Journal of Applied Microbiology

Journal of Applied Microbiology ISSN 1364-5072

ORIGINAL ARTICLE

Influence of acetate and propionate on sulphate-reducing bacteria activity

T.P.H. van den Brand[1], K. Roest[1], D. Brdjanovic[2,3], G.H. Chen[4] and M.C.M. van Loosdrecht[1,3]

1 KWR Watercycle Research Institute, Nieuwegein, the Netherlands
2 UNESCO-IHE, Delft, the Netherlands
3 TU Delft, Delft, the Netherlands
4 Hong Kong University of Science and Technology, Hong Kong, China

Keywords
acetate, competition, methanogens, propionate, sulphate-reducing bacteria, wastewater treatment.

Correspondence
Tessa P.H. van den Brand, KWR Watercycle Research Institute, Groningenhaven 7, 3433 PE Nieuwegein, the Netherlands.
E-mail: tessa.van.den.brand@kwrwater.nl

2014/1564: received 29 July 2014, revised 29 September 2014 and accepted 2 October 2014

doi:10.1111/jam.12661

Abstract

Aims: Sulphate-reducing bacteria (SRB) activity is generally considered as inconvenience in domestic wastewater treatment plants (WWTP), but could also be applied beneficially. The competition between SRB and methanogens is a point of concern for stable process design. As limited attention was given to the effect of varying acetate and propionate concentrations on SRB activity, this study focused specially on these substrates.

Methods and Results: The research was performed in sequencing batch reactors operated at 20°C and an SRT of 15 days. In the acetate-fed reactor, methanogens became dominant, while in the propionate reactor, SRB were the dominant population. In the mixed-substrate-fed reactor, both substrates were converted by SRB. The dominant SRB population in the mixed-substrate-fed reactor was different from the propionate-fed reactor, but all operational characteristics such as the substrate consumption rate, yield and growth rate were similar. The sludge adapted to propionate could easily switch to an acetate feed procedure.

Conclusions: These results indicate that under wastewater temperature of 20°C, the SRB are likely to outcompete methanogens more easily as inferred from pure substrate studies on acetate solely.

Significance and Impact of the Study: The present results show that the natural presence of propionate in wastewater allows stable sulphate reduction, which decreases the biogas production, but provides an opportunity for using SRB beneficially in wastewater treatment.

Introduction

Sulphate-reducing bacteria (SRB) are of importance for municipal wastewater treatment. The presence and activity of SRB can cause corrosion and odour problems, due to the formation of sulphide (Nielsen *et al.* 1992). Moreover, SRB activity reduces the methane formation potential during generation of biogas in anaerobic wastewater treatment. On the other hand, several studies point to the beneficial application of SRB in wastewater treatment (Lens *et al.* 1998; Lens and Kuenen 2001; Muyzer and Stams 2008) which can be summarized as follows: minimal sludge production due to a low growth yield of SRB (Lens *et al.* 2002), sulphide formation leading to substantial reduction of pathogens (Abdeen *et al.* 2010), opportunity to remove heavy metals by precipitation with sulphide (Lewis 2010), granulation ability of SRB (Lens *et al.* 2002) and the formation of sulphide to achieve nitrogen removal by autotrophic denitrification (Wang *et al.* 2009).

Knowledge on competition between SRB and methanogens is important for stable process design, for both the application of SRB, and for processes which need avoidance of SRB activity, such as biogas production. Volatile fatty acids (VFA) result from fermentation processes in the sewage. Acetate and propionate have shown to be the main VFA present in the sewage (Mino *et al.* 1998; Chen *et al.* 2004; López-Vázquez *et al.* 2008). The effect of

acetate and propionate on SRB performance got limited attention so far, and if discussed, it was for other objectives (Buisman *et al.* 1989; Omil *et al.* 1996; Finke *et al.* 2007). In van den Brand *et al.* (2014), an initial research on the application of SRB in domestic wastewater specific for the presence of acetate and propionate in moderate climates (20°C) was described, indicating that SRB can oxidize both acetate and propionate at solid retention times of 15 days. However, this research did not clearly indicate the effect of the substrate composition.

The combined effect of acetate and propionate on SRB activity is of interest as both VFA have a different role in the competition between SRB and methanogens. SRB can oxidize both acetate and propionate. Propionate oxidation by SRB can occur incomplete to acetate or complete to CO_2. In contrast, methanogens are only able to oxidize acetate, while propionate oxidation requires a separate population for propionate oxidation to hydrogen and acetate before conversion to methane. In the presence of both VFA and excess of sulphate, it is expected that SRB can effectively outcompete methanogens, as they are capable of oxidizing both substrates (Kovárová-Kovar and Egli 1998). The VFA composition can influence the SRB species, and thus the kinetic properties of the sulphate reduction process.

Therefore, this study focused on the role of short-chain fatty acids, acetate and propionate, on the competition of SRB and methanogens. The approach of this study was to apply three feed procedures: (i) acetate, (ii) propionate and (iii) both acetate and propionate in a sequencing batch reactor, and analyse the effluent quality and SRB population composition.

