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ORIGINAL PAPER

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Toxicity of nitrite toward mesophilic and thermophilic sulphate-reducing, methanogenic and syntrophic populations in anaerobic sludge

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Abstract The various problems associated with treating sulphate-containing wastewaters stem inherently from successful competitive interactions between sulphate reducing bacteria (SRB) and other bacteria involved in the process, resulting in the formation of H_2S. Prevention of in-reactor sulphide generation by use of specific SRB inhibitors presents a potential solution. Nitrite has been reported to be a specific inhibitor of SRB but its possible toxicity to syntrophic and methanogenic members of the anaerobic consortium has not been investigated. In batch activity and toxicity tests, under both mesophilic and thermophilic conditions, nitrite, at concentrations of up to 150 mg L^{-1}, was found to be ineffective as a specific inhibitor of SRB, and was also shown to have an inhibitory effect on the activity of syntrophic and methane-producing bacteria in mesophilic and thermophilic digester sludge samples.

Keywords Anaerobic · Toxicity · Nitrite · Methanogens · Sulphate-reducing population

Introduction

The anaerobic digestion of sulphate-rich wastewaters presents a number of problems due to the activity and growth of sulphate reducing bacteria (SRB). In the presence of sulphate, SRB use sulphate as the terminal electron acceptor during the oxidation of organic matter, resulting in the production of hydrogen sulphide. The presence of H_2S gives rise to a wide range of biological and physico-chemical problems, including toxicity to methanogens and SRB, precipitation of non-alkali metals, odour and corrosion of pumps and pipes.

Strategies for dealing with sulphate and its reduction to sulphide during the anaerobic digestion of sulphate waste streams can be divided into two categories:

1. Corrective measures: dealing with sulphide toxicity after it is produced
2. Preventative measures: averting sulphide production by suppression of sulphate reduction

If sulphide toxicity is not of major concern, the sulphide removal process can be introduced after the methanogenic stage. The selection of the most suitable method depends on factors, such as the operation and investment costs of the process, as well as the end objective of the treatment, i.e. either maximum biogas yield or removal of organic matter and sulphate [11], and will also depend on local legislation. As SRB compete with methane producing bacteria (MPB) and obligate hydrogen producing anaerobes (OHPA) for available substrates, resulting in a decrease in methane yield, selective inhibition of SRB would ensure optimal methane yields and might be more cost effective than other end-of-pipe solutions, such as effluent and flue-gas H_2S scrubbing.

Various unsuccessful attempts have been made in the past to selectively suppress sulphate reduction by using specific inhibitors. For example, attempts to specifically inhibit the in-reactor activity of SRB species using sulphate analogues, such as molybdate, have not been successful, due to the observed toxic effects of molybdate on other members of the complex anaerobic digestion (AD) microbial consortium [21]. Nitrite has been shown to inhibit sulphate reduction in various environments [5, 6, 14–16]. Nitrite has the effect of raising the redox potential of a medium and preventing the activity of SRB [7]. A limited study by Philpott [16], involving addition of nitrite to a thermophilic UASB reactor treating a synthetic wastewater with a COD/SO_4^{2-} ratio of 4:1, resulted in an immediate inhibition of sulphate reduction with a consequent dramatic improvement in the

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COD removal efficiency to 80%. Following the apparent suppression of SRB activity by nitrite at 50 ppm, effluent acetate levels decreased and effluent propionate and butyrate concentrations were reduced to non-detectable levels [16]. There was no indication, in this study, of inhibition of the syntrophic or methanogenic populations by nitrite, but toxic effects of nitrous oxides have been reported by other authors [2, 8, 9, 17, 19]. Kluber and Conrad [8] found that the addition of 5 mM (230 mg L^{-1}) nitrite in batch tests on anoxic rice field soil resulted in complete inhibition of methanogenesis with concurrent elevated levels of acetate and propionate, for at least 24 days. These authors found that decreasing the concentrations of nitrite added had a consequent reduction of the length of time of inhibition. In a different study, these authors reported that the denitrification products of nitrate (nitrite, N_2O and NO) could inhibit CH_4 production both reversibly and irreversibly, depending on the type of methanogenic bacteria involved and the applied concentration of the N-compound [9]. For example, *Methanosarcina barkeri* was shown to be more sensitive to nitrite than *Methanobacterium bryantii*. These authors concluded that nitrite was a more effective inhibitor of methanogenesis than nitrate due to the bactericidal effect of its reduced derivatives, NO or nitrosyl complexes. Kluber and Conrad [9] tested effects only on pure culture methanogens growing on H_2/CO_2, so different populations grown on different substrates may have different sensitivities to N-compounds and this needs to be considered.

