

Interactions between methanogenic and nitrate reducing bacteria during the anaerobic digestion of an industrial sulfate rich wastewater

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Abstract

The effect of nitrate addition on the anaerobic digestion of an industrial sulfate rich wastewater was investigated using batch cultures. A high chemical oxygen demand/NO₃-N ratio did not favor the dissimilatory nitrate reduction to ammonia. Denitrification was the main nitrate reduction pathway at all chemical oxygen demand/NO₃-N ratios tested. A lag phase, presumably caused by a high initial sulfide content, preceded nitrate reduction to molecular nitrogen. During this lag phase, the methane production was not affected by nitrate concentrations as high as 500 mg NO₃-N l⁻¹, except for cultures inoculated with a cold-stored consortium. Sulfate reduction in the cultures seemed to be directly prevented by nitrogen oxides. Methane production stopped as soon as denitrification started. Concurrently, an increase of the redox potential and a transient nitrite production were observed. These physical and chemical modifications would be responsible for methanogenesis inhibition. Furthermore, sulfide was consumed during denitrification. It was suggested that denitrification using this electron donor occurred in the system. Finally, it was shown that iron pre-treatment promoted both nitrate reduction to ammonia and the methanogenesis. It appeared that in a sulfide free environment, dissimilatory nitrate reduction to ammonia was the main nitrate reduction pathway. © 1999 Federation of European Microbiological Societies. Published by Elsevier Science B.V. All rights reserved.

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1. Introduction

Food and beverage industries which either use or produce molasses generate a significant pollution. The anaerobic digestion of such wastewaters has been intensively studied [1–4] and leads to the for-

mation of biomass, methane, carbon dioxide, hydrogen sulfide and ammonia. Sulfide under its free form (H₂S), produced by sulfate reduction, is a very toxic compound [5]. Ammonia, the product of protein digestion, has been found to inhibit the methanogenesis at high pH values [6,7]. Furthermore, its removal from digested wastewater is required since its discharge into the aquatic environment results in ecological and public health problems.

Biological treatment by nitrification and denitrifi-

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cation is the most widely used process for this purpose. Nitrite and nitrate produced by the nitrifying bacteria under aerobic conditions are reduced to molecular nitrogen (N_2) by denitrifying organisms under anoxic conditions. The denitrifying microorganisms are aerobic bacteria able to use nitrogen oxides as final electron acceptors in the absence of oxygen [8]. Moreover, the occurrence of denitrifying organisms was demonstrated in anaerobic nitrate free environments such as deep marine sediment [9] or digested sludge [10–15]. These microorganisms would be able to provide for their maintenance by carrying on a low level of fermentation when oxygen or nitrate are not available [14,15].

To remove both carbon and nitrogen from wastewaters, it would be very interesting to combine denitrification and anaerobic digestion in an integrated process coupled to a nitrification stage. But dissimilatory nitrate reduction to ammonia (DNRA) has been found to be the main nitrate reduction pathway in anaerobic digesters [8,15] and in other methanogenic environments [16,17]. However, using batch cultures, Akunna et al. showed recently that a low electron donor/electron acceptor ratio and/or a high initial nitrate load increased the denitrification activity of an anaerobic sludge with respect to its DNRA capacity [10,12]. The nature of the carbon source also plays an important role in the shift towards one of the two different pathways [11,18].

In addition, nitrate was found to strongly inhibit the methanogenesis [11,16,17,19–24]. This inhibitory effect is still unclear and cannot be only attributable to a redox change [3,9,16,24].

However, several single integrated denitrification/methane production processes have been proposed [13,25–27]. Using synthetic wastewaters and anaerobic upflow filters, it has been shown that denitrification took place in the bottom of the digester while methanogenesis occurred in the upper part, where nitrate was completely exhausted [11,13]. Hendriksen and Ahring observed the same phenomenon using up-flow anaerobic sludge blanket (UASB) reactors [19,28]. Garuti et al. proposed the ANANOX process, a combined anaerobic-anoxic-oxic municipal wastewater treatment system which removed more than 80% of the chemical oxygen demand (COD), suspended solids and nitrogen [29]. Lin and Chen developed a co-immobilized mixed culture system

of denitrifying bacteria and methanogenic organisms included in polyvinyl alcohol gel beds [22]. It seems that methanogens are active inside the beads where nitrate is absent, while nitrate reducers grew at the surface.

Most of these previous studies have used synthetic media, but little information is available on industrial effluents. We evaluated the feasibility of a combined denitrification/methanization process using an industrial wastewater from a food processing plant.