Material and methods

Long-term sequencing batch reactors

Three SBR reactors were operated under the same conditions to the study described in van den Brand *et al.* (2014), except that three different substrate feeds were applied. The characteristics of the substrate used in the influent of each reactor are given in Table 1. The reactors were operated in repeated cycles which lasted 6 h with 1·5 l influent addition every cycle in a working volume of 2·5 l (thus, 1 l remained in the reactor after effluent discharge). The cycle was divided in three phases: (i) 5-h-and-20-min feed-reaction phase, with continuous feeding of 1·5 l in the first 110 min, (ii) 20-min settling and (iii) 20-min effluent withdrawal. The following parameters were controlled: temperature (20°C), pH (7·6 ± 0·2), no aeration (DO; 0%), mixing (300 rpm), solid retention time (SRT, 15 days) and hydraulic retention times (HRT, 10 h). Every cycle, which lasts 6 h, treats 1·5 l wastewater, resulting in an average inflow and outflow of 0·25 l h^{-1}.

Table 1 Substrate characteristics in the influent of each reactor discussed in this study

Reactor	Mixed substrates	Acetate	Propionate
Substrate	Acetate and propionate	Acetate	Propionate
COD concentration (mg l^{-1})	300	300	300
Acetate concentration (mmol l^{-1})	2·68	3·57	0
Propionate concentration (mmol l^{-1})	1·15	0	1·53

All reactors were inoculated with a similar inoculum: activated sludge from wastewater treatment plant (WWTP) Amsterdam-West (the Netherlands) and sediment from a pond in the ecological garden of KWR Watercycle Research Institute (the Netherlands). To maintain a most adapted microbial community in the culture, every 2 weeks, the population in all reactors were enriched by addition of new biomass (approx. 5%v/v) of composition similar to the original inoculum. Over the last 15 days, however, no enrichment of the culture within the reactor occurred, to avoid disturbance of the SRB population analyses.

The reactor was fed with synthetic saline wastewater with a COD/SO_4^{2-} ratio of 0·6 g/g and consisting of: 7·32 g l^{-1} aquarium salt corresponding to 500 mg SO_4^{2-} l^{-1} (Reef Crystals™), 0·09 mmol l^{-1} K_2HPO_4, 0·04 mmol l^{-1} KH_2PO_4, 2·89 mmol $l^{-1}NH_4Cl$ (40 m gN l^{-1}), 0·34 mmol l^{-1} $MgCl_2{\cdot}6H_2O$, 0·39 mmol l^{-1} $CaCl_2$ and 1 ml l^{-1} trace elements solution as described by Lau *et al.* (2006). The COD content in influent was 300 mg l^{-1}, corresponding to 3·57 $NaCH_3COO{\cdot}3H_2O$ and 1·53 mmol l^{-1} $NaC_3H_5O_2$ for, respectively, the acetate- and propionate-fed reactor.

Steady-state operation was evaluated based on the stable effluent quality and constant profile of conversions in the reactor. Steady state was reached after approx. 21 days. After reaching steady state, the reactor was ran for three SRTs before the SRB population analysis and substrate activity tests were executed. The detailed description of the analytical methods is described in van den Brand *et al.* (2014).

After stable operation and detailed cycle and population analyses, the media composition (acetate) was switched to propionate, and *vice versa*.

Activity analyses

Batch tests for evaluating substrate conversion potential were executed by incubating sludge from an SBR. In a 100-ml serum bottle, 50 ml of the media was added and

30 ml of sludge from the reactor. The media had the same composition as the media used in the long-term reactors with the same variations in substrate composition, such as acetate and propionate, solely acetate and solely propionate, but all with a COD concentration of 300 mg l^{-1}. To obtain anaerobic conditions, the media was flushed with N_2 gas for 1 min. The SRB activity was measured as the increasing concentration of sulphide in time. The sulphide concentration in the liquid phase was measured according to the methylene blue method (APHA 1995); to assure no sulphide losses during analyses, the sulphide was upon sampling immediately fixated with 1 drop of 1 mol l^{-1} NaOH. Moreover, also the $COD_{substrates}$ concentration of each batch tests was checked by the spectrophotometer with a standard test kit (Hach-Lange LCK 514) after removal of the sulphide by adding $ZnSO_4$.

SRB population analyses

The SRB population was analysed for two different goals: first to demonstrate the presence of SRB in each of three reactors and second to observe the population difference between the acetate, propionate and mixed-substrate-fed reactors. Reactor samples and the inoculum were analysed using terminal restriction fragment length polymorphism (TRFLP) and sequencing of the partial dsrA gene. Immediately after sampling, DNA was isolated using the power-biofilm DNA isolation kit. After PCR amplification with the primer set DSR1334R and DSR1Fmix (Table 2), TRFLP was performed as described in (Santillano *et al.* 2010). The PCR products after 70 cycles were cloned with the pGEM®-T Easy Vector kit. Colonies were picked and analysed by Macrogen®.

Results

Long-term operation

In the reactor fed with acetate, no sulphate reduction occurred, while complete COD removal (98%) was achieved after 3 weeks of operation (Fig. 1a). Methane was observed in the off-gas (data not shown), indicating the activity of methanogens, which was confirmed by the dominant detection of methanogens by their autofluorescence. The biomass concentration within the reactor was gVSS·l^{-1} (Table 3). Sulphide formation was not observed.