Roy and Conrad [17] found that addition of nitrate to a methanogenic rice soil slurry completely suppressed CH_4 production, and concluded that the main mechanism involved in the suppression of CH_4 production by nitrate was the inhibition of methanogenesis by denitrification intermediates rather than the competition between denitrifiers and methanogens for substrates. This study also determined that methanogenesis resumed at a lower rate than that prior to the addition of the N-compound [17].

Kluber and Conrad [8] reported the inhibition of SRB by nitrite. Percheron et al. [14] also found that sulphate reducers were completely inhibited by nitrogenous oxides. In batch cultures using molasses wastewater, nitrite denitrification began immediately without any significant lag phase, and sulphate reduction was inhibited. Methane production was also inhibited but the authors could not conclude whether nitrite or its reduction products were responsible. Nitrite reduction used sulphides, probably as electron donors, and the formation of elemental sulphur was assumed.

In the present study, biomass was obtained from two laboratory-scale UASB reactors. These were operated on a synthetic volatile fatty acid (VFA)/ethanol/glucose wastewater under mesophilic conditions (37°C) initially and subsequently at 55°C. One reactor served as a control whereas the second reactor influent contained 4 g sulphate L^{-1} throughout the trial. Nitrite was included at increasing concentrations to the influent of both reactors when operating under thermophilic conditions. The potential of nitrite as a selective inhibitor of SRB species was investigated in batch toxicity tests using sludge removed from the reactor at various stages during the 850 day trial. Nitrite toxicity against methanogenic and syntrophic, as well as SRB populations, was investigated in order to determine the potential selectivity of nitrite inhibition.

Materials and methods

Source of bacterial biomass

The sludge samples used in these investigations were obtained from two UASB reactors operated in the absence and presence of sulphate (S1 and S2) [12]. The reactor influent was a synthetic wastewater containing a VFA (acetate, propionate and butyrate) and ethanol mixture (1:1:1:1 COD basis) and 15% glucose, based on COD values. The total influent COD was 12 g L^{-1} . The COD:N:P ratio was maintained at 1,000:5:0.5 by supplementation with NH_4Cl and KH_2PO_4 to the required concentrations. Buffering was carried out by addition of $NaHCO_3$ (12 g L^{-1}) and feed was supplemented with micronutrients (1 ml L^{-1}), as recommended by Shelton and Tiedje [20].

The first reactor (S1) was maintained as a control, i.e. without sulphate supplementation. Reactor 2 (S2) was supplemented with 4 g sulphate L^{-1} in the form of $MgSO_4{\cdot}7H_2O$ from the beginning of the trial, resulting in an influent COD/SO_4^{2-} ratio of 3:1. Feeding was commenced to S1 and S2 at a hydraulic retention time (HRT) of 2 days and a volumetric loading rate (VLR) of 6 kg COD m^{-3} day^{-1} . Both reactors were converted to thermophilic operation on day 287 of the trial by a single-step change in operating temperature to 55°C. Nitrite (50 mg L^{-1}) was included in the influent to both reactors from day 544 onwards and increased to 75 mg L^{-1} and 100 mg L^{-1} on days 603 and 655, respectively.

Biomass samples for analysis were taken from the seed sludge and from the reactors after adaptation to both operational temperatures.

