E. 1997 Production, treatment and disposal of wastewater sludge in Europe from a UK perspective. *European Water Pollution Control* **7**(2), 9–17.

Harada, H., Uemura, S. & Momonoi, K. 1994 Interation between sulfate-reducing bacteria and methane-producing bacteria in UASB reactors fed with low strength wastes containing different levels of sulfate. *Water Research* **28**(2), 355–367.

Hellinga, C., Schellen, A. A. J. C., Mulder, J. W., van Loosdrecht, M. C. M. & Heijnen, J. J. 1998 The sharon process: an innovative method for nitrogen removal from ammonium-rich wastewater. *Water Science & Technology* **37**(9), 135–142.

Henze, M., van Loosdrecht, M. C. M., Ekama, G. A. & Brdjanovic, D. 2008 *Biological Wastewater Treatment: Principles, Modelling and Design*. IWA publishing, London, ISBN: 1843391880.

Lau, G. N., Sharma, K. R., Chen, G.-H. & van Loosdrecht, M. C. M. 2006 Integration of sulphate reduction, autotrophic denitrification and nitrification to achieve low-cost excess sludge minimization for Hong Kong sewage. *Water Science & Technology* **53**(3), 227–235.

Lens, P. N. L., Visser, A., Jannsen, A. J. H., Hulshoff Pol, L. W. & Lettinga, G. 1998 Biotechnological treatment of sulfate-rich wastewaters. *Critical Reviews in Environmental Science and Technology* **28**(1), 41–88.

Lens, P. N. L. & Kuenen, J. G. 2001 The biological sulfur cycle: novel opportunities for environmental biotechnology. *Water Science & Technology* **44**(8), 57–66.

Lens, P., Vallero, M., Esposito, G. & Zandvoort, M. 2002 Perspectives of sulfate reducing bioreactors in environmental biotechnology. *Reviews in Environmental Science and Bio/Technology* **1**(4), 311–325.

Lewis, A. E. 2010 Review of metal sulphide precipitation. *Hydrometallurgy* **104**, 222–234.

Lu, H., Wu, D., Tang, D. T. W., Chen, G.-H., van Loosdrecht, M. C. M. & Ekama, G. 2011 Pilot scale evaluation of SANI process for sludge minimization and greenhouse gas reduction in saline sewage treatment. *Water Science & Technology* **63**(10), 2149–2154.

Metcalf and Eddy 1991 *Wastewater Engineering; Treatment, Disposal and Reuse*, 3rd edn. McGraw-Hill, New York, ISBN 978-0070416901, pp. 529–591.

Mizuno, O., Li, Y. Y. & Noike, T. 1994 Effects of sulfate concentration and sludge retention time n the interaction between methane production and sulfate reduction for butyrate. *Water Science & Technology* **30**(8), 45–54.

Muyzer, G. & Stams, A. J. M. 2008 The ecology and biotechnology of sulphate-reducing bacteria. *Nature Reviews Microbiology* **6**, 441–454.

Nicolella, C., van Loosdrecht, M. C. M. & Heijnen, J. J. 2000 Wastewater treatment with particulate biofilm reactors. *Journal of Biotechnology* **80**(1), 1–33.

Omil, F., Lens, P., Visser, A., Hulshoff Pol, L. W. & Lettinga, G. 1998 Long-term completion between sulfate recucing and methanogenic bacteria in UASB reactor treating volatile fatty acis. *Biotechnology & Bioengineering* **57**(6), 676–685.

Oude Elferink, S. J. W. H., Visser, A., Hulshoff Pol, L. W. & Stams, A. J. M. 1994 Sulfate reduction in methanogenic bioreactors. *FEMS Microbiology Reviews* **15**(2–3), 199–136.

Poinapen, J., Ekama, G. & Wentzel, M. C. 2009 Biological sulphate reduction with primary sewage sludge in an upflow anaerobic sludge bed (UASB) reactor – Part 2: modification of simple wet chemistry analytical procedures to achieve COD and S mass balances. *Water S.A.* **35**(5), 525–534.

Rubio-Rincón, F. J., Lopez-Vazquez, C. M., Welles, L., van den Brand, T., Abbas, B., van Loosdrecht, M. C. M. & Brdjanovic, B. 2017a Effects of electron acceptors on sulphate reduction activity in activated sludge processes. *Applied Microbiology and Biotechnology* **101**(4), 1661–1672.

Rubio-Rincón, F. J., Lopez-Vazquez, C. M., Welles, L., van Loosdrecht, M. C. M. & Brdjanovic, B. 2017b Sulphide effect on the physiology of Candidatus Accumulibacter phosphatis type I. *Applied Microbiology and Biotechnology* **101**(4), 1661–1672.

Sallanko, J. & Pekkala, M. 2008 Wastewater temperature decrease in pressure sewers. *Water Environment Research* **80**(12), 2247–2252.

Spinosa, L. & Vesilind, P. A. (eds) 2001 *Sludge into Biosolids: Processing, Disposal and Utilization*. IWA publishing, London, ISBN: 9781900222082.

Strous, M. & Jetten, M. S. M. 2004 Anaerobic oxidation of methane and ammonium. *Annual Review of Microbiology* **58**, 99–117.

Thauer, R. K., Jungermann, K. & Decker, K. 1977 Energy conservation in chemotrophic anaerobic bacteria. *Bacteriology Reviews* **41**(1), 100–180.

van den Brand, T. P. H., Roest, K., Chen, G. H., Brdjanovic, D. & van Loosdrecht, M. C. M. 2014a Influence of acetate and propionate on sulphate-reducing bacteria activity. *Journal of Applied Microbiology* **117**(6), 1839–1847.

van den Brand, T. P. H., Roest, K., Chen, G. H., Brdjanovic, D. & van Loosdrecht, M. C. M. 2014b Temperature effect on acetate and propionate consumption by sulfate-reducing bacteria in saline wastewater. *Applied and Environmental Microbiology* **98**(9), 4245–4255.

Water Practice & Technology Vol 13 No 3
doi: 10.2166/wpt.2018.068

van den Brand, T. P., Roest, K., Chen, G. H., Brdjanovic, D. & van Loosdrecht, M. C. 2015a Long-term effect of seawater on sulfate reduction in wastewater treatment. *Environmental Engineering Science* **32**(7), 622–630.
van den Brand, T. P. H., Roest, K., Chen, G. H., Brdjanovic, D. & van Loosdrecht, M. C. M. 2015b Occurrence and activity of sulphate reducing bacteria in aerobic activated sludge systems. *World Journal of Microbiology and Biotechnology* **31**(3), 507–516.
van den Brand, T. P. H., Roest, K., Chen, G. H., Brdjanovic, D. & van Loosdrecht, M. C. M. 2015c Potential for beneficial application of sulfate reducing bacteria in sulfate containing domestic wastewater treatment. *World Journal of Microbiology and Biotechnology* **31**(11), 1675–1681.
van den Brand, T. P. H., Roest, K., Chen, G. H., Brdjanovic, D. & van Loosdrecht, M. C. M. 2015d Effects of chemical oxygen demand, nutrients and salinity on sulfate-reducing bacteria. *Environmental Engineering Science* **32**(10), 858–864.
Wang, J., Lu, H., Chen, G.-H., Lau, G. N., Tsang, W. L. & van Loosdrecht, M. C. M. 2009 A novel sulfate reduction, autotrophic denitrification, nitrification integrated (SANI) process for saline wastewater treatment. *Water Research* **43**(9), 2363–2372.