In contrast to the acetate-fed reactor, SRB activity was observed for the reactor fed with propionate (Fig. 1b and Table 3). The effluent values became stable after 4 weeks of operation, complete $COD_{subsrates}$ removal was achieved (99%), and the COD/SO_4^{2-} consumption ratio of 0·68 (g/g) indicates that this was accomplished by SRB activity. This was confirmed by the absence of methane in the off-gas. The sulphate reduction rate within the reactor was 1·25 mmolSO_4^{2-} $gVSS^{-1}$ h^{-1}. Throughout the cycle, no acetate production was observed within the reactor. The biomass concentration in the reactor was 0·85 gVSS l^{-1} and the yield 0·023 ($gVSS/gCOD_{consumed\ substrates}$), both lower than the values for the acetate-fed reactor (Table 3). The characteristic values of the sludge adapted to propionate feeding, such as yield and growth rate, were similar to that from the reactor fed with acetate and propionate (Table 3).

Table 2 Primers used for PCR amplification in the TRFLP and sequencing technique

	Sequence (5′–3′)	Reference
DSR1334R	TYT TCC ATC CAC CAR TCC	Santillano *et al.* (2010)
DSR1Fmix		
DSR1F	ACS CAC TGG AAG GAC G	Wagner *et al.* (1998)
DSR1Fa	ACC CAY TGG AAA CAC G	Loy *et al.* (2004)
DSR1Fb	GGC CAC TGG AAG CAC G	Loy *et al.* (2004)
DSR1Fc	ACC CAT TGG AAA CAT G	Zverlov *et al.* (2005)
DSR1Fd	ACT CAC TGG AAG CAC G	Zverlov *et al.* (2005)

SRB population

There was no SRB population observed by TRFLP in the acetate-fed reactor, in line with the observed absence of sulphide formation in that reactor. The SRB community profile from the propionate reactor had a low similarity with the inoculum; 45 and 19% for respectively the sludge from WWTP Amsterdam-West and the pond sediments from the ecological garden of KWR (Nieuwegein, the Netherlands) (Fig. 5). The sequencing results demonstrated that in the mixed-substrate-fed reactor, two SRB species were dominant (Accession Numbers: KF921953 and KF921955) (Fig. 5), while in the reactor fed with solely propionate, only 1 dominant species was observed (Accession Number: KJ546432), which was not equal to any of the species obtained in the mixed-substrate-fed reactor. The detected sequences of dominant species were most closely related to sequences in the genetic databank of uncultured SRB species. The first cultured species related to the strain obtained from the propionate-fed reactor was *Desulfobulbus propionicus* (86%); the first uncultured related sequence had Accession Number EU188841.1 (99%). In the mixed-substrate-fed reactor, the first cultured related species were *Desulfotalea arctica* (79%) and *Desulfofustis glycolicus* (78%), and the closest uncultured related species were for both sequences AY741558 (respectively

Table 3 Operational results and characteristics of the reactors fed with acetate and propionate, and the reactors fed with equal amounts of COD, but only acetate or propionate as carbon source

		Mixed-substrate reactor	Acetate reactor	Propionate reactor
Reactor	Feed composition	Acetate and propionate	Acetate	Propionate
	COD concentration feed (mg l^{-1})	300	300	300
	Duration (days)	131	90	90
	COD balance (%)	106	n.d.	96
	Sulphur balance (%)	91	100	102
Effluent	$COD_{substrates}$ (mg $l^{-1)}$	1 ± 1	5 ± 4	4 ± 3
	Sulphate (mg l^{-1})	51 ± 2	500 ± 15	62 ± 4
	Sulphide (mg l^{-1})	148 ± 7	0 ± 0	146 ± 5
Conversion	$COD_{substrates}$ removal (%)	100	98	99
	SO_4 removal (%)	89	0	88
	Maximal rate (mmolSO_4^{2-} $gVSS^{-1}$ h^{-1})	1·26	0	1·25
	Maximal rate (mgCOD $gVSS^{-1}$ h^{-1})	80·9	n.d.	80·1
Growth	Yield (gVSS/$gCOD_{substrates}$)	0·028	0·030	0·023
	VSSreactor (g l^{-1})	0·72	1·12	0·85

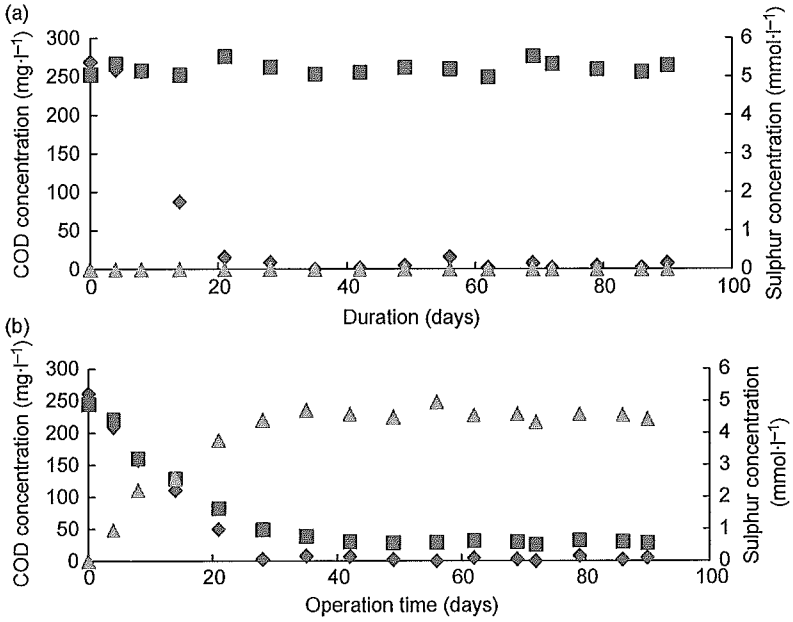

Figure 1 (a) Effluent quality profile of $COD_{substrates}$ (◆), sulphate (■) and sulphide (▲), of the sequencing batch reactor fed with solely acetate as carbon source. (b) Effluent quality profile of $COD_{substrates}$ (◆), sulphate (■) and sulphide (▲), of the sequencing batch reactor fed with solely propionate as carbon source.