Nitrite toxicity tests

Toxicity thresholds were determined for nitrite against control and sulphate adapted mesophilic and thermophilic UASB reactor sludges to establish IC_{50} values based on the inhibition of the specific methanogenic activity (SMA) and substrate utilisation rates (SUR) for SRB species. SMA and SUR values were determined against the appropriate substrate in the presence of a range of nitrite concentrations. SMA and SUR values were also determined for blank and control vials. All tests were carried out in triplicate and stock solutions of $NaNO_2$ were made up in anaerobic buffer and added to

test vials at the required concentrations. Toxicity was defined in terms of the IC_{50} value, i.e. the concentration of nitrite that resulted in 50% inhibition of the control vial activities.

The SMA toxicity test procedure used was that described by Colleran and Pistilli [3]. Direct substrate degradation and sulphide production in batch test vials were monitored in order to determine the activity of SRB. Bromoethane sulphonic acid (BES) and sulphate were added to test vials (60 mL) at concentrations of 60 mM and 30 mM, respectively. BES is reported to be a specific inhibitor of methanogenic bacteria [13]. Substrate degradation and sulphide production were monitored by sampling the supernatant of the test vial as a function of time. Samples were centrifuged at 10,000 *g* (rcf) for 10 min, analysed for VFA and sulphide and SRB SUR rates calculated.

Test for the potential presence of nitrite reducers in reactor sludges

Tests were carried out to establish the presence of nitrite reducers in the anaerobic sludge of reactors containing NO_2^- in the influent. Direct substrate depletion tests were carried out in the presence of BES and molybdate as inhibitors of methanogens and SRB, respectively, using either acetate and H_2/CO_2 as substrate (electron donors) and NO_2^- as electron acceptor. All tests were set up in triplicate. The sludge was washed prior to inoculation into test vials to remove any remaining substrates. The sludge volatile suspended solids (VSS) concentration was maintained between 2 g and 5 g VSS L^{-1} in all test vials. Anaerobic buffer was included along with either BES, molybdate, NO_2^- and combinations thereof, in test vials to give a final volume of 30 mL in the acetate vials and 10 mL in the H_2/CO_2 vials. Helium gas was used to flush the vial headspace to remove any air. Vials were placed on an orbital shaker at 100 rpm in a temperature controlled room at 37°C. Acetate depletion was measured throughout the test by removal of samples from test vials and gas chromatography (GC) analysis of the supernatant was carried out using a Shimadzu GC-14B chromatograph with a hydrogen flame ionisation detector, fitted with a carbopack glass column. The column temperature was maintained at 175°C and the injection port and detector temperatures were 200°C and 250°C, respectively. The pressure decrease in millivolts was measured in vials pressurised to 1 atm (101 kPa)-pressure with the gaseous substrate, H_2/CO_2 [3]. Samples were taken periodically for nitrite depletion in the acetate vials and at the beginning and end of the H_2/CO_2 activity tests.

Analysis

The CH_4 content of the batch test vial headspace was analysed by GC. Headspace samples were extracted using 5 mL gas-tight syringes and analysed in triplicate. The GC configuration used was; a glass column (1.8 m ×4.0 mm i.d., 6.0 mm o.d.), packed with Poropak Q1000-120 mesh, in a Pye Unicam Series 304 chromatograph fitted with a gas sampling port and a hydrogen flame ionisation detector. Nitrogen was used as the carrier gas at a flow rate of 60 mL min^{-1} . The operating temperatures of the column, injection port and flame ionisation detector were 35°C, 100°C and 105°C, respectively.

The VSS content of test vials was analysed according to standard methods [1]. Nitrite analysis was carried out using a HACH DR 4000 spectrophotometer, according to the HACH ferrous sulphate method (program number 2600). The detection limit for the program is 1 mg L^{-1} NO_2^- and the detectable range is between 0 mg L^{-1} and 250 mg L^{-1} NO_2^-.