First, the influence of the COD/NO_x-N ratio upon the shift towards denitrification and nitrate dissimilation to ammonia at NO₃-N concentrations within the range 500–1500 mg l⁻¹ was investigated. In a second part, the effect of nitrate and its reduction to N₂ or/and to NH₄⁺ on the methanogenesis has been carried out. In addition, since the effluent has a high sulfate content, its reduction in the presence of nitrate was also evaluated. Finally, the redox potential was monitored and used to try to explain the results obtained.

2. Materials and methods

2.1. Biomass and culture conditions

The methanogenic consortium originated from a 20-l laboratory scale digester fed with diluted molasses wastewater [30]. In the study of the influence of the COD/NO_x-N ratio on nitrate reduction, this consortium was stored at 4°C for 15 days before inoculation. Experiments were performed in 1.2-l batch reactors containing 1 l of liquid medium, bubbled with argon to remove air and sealed. They were agitated with a magnetic stirrer (stirrer speed at 400 rpm) and placed in a temperature-controlled room (34 ± 1°C). Gas production was periodically determined using a 100-ml air-tight syringe and the pH was monitored with an Ingold pH meter (2301) in each sample.

24 g of concentrated molasses wastewater ($d=1.318$) was introduced in each reactor. This substrate contained a high COD content (Total COD = 460 g l⁻¹ and soluble COD = 430 g l⁻¹). Its total organic carbon (TOC), total Kjeldahl nitrogen (TKN) and sulfate concentrations were respectively 260, 36 and 63 g l⁻¹. The pH was adjusted to 7.3

through addition of HCl. Initial conditions of these batch cultures are described in Table 1.

In the study of the nitrate effect on the methanogenesis (Table 2, cultures 4–7), the same consortium was used, but the sludge (1 l) was anaerobically transferred from the 20-l digester to each reactor and de-oxygenated through argon bubbling, without any cold storage.

The nitrate reduction in a sulfide free environment was studied after sulfide precipitation by FeCl_2 addition (Table 2, culture 8).

The redox potential (E_h) was continuously monitored using an Ingold pH transmitter (2400) and a combination redox electrode (Ag/AgCl reference system, KCl 3 M, $E_{h,\text{ref}}^{35^\circ\text{C}} = 199.8$ mV).

2.2. Calculations of nitrogen balances

Nitrogen balances were calculated on nitrate conversion, to determine the reduction pathway. The produced $\text{N}_2\text{-N}$, $\text{N}_2\text{O-N}$, the accumulation of nitrogen gas in the headspace of reactors, the nitrogen oxides ($\text{NO}_x\text{-N}$) sampled and the $\text{NO}_x\text{-N}$ consumed were considered to estimate the percentage of denitrification. The difference was attributed to DNRA.

The variation of the TKN content, including organic nitrogen and ammonia, was compared to the control to confirm the results obtained with the previous balance. An increase of the TKN content of a flask, compared to the control without any nitrogen oxide, indicated a probable nitrate reduction to ammonia.

2.3. Analyses

Samples were collected from each culture. After centrifugation (10 min, $8000 \times g$), the TOC content was determined through UV oxidation with a Dohrman DC 80 apparatus. Suspended solids (SS) and volatile suspended solids (VSS) analyses were carried out according to standard methods [31]. Acetate was measured by gas chromatography (Chrompack CP 9000) using a capillary column (FFAP), a flame ionization detector and ethyl-2-butyric acid as an internal standard.

Sulfate, nitrate and nitrite were measured by ion chromatography (Dionex 100). Separation and elution were carried out on a IonPac AS4A analytical

column using carbonate/bicarbonate eluent ($1.8 \text{ mmol l}^{-1} \text{ Na}_2\text{CO}_3/1.7 \text{ mmol l}^{-1} \text{ NaHCO}_3$) and a sulfuric regenerant (H_2SO_4 25 mmol l^{-1}). The COD was titrated by the potassium dichromate ferrous ammonia sulfate method [31].

Dissolved sulfide determination was carried out using the method developed by Percheron et al. [32].

Ammonia and TKN were determined by the titrimetric method after distillation according to standard methods [31].

The gas composition (CO_2 , CH_4 , O_2 , H_2 , N_2O and N_2) was determined by gas chromatography (Shimadzu CR3A) using a Hayesep 80-100 mesh column, a molecular sieve column and a katharometer detector.