83 and 85%). Clearly, the enriched SRB organisms had no relation to known cultured species.

Morphology

In all three reactors, a granular type of sludge evolved. The average diameter of granules from the propionate-fed reactor was larger than those from the acetate-fed reactor (Fig. 2). The diameter size of granules from the propionate reactor had a wide range of 200–600 1m, but all had a smooth surface. The largest granules from the propionate reactor had similar size as those from the mixed-substrate-fed reactor.

Adaptation to carbon sources

In the batch activity test fed with acetate and propionate, the amount of sulphate reduced corresponded to a case in which only propionate would have been oxidized (Fig. 3). Since for sludges from the propionate-fed reactor, the short-term activity test showed that the sludge could not consume acetate within 5 h after incubation (Fig. 3), it was concluded that only propionate and no acetate was oxidized in the batch test fed with acetate and propionate. In the batch test fed with both acetate and propionate, solely propionate was oxidized by SRB. The sulphate reduction rate over the first 0·5 h is equal

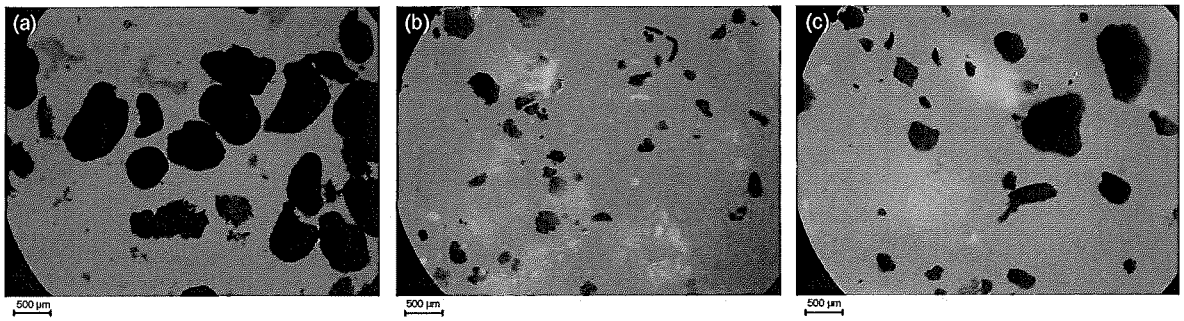

Figure 2 Microscopic pictures from the sludge present in the sequencing batch reactors fed with equal amounts of COD, but fed with acetate and propionate (a), acetate (b) and propionate (c) as carbon source.

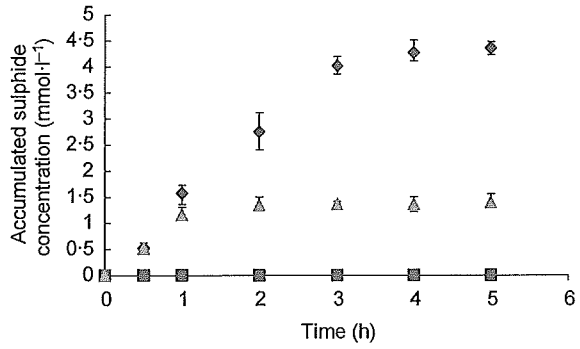

Figure 3 The sulphide production in time for sludge enriched on propionate, converting propionate (◆), acetate (■) and a mixture of acetate and propionate (▲).

in the batch test fed with propionate and propionate and acetate. After 1 h, no sulphate reduction occurred as the propionate portion of the organics was depleted, while acetate could not be oxidized as a sole substrate by SRB adapted to propionate feeding.

Sludges from the acetate-fed reactor did not show any sulphate-reducing activity using acetate or propionate (data not shown). In batch tests inoculated with acetate-adapted sludge (methanogenic activity), acetate was consumed, while propionate was not converted.

Long-term switch of media

The feed of the acetate and propionate reactors was switched after 90 days of operation. The sludge adapted to propionate could not consume acetate within 5 h (Fig. 3), but after 2 days, acetate-based sulphate reduction was observed (Fig. 4). Complete acetate removal (99%) was recorded after 4 days of adaption to the new acetate feed procedure. Sulphate (63 $mgSO_4^{2-}\ l^{-1}$) and sulphide (146 $mgS^{2-}\ l^{-1}$) concentration in the effluent showed that this acetate removal was due to SRB activity. The sludge enriched with acetate had no SRB activity, and the change to propionate feed did not initiate any sulphate-reducing activity within 2 weeks.