Results

Effect of nitrite on individual microbial populations in the sludge

Nitrite toxicity to the individual sulphate reducing, methanogenic and syntrophic populations involved in the anaerobic digestion process was investigated in batch tests at mesophilic and thermophilic temperatures with sludge removed from S1 and S2. Table 1 summarises the nitrite IC_{50}values for the syntrophic and methanogenic populations in S1 and S2 at 37°C and 55°C. Table 2 summarises the nitrite IC_{50} values for the SRB species present in S2 sludge only.

IC_{50} values were unexpectedly low for some substrates, i.e. acetate, butyrate and ethanol in S1 (Table 1) and this was found to be influenced in some cases by a lag phase prior to substrate degradation or biogas production (Fig. 1). It was therefore necessary to examine the patterns of substrate utilisation for the individual substrates.

Table 1 Summary of nitrite IC_{50} values (mg NO_2 L^{-1}) obtained for propionate, butyrate and ethanol syntrophs and acetate and H_2-utilising methanogenic populations from S1 and S2 mesophilic and thermophilic sludge sampled prior to nitrite inclusion in reactor influents. No specific methanogenic activity (SMA) activity was recorded against propionate and butyrate in the absence of nitrite

Day	IC_{50} values against test substrates (mg NO_2 L^{-1})				
	Acetate	Propionate	Butyrate	Ethanol	H_2/CO_2
S1 Mesophilic (day 248)	13	>150	17	23	>150
S2 Mesophilic (day 235)	21	>150	>150	>150	138
S1 Thermophilic (day 430)	18	5	21	21	73
S2 Thermophilic (day 430)	12	–	–	32	17

Table 2 Summary of nitrite IC_{50} values (mg NO_2 L^{-1}) obtained for propionate, butyrate, ethanol, acetate and H_2-utilising sulphate reducing bacteria (SRB) populations from S2 sludge at mesophilic and thermophilic temperatures, prior to nitrite inclusion in the S2 influent. There was no detectable SRB substrate utilisation rate (SUR) acetate value in control vials

Day IC_{50} values against test substrates (mg NO_2 L^{-1})					
	Acetate	Propionate	Butyrate	Ethanol	H_2/CO_2
S2 Mesophilic (day 235)	–	50	>150	–	>150
S2 Thermophilic (day 430)	10	20	20	10	50

Under mesophilic conditions, the microbial populations in the control reactor, S1, appeared to be more sensitive to nitrite toxicity than the populations of the sulphate-adapted reactor, S2, despite the fact that neither reactor was exposed to influent nitrite. The IC_{50} values for the butyrate- and ethanol-utilising syntrophs in S1 were considerably lower than the corresponding S2 IC_{50} values (Table 1), and all groups exhibited a low tolerance for nitrite except for the propionate-utilising syntrophs and the hydrogenotrophic methanogens. The mesophilic acetoclastic methanogens were found to be the most sensitive to nitrite inhibition in both S1 and S2 sludges, with IC_{50} values of 13 mg L^{-1} and 21 mg L^{-1}, respectively (Table 1).

This situation was somewhat reversed under thermophilic conditions, with the S2 acetoclastic, hydrogenotrophic, propionate- and butyrate-degrading populations being apparently more sensitive to nitrite inhibition than S1 populations at 55°C (Table 1). The SMA values for the S2 sludge against acetate, propionate and butyrate during thermophilic operation were very low compared to those reported under mesophilic conditions (Table 3), due to the effects of increased temperature, so the validity of the IC_{50} values presented in Table 1 for S2 are open to question. However, the SMA value recorded for the S2 hydrogenotrophic population during thermophilic operation was relatively high, i.e. 269 mL CH_4 (STP) g^{-1} VSS day^{-1} after