3. Results

3.1. Influence of the COD/ $\text{NO}_x\text{-N}$ ratio on denitrification

Four batch reactors (1 l) were run at various nitrate concentrations to check the effect of the COD/ $\text{NO}_x\text{-N}$ ratio on the denitrification efficiency (Table

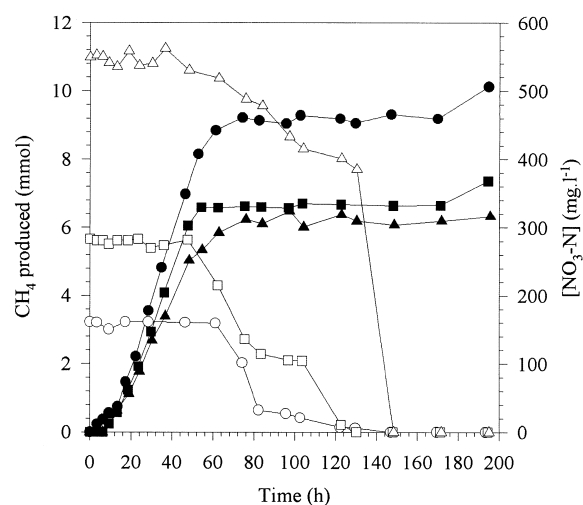


Fig. 1. Evidence of methane production in the presence of nitrate (cultures 4, 5 and 6), ● CH_4 produced in the presence of $161 \text{ mg NO}_3\text{-N l}^{-1}$, ■ CH_4 produced in the presence of $281 \text{ mg NO}_3\text{-N l}^{-1}$, ▲ CH_4 produced in the presence of $550 \text{ mg NO}_3\text{-N l}^{-1}$, open symbols represent the $\text{NO}_3\text{-N}$ concentration of the corresponding culture.

Table 1

Influence of the $\text{NO}_x\text{-N}$ ratio on denitrification: initial conditions and the final nitrogen balance during the study of the COD/ $\text{NO}_3\text{-N}$ ratio effect on nitrate reduction at a high nitrate concentration

	Control	Culture 1	Culture 2	Culture 3
<i>Initial</i>				
^sCOD (mg)	15160	14610	15110	15160
TOC (mg)	6180	6040	6110	6000
$\text{NO}_3\text{-N}$ (mg)	0	500	970	1630
E_h (mV)	-400	-	-	-380
COD/ $\text{NO}_3\text{-N}$ ratio	-	29.2	15.5	9.3
<i>Nitrogen balance</i>				
Reduced $\text{NO}_3\text{-N}^a$ (mg)	0	493	914	1553
Produced $\text{N}_2\text{O-N}$ (mg)	0	98	204	767
Produced $\text{N}_2\text{-N}$ (mg)	0	361	511	468
$\Delta\text{N-gas}$ in the headspace ^b (mg)	0	29	2030	444
Total recovered N (mg)	0	489	918	1679
% of $\text{NO}_3\text{-N}$ de-nitrified	-	99.1	100.5	108.1
$\text{NH}_4\text{-N}$ variation (mg)	+140 (35 h)	+138 (35 h)	-	-
	+260 (84 h)	-	+140 (60 h)	+40 (60 h)
TKN variation (mg)	-200 (60 h)	-245 (35 h)	-290 (60 h)	-360 (84 h)

In each 1-l culture, the initial VSS concentration and pH value were respectively 2230 mg l^{-1} and 7.3. The inoculum has been stored at 4°C for 2 weeks before inoculation.

^aReduced $\text{NO}_x\text{-N}$ was evaluated considering the amount of nitrogen oxides removed from the broth at each sampling and thus not available for reduction.

^b $\Delta\text{N-gas}$ in the headspace is the total of $\text{N-N}_2\text{O}$ and N-N_2 in the headspace of the reactors.

1). The inoculum was previously stored at a cold temperature (4°C).

In all cases, nitrate was completely reduced to N_2O and N_2 . The nitrogen balance showed a good recovery of reduced nitrate. In the culture containing $1625 \text{ mg NO}_3\text{-N}$ (culture 3), the total nitrogen recovered slightly exceeded the total nitrogen reduced. The high N_2O production in this last culture led to an overestimation of the overall nitrogen gas produced, since N_2O contents up to 10^6 ppm were found.

The $\text{NH}_4\text{-N}$ and TKN contents were determined in order to check whether nitrate was truly denitrified and not reduced to ammonia.

In culture 1, nitrate was consumed within 35 h, during which the ammonia concentration increased from 750 to $888 \text{ mg NH}_4\text{-N l}^{-1}$. A similar increase was observed in the control culture within the same period. This can be explained by the organic nitrogen mineralization. In cultures 2 and 3, the nitrogen oxide was reduced within 60 and 84 h, respectively, without any ammonia production, compared to the control reactor (Table 1).