Discussion

Sulphate-reducing bacteria and methanogens both rely mainly on fermentation products as substrate. SRB have a rather wide substrate spectrum (among others propionate, ethanol, propanol, pyruvate, lactate, Widdel and Pfennig 1982), whereas methanogens have a more narrow VFA substrate spectrum (mainly hydrogen and acetate (Oude Elferink *et al.* 1994), and most of the VFA can only be oxidized in syntrophy with acetogens. Typical factors affecting the competition between SRB and methanogens are temperature (Visser *et al.* 1993a,b; Shin *et al.* 1996), pH (McCartney and Oleszkiewicz 1993; Visser *et al.* 1993a,b), sulphide (Oude Elferink *et al.* 1994), substrate composition (Oude Elferink *et al.* 1994) and sulphate concentration (Rebac *et al.* 1996). In this study, the main fermentation products in wastewater treatment systems (acetate and propionate) were used to evaluate the competition between both microbial groups. Acetate can be a substrate for both groups, while propionate is only directly consumed by SRB. For methanogens, propionate first needs to be fermented into acetate and hydrogen. Hydrogen could also be used by methanogens or SRB.

Methanogens dominant in acetate-fed reactor

In the acetate-fed reactor, complete $COD_{substrates}$ removal was achieved, but no sulphate reduction occurred (Fig. 1a). The methanogenic presence and activity was confirmed by methane detection in the off-gas and the observation of autofluorscence microbes. A slow-feed procedure was applied in the sequencing batch reactor, resulting in a very low actual acetate concentration in the reactor. Therefore, the affinity for acetate of these micro-organisms is determining the outcome of the

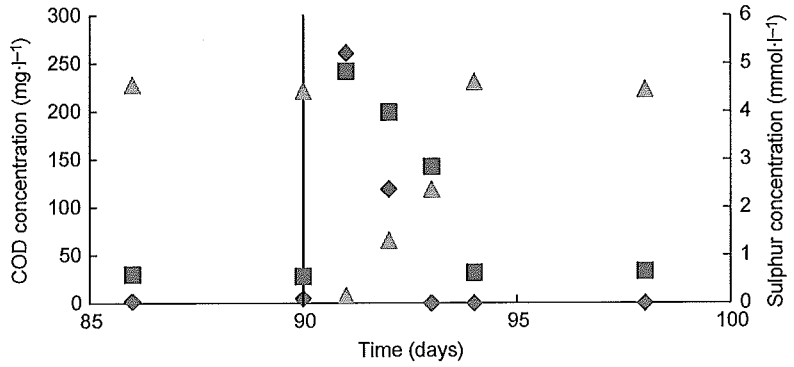

Figure 4 Profile for effluent quality analyses of $COD_{substrate}$ (◆), sulphate (■) and sulphide (▲) for the reactor initially fed with propionate. After 90 days, the feed procedure was changed to solely acetate as carbon source, and SRB activity recovered within a week. As the growth rate is very slow, no significant change in SRB population over the first 5 days is expected.

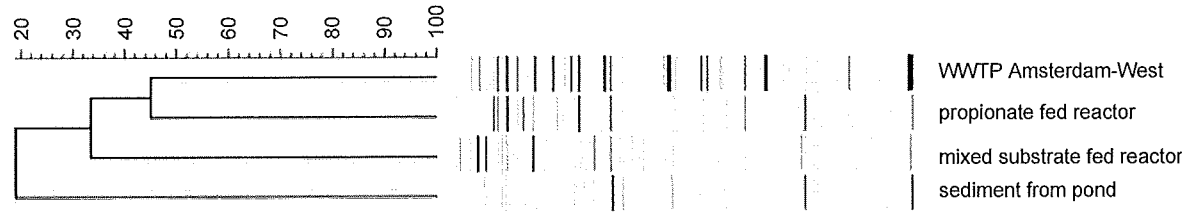

Figure 5 Comparison of TRFLP profiles of SRB communities from the reactors fed with acetate and propionate, only propionate as carbon source and the inoculum; WWTP Amsterdam-West and ecological garden. In the acetate-fed reactor, no SRB were detected.

competition. Reported affinity constants are rare, with values of 0·2 mmol l^{-1} for *Desulfobacter postgatei* (Oude Elferink *et al.* 1994), and 0·4 mmol l^{-1} for *Methanosaeta* sp. (Jetten *et al.* 1992). In general, SRB are assumed to have a kinetic advantage over methanogens for the competition on acetate; also more energy is released per mol of acetate by SRB. (Parkin *et al.* 1990; Lens *et al.* 1998; Wang *et al.* 2009). Although then a dominance of SRB might be expected, methanogens became dominant in the acetate-fed reactor, which might be caused by the different conditions, such as low temperature, as compared to the previous studies. It was observed that the acetate concentration in the mixed-substrate-fed reactor, with the SRB culture, was above 0·3 mmol l^{-1} (van den Brand *et al.* (2014)). This value is in the range of the reported Km values for methanogens and SRB. A high sulphate concentration has been reported as favouring SRB over methanogens (Harada *et al.* 1994; Omil *et al.* 1998). As the COD/SO_4^{2-} ratio of the influent was 0·6 gCOD/gSO4, the sulphate was not limiting and this methanogenic dominance was not caused by sulphate limitation. So far, limited research was performed on the competition between SRB and methanogens on solely acetate as carbon source. The few studies which did so, indicate, similar to the results of this study, that methanogens are in favour, especially if sulphate is limiting (Yoda *et al.* 1987; Bhattacharya *et al.* 1996).