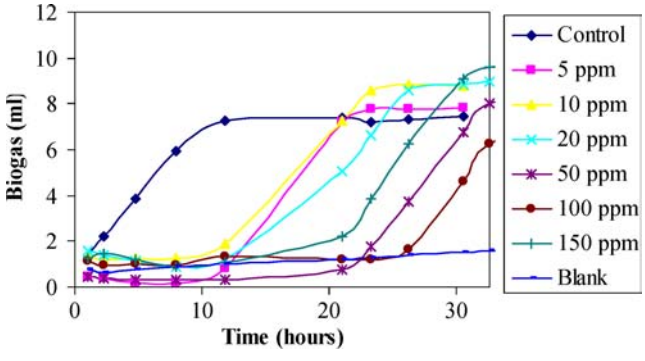

Fig. 1 The effect of increasing nitrite concentrations (5, 10, 20, 50, 100 and 150 mg NO_2-N L^{-1}) on the patterns of methanogenic substrate utilisation of acetate in mesophilic sulphate-adapted sludge (S2) (day 235)

Table 3 SMA profiles of the syntrophic and methanogenic populations in the S1 and the S2 sludges sampled on day 148 at 37°C and on day 430 at 55°C [mL CH_4 (STP) g^{-1} VSS day^{-1}]. All SMA values are the mean of triplicates. No SMA activity was recorded against propionate (i.e. activity below detectable level)

SMA profiles [mL CH_4 (STP) g^{-1} VSS day^{-1}]					
	Acetate	Propionate	Butyrate	Ethanol	H_2/CO_2
S1 (day 148) (37°C)	278	179	204	472	108
S2(day 148) (37°C)	469	13	198	195	137
S1 (day 430) (55°C)	219	9	544	116	308
S2 (day 430) (55°C)	10		3	29	269

adaptation to 55°C and, therefore, the IC_{50} value could be calculated more accurately. In batch tests, the presence of nitrite exerted an inhibitory effect on the hydrogenotrophic methanogenic population in S2, with an IC_{50} value of 17 mg L^{-1} (Table 1). SMA values for the S1 sludge at 55°C were found to be quite high for all substrates except propionate (Table 3), therefore nitrite toxicity testing against the populations in this sludge was also more unambiguous. Nitrite exerted an inhibitory effect on all the S1 microbial populations tested, with the hydrogenotrophic methanogens found to be the least susceptible group, with an IC_{50} value of 73 mg L^{-1} (Table 1).

A similar situation was found for the SRB species in the S2 sludge. All populations tested were more sensitive to nitrite toxicity at thermophilic than at mesophilic temperatures (Table 2). At mesophilic temperatures, the propionate-utilising SRB were more sensitive than the H_2/CO_2-utilising SRB, with an IC_{50} value of 50 mg L^{-1} found for propionate, compared to >150 mg L^{-1} for H_2/CO_2. At 55°C, IC_{50} values for all groups were quite low, with the hydrogen-utilising SRB again found to be the most tolerant population.

Lag phases were observed prior to substrate utilisation for some substrates, for example, the pattern of acetate utilisation in S2 sludge sampled on day 235 illustrated in Fig. 1. The length of the lag phase seemed to be deter-

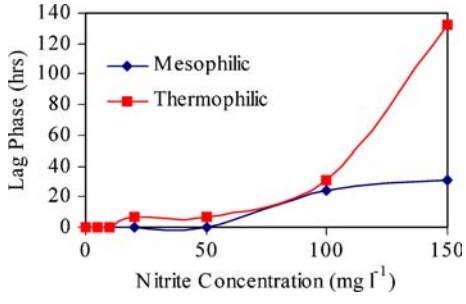

Fig. 2 The relationship between the nitrite concentration and the lag phase before substrate conversion in the S1 hydrogenotrophic methanogenic toxicity tests at mesophilic (day 248) and thermophilic (day 430) temperatures

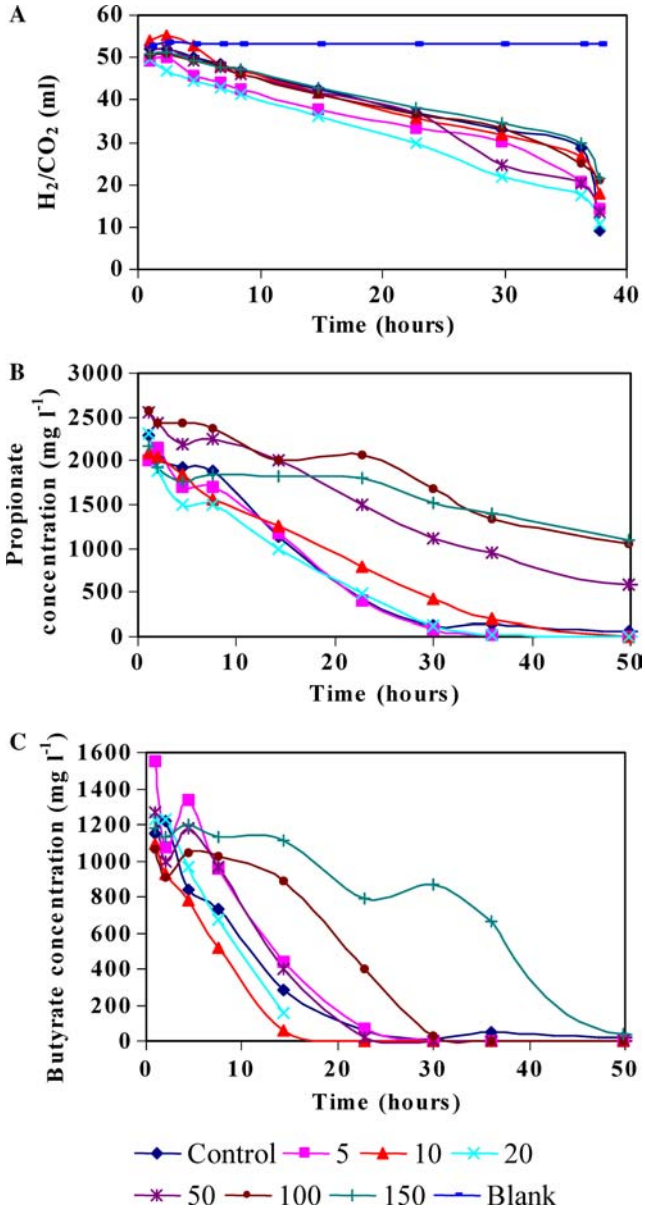

Fig. 3a–c The effect of increasing nitrite concentrations (5, 10, 20, 50, 100 and 150 mg NO_2-N L^{-1}) on the patterns of sulphate reducing bacteria (SRB) substrate utilisation for S2 sludge at mesophilic temperatures (day 235). **a** H_2/CO_2, **b** propionate, **c** butyrate

mined by the concentration of nitrite in the test vial, i.e. the length of the lag phase increases with increasing in-vial nitrite concentration. Figure 2 illustrates the relationship between the nitrite concentration and the lag phase, before substrate conversion in the S1 hydrogenotrophic methanogenic toxicity tests at mesophilic (day 248) and thermophilic (day 430) temperatures. At the highest concentration of nitrite used (150 mg L^{-1}), a significantly longer lag phase was noted in the thermophilic than in the mesophilic toxicity test vials (Fig. 2).

No apparent lag phase was observed before substrate degradation in SRB toxicity tests (Fig. 3).

Analysis of reactor effluents revealed that nitrite was being depleted in the reactor (effluent NO_2 levels of < 0.017 mg L^{-1}). If it is assumed that nitrite is also converted in the batch tests, then the observed pattern of substrate degradation following a lag phase may be as a result of initial nitrite inhibition, followed by nitrite utilisation and therefore lifting of inhibition and subsequent substrate degradation by MPB and SRB. To test this theory, and to establish the presence of nitrite utilisers in the sludge, a test was designed to investigate substrate degradation in the presence of nitrite (400 mg NO_2 L^{-1}) and specific inhibitors of MPB and SRB, namely 2 bromo-ethane sulfonate (BES) and molybdate (Mo). The substrates tested were acetate and H_2/CO_2 using S1 sludge. Acetate degradation only occurred in vials with acetate on its own and vials containing nitrite (Fig. 4a). The presence of nitrite resulted in a lag phase and a reduced rate of substrate depletion. Acetate degradation did not occur in vials in which the AMPB and ASRB were inhibited (Fig. 4a). The in-vial nitrite concentration was reduced from 400 mg L^{-1} to < 200 mg NO_2 L^{-1} after 60 h in all test vials containing nitrite (Fig. 4b) and this corresponded to the lag phase prior to onset of rapid acetate depletion in vials with nitrite (acetate + NO_2 only; Fig. 4a). Due to the nature of the test involving the gaseous substrate, H_2/CO_2, samples for nitrite analysis could not be removed during the test, but analysis at the end found that nitrite concentrations were negligible. H_2/CO_2 utilisation appeared to be mediated by the HSRB species as substrate utilisation occurred in vials in the presence and absence of the specific methanogenic inhibitor, BES, but not in vials with molybdate (Fig. 5). Again, a lag phase of ca.100 h