SRB dominant in propionate-fed reactor

Since methanogens are not reported to oxidize propionate, the activity and dominance of SRB in the propionate-fed reactor (Fig. 1a) was according to expectations. Characteristic values, such as the maximal sulphate reduction rate, yield and growth, were similar for the propionate-fed reactor and the mixed-substrate reactor (Table 3). The SRB population, however, was different in these two reactors, as the TRFLP profile showed that the similarity was <40% (Fig. 5). Also the sequencing results showed that different species became dominant in the propionate reactor and the mixed-substrate reactor. The closest cultured species related to the dsrA gene of the dominant strain in the propionate-fed reactor was *D. propionicus* (86%). *Desulfobulbus propionicus* has been shown to be deficient for acetate oxidation, but could use propionate as electron donor (Widdel and Pfennig 1982). *Desulfobulbus propionicus* has been reported to show incomplete propionate oxidation, resulting in the accumulation of acetate. This is in contrast with the results of this study, which shows that in the propionate-fed reactor, no acetate formation was observed and the sludge could oxidize acetate after a short adaptation period; it was assumed that complete propionate oxidation occurred. The contrasting results could be explained by the fact that the similarity of the obtained SRB species in the propionate-fed reactor compared to

D. propionicus is low (86%), and therefore, other characteristics can be expected.

SRB and methanogens competition for mixed substrate

With pure acetate feeding, methanogens outcompeted the SRB. However, on the mixed substrate of acetate and propionate, a culture of SRB dominated. In this reactor, two SRB species seem to have become dominant. This seems to indicate initially occurrence of two specialist SRB (one growing on acetate and one on propionate); however, it would then be expected that also in the only acetate-fed reactor a SRB would have become dominant. There may be several reasons for SRB dominancy in the mixed-substrate reactor such as SRB consume both substrates and thereby have more substrate available, propionate suppresses the methanogenic activity, and the produced sulphide is more inhibitory to methanogens than SRB or competition for other essential nutrients.

The sludge adapted to solely propionate feed was after switching feed procedure into solely acetate not able to oxidize acetate on short term of 6 h (Fig. 3). After four days, however, the SRB adapted to the new feed procedure of solely acetate, indicating they could switch their metabolism to acetate utilization (Fig. 4). The growth rate of these SRB species was low, and ingrowing of a new dominant population can only be achieved after >15 days, when the SRT is 15 days. As the present population could achieve complete acetate removal within 5 days, it was therefore assumed that the population firstly adapted to propionate could also oxidize acetate completely. This suggests that the disability to oxidize acetate in the first 5 h after changing the feed procedure from acetate to propionate was a result of an inactive acetate transport system, which could become active within soon afterwards. Thus, it seems that the present SRB can oxidize two substrates, both acetate and propionate. The ability to grow on both substrates favours the SRB over methanogens which is only able to grow on acetate (Kovárová-Kovar and Egli 1998), enabling the SRB to outcompete the methanogens in the mixed-substrate-fed reactor. van den Brand *et al.* (2014) revealed that the acetate and propionate consumption rates in terms of COD were practically identical, indicating that SRB could grow equally well on both substrates. In general, when bacteria grow on a substrate mixture, the resulting concentration of the individual substrates will be lower than when growth occurs on a single substrate, if in both cases, the COD concentration was equal. Because methanogens as specialists cannot grow on mixed substrates, they are effectively outcompeted in more complex systems. Thus, as SRB lower in a mixed-substrate influent effectively the acetate concentration, the growth rate of methanogens is reduced and easily becomes slower than the dilution rate of the reactor. Propionate has shown to decrease the methanogenic activity (Barredo and Evison 1991; Dhaked *et al.* 2003). Propionate is, however, fed at a rate slower than the maximal uptake rate, and therefore, in the reactor, the concentration is always low; inhibition of methanogens by the propionate is therefore in this particular situation not likely.

Application in wastewater treatment

In all three reactors, granules were formed (Fig. 2). Granular sludge is an effective way to increase volumetric capacity and decrease reactor footprint (Nicolella *et al.* 2000). Methanogenic sludge is known to easily granulate. Also for sulphate-reducing bacteria, granular sludge formation has been reported (Lens *et al.* 2002; Hao *et al.* 2013). The driving forces behind granular sludge formation are diverse organic load rate, hydrodynamics shear force, settling time, reactor configuration and calcium content (Yu *et al.* 2001; Liu and Tay 2004). All these factors are likely not applicable in this study, especially the settling time is rather long. A low intrinsic growth rate has also been reported as a factor for granular sludge formation (Van loosdrecht *et al.* 1995); indeed, the low growth rate is responsible here for the granule formation. Application of SRB in wastewater treatment can therefore lead to stable granular sludge formation without the need for extra process adaptations. Indeed, in the upflow SRB rector of the SANI concept spontaneous granulation has been reported despite a relatively low upflow velocity in the SANI reactor of 0·39 m h^{-1} (Hao *et al.* 2013).

In domestic wastewater, there will always be a mixed substrate present. Fermentation of the COD will lead to a range of volatile fatty acids (Mino *et al.* 1998; Chen *et al.* 2004; López-Vázquez *et al.* 2008). Propionate and acetate are the main fermentation products and are reported in concentration ranges of 28–93 mg l^{-1} for acetate and 5–82 mg l^{-1} for propionate (López-Vázquez *et al.* 2008). Based on the results of this study, it is therefore expected that in municipal wastewater SRB will win the competition from methanogens unless sulphate is present in limited amounts. Indeed, in the saline wastewater of Hong Kong, full sulphate reduction occurs without methanogenesis (Lu *et al.* 2011). In many drinking water systems, sulphate can be present or sulphate can be introduced in the sewer by intrusion of seawater or brackish water. The effective competition by SRB when sulphate is present makes that potential for biogas production on such wastewater limited, but beneficial stable use of SRB in wastewater treatment concepts (without interference of methane formation) is feasible.

This study revealed that SRB outcompete methanogens in the presence of both acetate and propionate, which are typical products of fermentation in the sewer. Therefore, if wastewater contains significant amount of sulphate, a COD/SO_4^{2-} ratio below 0·67, a sufficient anaerobic wastewater treatment process other than SRB will likely not succeed. In the presence of acetate, propionate and sulphate, methanogens will likely not become dominant, and therefore, the biogas production is limited and with a lower methane content.

Final remarks

In the present study, the effect of substrate composition on the competition of SRB and methanogens at 20°C and a SRT of 15 days was investigated. The main conclusions drawn from the current study are the following: (i) on pure acetate substrate, methanogens become dominant, while on pure propionate, SRB become dominant; (ii) SRB sludge enriched on propionate could not convert acetate immediately (<5 h), but the population could adapt in a few days to acetate consumption; prolonged acetate and propionate addition led to a shift in the microbial population after 2 weeks; (iii) methanogens were effectively outcompeted when a mixed substrate of acetate and propionate was fed to the reactor; and (iv) the presence of mixed substrates in wastewater is giving advantage to SRB over methanogens, limiting the potential for biogas production but providing opportunity for using SRB in wastewater treatment plant design.

Conflict of Interest

No conflict of interest declared.

References

Abdeen, S., Di, W., Hui, L., Chen, G.-H. and 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 Sci Technol* 62, 2564–2570.

APHA (1995). *Standard Methods for the Examination of Water and Wastewater*, 19th edn. Washington: APHA. ISBN:0-87553-223-3.

Barredo, M.S. and Evison, L.M. (1991) Effect of propionate toxicity on methanogen-enriched sludge, *Methanobrevibacteri smithii*, and *Methanosprillum hungatii* at different pH values. *Appl Environ Microbiol* 57, 1764–1769.

Bhattacharya, S.K., Uberoi, V. and Dronamraju, M.M. (1996) Interaction between acetate fed sulfate reducers and methanogens. *Water Res* 30, 2239–2246.

van den Brand, T.P.H., Roest, K., Chen, G.H., Brdjanovic, D. and van Loosdrecht, M.C.M. (2014) Temperature effect on acetate and propionate consumption by sulphate reducing bacteria in saline wastewater. *Appl Microbiol Biotechnol* 98, 4245–4255.

Buisman, C.J.N., Stams, A.J.M., Meijer, H. and Lettinga, G. (1989) Sulphur and sulphate reduction with acetate and propionate in an aerobic process for sulphide removal. *Appl Microbiol Biotechnol* 32, 363–370.

Chen, Y., Randall, A.A. and McCue, T. (2004) The efficiency of enhanced biological phosphorus removal from real wastewater affected by different ratios of acetic to propionic acid. *Water Res* 38, 27–36.

Dhaked, R.K., Waghmare, C.K., Alam, S.I., Kamboj, D.V. and Singh, L. (2003) Effect of propionate toxicity on methanogenesis of night soil at phychrophilic temperature. *Bioresour Technol* 87, 299–303.

Finke, N., Vandieken, V. and Jørgensen, B.B. (2007) Acetate, lactate, propionate, and isobutyrate as electron donors for iron and sulfate reduction in Arctic marine sediments, Svalbard. *FEMS Microbiol Ecol* 59, 10–22.

Hao, T., Wei, L., Lu, H., Chui, H., Mackey, H.R., van Loosdrecht, M.C.M. and Chen, G. (2013) Characterization of sulfate-reducing granular sludge in the SANI® process. *Water Res* 47, 7042–7052.

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

Jetten, M.S.M., Stams, A.J.M. and Zehnder, A.J.B. (1992) Methanogenesis from acetate: a comparison of the acetate metabolism in *Methanothrix soehngenii* and *Methanosarcina* spp. *FEMS Microbiol Rev* 88, 181–197.

Kovárová-Kovar, K. and Egli, T. (1998) Growth kinetics of suspended microbial cells: from single-substrate-controlled growth to mixed-substrate kinetics. *Microbiol Mol Biol Rev* 62, 646–6668.

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

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

Lens, P.N.L., Visser, A., Janssen, A.J.H., Hulshoff Pol, L.W. and Lettinga, G. (1998) Biotechnological treatment of sulfate-rich wastewaters. *Crit Rev Environ Sci Technol* 28, 41–88.

Lens, P., Vallero, M., Esposito, G. and Zandvoort, M. (2002) Perspectives of sulfate reducing bioreactors in environmental biotechnology. *Rev Environ Sci Biotechnol* 1, 311–325.

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

Liu, Y. and Tay, J.-H. (2004) State of the art of biogranulation technology for wastewater treatment. *Biotechnol Adv* 22, 533–563.

López-Vázquez, C.M., Hooijmans, C.M., Brdjanovic, D., Gijzen, H.J. and van Loosdrecht, M.C.M. (2008) Factors affecting the microbial populations at full-scale enhanced biological phosphorus removal (EBPR) wastewater treatment plants in The Netherlands. *Water Res* 42, 2349–2360.