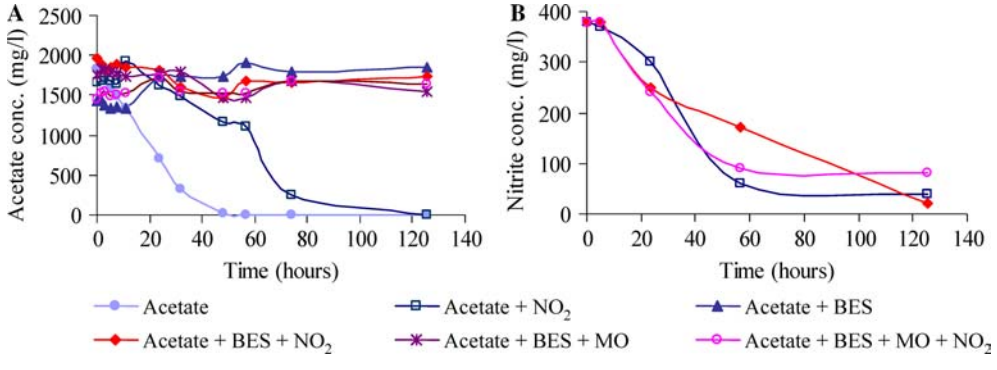

Fig. 4 Depletion of acetate (**a**) (mg L^{-1}) and nitrite (**b**) (mg L^{-1}) in vials containing S1 sludge (day 630), acetate and combinations of nitrite, bromoethane sulphonic acid (BES) and molybdate (MO)

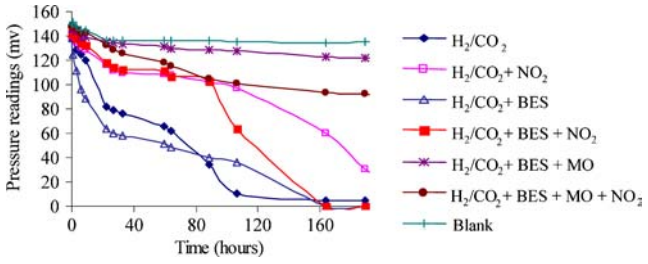

Fig. 5 Depletion of H_2/CO_2 in vials containing S1 sludge (day 630), H_2CO_2 and combinations of nitrite, BES and MO. In-vial nitrite concentrations at the end of the test were negligible (results not shown)

prior to H_2/CO_2 utilisation was noted in the presence of nitrite, which probably corresponded to the length of time taken for nitrite utilisation in the vials. Experimental constraints did not allow for the evaluation of other nitrogen compounds. There appeared to be some H_2 utilisation in vials where both the MPB and SRB were inhibited and where nitrite was included, suggesting the presence of a nitrite utilising population using H_2 as substrate.

Discussion

This study indicates that nitrite cannot act as a specific inhibitor of SRB activity in anaerobic batch tests at mesophilic and thermophilic temperatures. Nitrite also had a negative effect on the syntrophic and methanogenic populations in the reactor sludges. Inhibition of the various populations tested appeared to be temporary and was lifted after a lag phase, the length of which was determined by the concentration of nitrite in the test vials. Consequently, nitrite cannot be considered as a specific inhibitor of sulphate reduction under the conditions imposed in this study.

These results contradict those reported by Philpott [16], who found that addition of nitrite at 50 mg L^{-1} to a thermophilic UASB reactor (COD/SO_4^{2-} of 4) resulted in an immediate inhibition of sulphate reduction, with a concurrent improvement in the COD removal efficiency. Unlike the present study, no evidence was reported for syntrophic or methanogenic inhibition by nitrite in the Philpott study. However, the toxic effects of nitrous oxides have been reported in the literature. As well as toxicity, another hypothesis for the observed suppression, albeit temporary, of methanogenesis and sulphate reduction in the batch tests, is competition between the denitrifiers and the methanogens, syntrophs and SRB species for available substrates. Denitrification is a biological process often applied to remove nitrates or nitrites from wastewaters by their reduction to molecular nitrogen. A large number of heterotrophic bacteria are able to denitrify wastewaters under anoxic conditions (*Pseudomonas*, *Paraccocus*, *Alcaligenes*, *Thiobacillus*, *Bacillus*) [18]. The presence of denitrifiers in the biomass tested would explain nitrite conversion in toxicity tests and the subsequent lifting of inhibition of substrate utilisation once the nitrite was reduced. Competition between the denitrifiers and the sulphate reducers and methanogens may also be a contributing factor to the observed inhibition. The standard free-energy ($\Delta G°$) yields for the processes of denitrification are summarised in Table 4 [22]. Assuming H_2 as a standard electron donor, the amount of free energy produced per reaction is much higher when using nitrite rather than either sulphate or carbon dioxide as electron acceptor.

This would place denitrifiers at an energetic advantage over both methanogens and sulphate reducers in the competition for H_2. It has also been observed that use of acetate as the carbon source ensures high denitrification rates [4, 10]. Kluber and Conrad [8] found that addition of nitrate, nitrite and N_2O caused a complete, but largely reversible, inhibition of methanogenesis. Addition of each of the N-compounds significantly decreased the H_2 partial pressure below the threshold level of methanogens. H_2 was utilised so effectively by the nitrate- and nitrite-reducing bacteria that the resulting H_2 partial pressure could no longer support exergonic methanogenesis. Methanogenic activity did not resume until all electron acceptors were reduced and, as a consequence, H_2 had reached the methanogenic threshold. These authors concluded that competition for H_2 with denitrifying bacteria, iron- and sulphate-reducing bacteria seemed to be an important factor in inhibition of methanogenesis but inferred that, after addition of nitrite and NO, toxic effects may have been more important than competition.

Conclusions

Although nitrite was shown to inhibit SRB activity in anaerobic batch tests, under both mesophilic and thermophilic conditions, at the higher concentrations utilised in this study, the inhibition was incomplete. Inhibitory effects were also observed for the syntrophic and methanogenic populations of the test sludges. However, this inhibition was temporary and was lifted after a lag phase, the length of which was determined by the concentration of nitrite in the test vials. Nitrite is, evidently, not selectively inhibitory to the SRB species of anaerobic digester sludges. It may also be concluded that the activity of nitrite-utilising bacteria present in the reactor sludges converts nitrite, after a lag phase, to non-toxic nitrogen derivatives that allow resumption of the activity of SRB and non-SRB species in the reactor

Table 4 Standard free-energy changes for the reactions of denitrification [21]

	Reaction	$\Delta G°$ kJ mol^{-1}
1	$4H_2 + SO_4^{2-} + H^+ \rightarrow HS + 4H_2O$	−38
2	$4H_2 + HCO_3^- + H^+ \rightarrow CH_4 + 3H_2O$	−32.7
3	$2H_2 + 2H^+ + 2NO_2^- \rightarrow N_2O + 3H_2O$	−454
4	$3H_2 + 2H^+ + 2NO_2^- \rightarrow N_2 + 4H_2O$	−795

sludges. Consequently, nitrite cannot be considered as a potential in-reactor specific inhibitor of SRB species in anaerobic digesters treating sulphate-containing wastewaters.

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