Loy, A., Kusel, K., Lehner, A., Drake, H.L. and Wagner, M. (2004) Microarray and functional gene analyses of sulfate-reducing prokaryotes in low-sulfate, acidic fens reveal cooccurrence of recognized genera and novel lineages. *Appl Environ Microbiol* 70, 6998–7009.

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

McCartney, D.M. and Oleszkiewicz, D.M. (1993) Competition between methanogens and sulfate reducers: effect of COD: sulfate ratio and acclimation. *Water Environ Res* 65, 655–664.

Mino, T., van Loosdrecht, M.C.M. and Heijnen, J.J. (1998) Microbiology and biochemistry of the enhanced biological phosphate removal process. *Water Res* 32, 3193–3207.

Muyzer, G. and Stams, A.J.M. (2008) The ecology and biotechnology of sulphate-reducing bacteria. *Nature* 6, 441–455.

Nicolella, C., van loosdrecht, M.C.M. and Heijnen, J.J. (2000) Wastewater treatment with particulate biofilm reactors. *J Biotechnol* 80, 1–33.

Nielsen, P.H., Raunkjaer, K., Norsker, N.H., Jensen, L.A. and Hvitved-Jacobsen, T. (1992) Transformation of wastewater in seqer systems - A review. *Water Sci Technol* 25, 17–31.

Omil, F., Lens, P., Hulshoff Pol, L. and Lettinga, G. (1996) Effect of upward velocity and sulphide concentration on volatile fatty acid degradation in a sulphidogenic granular sludge reactor. *Process Biochem* 31, 699–710.

Omil, F., Lens, P., Visser, A., Hulshoff Pol, L.W. and Lettinga, G. (1998) Long-term competition between sulfate reducing and methanogenic bacteria in UASB reactors treating volatile fatty acids. *Biotechnol Bioeng* 57, 676–685.

Oude Elferink, S.J.W.H., Visser, A., Hulshoff Pol, L.W. and Stams, A.J.M. (1994) Sulfate reduction in methanogenic bioreactors. *FEMS Microbiol Rev* 15, 119–136.

Parkin, G.F., Lynch, N.A., Kuo, W.C., Vankeuren, E.L. and Bhattacharya, S.K. (1990) Interaction between sulfate reducers and methanogens fed acetate and propionate. *Res J Water Pollut Control Fed* 62, 780–788.

Rebac, S., Visser, A., Gerbens, S., van Lier, J.B., Stams, A.J.M. and Lettinga, G. (1996) The effect of sulphate on propionate and butyrate degradation in a psychrophilic anaerobic expanded granular sludge bed (EGSB) reactor. *Environ Technol* 17, 997–1005.

Santillano, D., Boetius, A. and Ramette, A. (2010) Improved dsrA-based terminal restriction fragment length polymorphism analysis of sulfate-reducing bacteria. *Appl Environ Microbiol* 76, 5308–5311.

Shin, H.S., Oh, S.E. and Bae, B.U. (1996) Competition between SRB and MPB according to temperature change in the anaerobic treatment of tannery wastes containing high sulfate. *Environ Technol* 17, 361–370.

Van loosdrecht, M.C.M., Eikelboom, D., Gjaltema, A., Mulder, A., Tijhuis, L. and Heijnen, J.J. (1995) Biofilm structures. *Water Sci Technol* 32, 35–43.

Visser, A., Gao, Y. and Lettinga, G. (1993a) Effects of pH on methanogenesis and sulphate reduction in thermophilic (55°C) UASB reactors. *Bioresour Technol* 44, 113–121.

Visser, A., Gao, Y. and Lettinga, G. (1993b) Effects of short-term temperature increases on the mesophilic anaerobic breakdown of sulfate containing synthetic wastewater. *Water Res* 27, 541–550.

Wagner, M., Roger, A.J., Flax, J.L., Brusseau, G.A. and Stahl, D.A. (1998) Phylogeny of dissimilatory sulfite reductases supports an early origin of sulfate respiration. *J Bacteriol* 180, 2975–2982.

Wang, J., Lu, H., Chen, G.-H., Lau, G.N., Tsang, W.L. and van Loosdrecht, M.C.M. (2009) A novel sulfate reduction, autotrophic denitrification, nitrification integrated (SANI) process for saline wastewater treatment. *Water Res* 43, 2363–2372.

Widdel, F. and Pfennig, N. (1982) Studies on dissimilatory sulfate-reducing bacteria that decompose fatty acids II. Incomplete oxidation of propionate by *Desulfobulbus propionicus* gen. nov., sp. nov. *Arch Microbiol* 131, 360–365.

Yoda, M., Kitagawa, M. and Miyaji, Y. (1987) Long term competition between sulfate-reducing and methane-producing bacteria for acetate in anaerobic biofilm. *Water Res* 21, 1547–1556.

Yu, H.Q., Tay, J.H. and Fang, H.H.P. (2001) The roles of calcium in sludge granulation during uasb reactor start-up. *Water Res* 35, 1052–1060.

Zverlov, V., Klein, M., Lucker, S., Friedrich, M.W., Kellermann, J., Stahl, D.A., Loy, A. and Wagner, M. (2005) Lateral gene transfer of dissimilatory (Bi)sulfite reductase revisited. *J Bacteriol* 187, 2203–2208.