Analyses of the $\text{NH}_4\text{-N}$ concentrations showed that ammonia was produced from the organic matter

through ammonification, but also that TKN consumption occurred, especially in cultures containing nitrate. In this case, the higher the initial nitrate content, the lower $\Delta\text{NH}_4\text{-N}$. TKN concentrations decreased more severely in the presence of nitrate. This could suggest a significant incorporation of organic nitrogen into the bacterial biomass, e.g. denitrifying microorganisms (Table 1).

It can be concluded from this experiment that for all COD/ $\text{NO}_x\text{-N}$ ratios tested, nitrate was completely de-nitrified and no DNRA occurred.

3.2. Interactions between methanogenesis and denitrification

An active consortium originated from a 20-l digester fed with molasses wastewater, without any preliminary cold storage, was cultured in the presence of different nitrate concentrations (cultures 4, 5, 6). As shown in Fig. 1, an unexpected methane production was observed in the three flasks, despite $\text{NO}_3\text{-N}$ concentrations up to 500 mg l^{-1} (Fig. 1).

The higher the nitrate content, the lower the maximum methane production rate (0.15 mmol h^{-1} at

550 mg $\text{NO}_3\text{-N l}^{-1}$, 0.18 mmol h^{-1} at $281 \text{ mg NO}_3\text{-N l}^{-1}$ and 0.2 mmol h^{-1} at $161 \text{ mg NO}_3\text{-N l}^{-1}$.

The methanogenesis stopped as soon as nitrate reduction began (Fig. 1). The nitrogen balance shows that NO_3 was truly de-nitrified, since a good recovery of nitrogen was observed (Table 2).

The high sulfide content in the sludge ($12\text{--}14 \text{ mmol l}^{-1}$) compared to the 4°C stored consortium used in the previous experiment (only $2\text{--}4 \text{ mmol l}^{-1}$) could explain this observation. A linear correlation between the initial sulfide content and the length of the lag phase before nitrate reduction was found (Fig. 2). Data from all the experiments presented in this paper were used in this figure. This suggested an important role of dissolved sulfide on the start-up of denitrification.

Similar results were observed with culture 7, carried out with a control culture without nitrate (Table 2). Methanogenesis was not affected by nitrate at an initial concentration of $230 \text{ mg NO}_3\text{-N l}^{-1}$. Indeed, the methane production rate was similar to the control culture during 45 h (Fig. 4a). Once again, the methanogenesis stopped at the beginning of nitrate reduction.

The nitrogen balance in Table 2 shows that nitrate was completely de-nitrified to N_2 .

Fig. 3 presents a comparison of the methane pro-

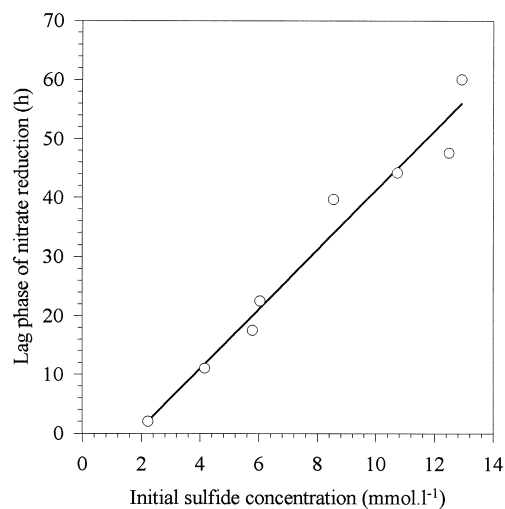


Fig. 2. Correlation between the initial sulfide content and the length of the lag phase before denitrification in the methanogenic reactors carried out in this work.

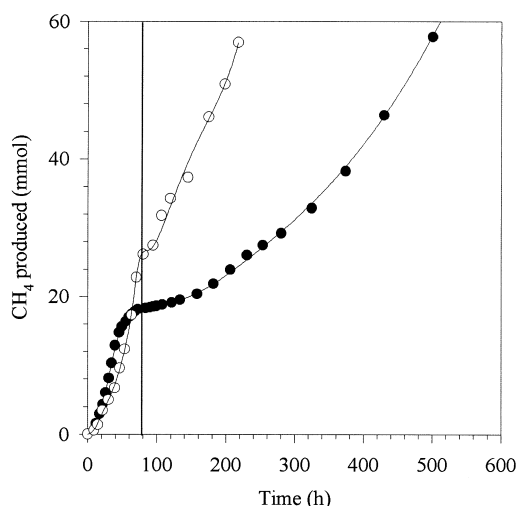


Fig. 3. Comparison of the methane production in the reactors without nitrate, after cold storage (●) (control reactor, Table 1) and without any storage (○) (control reactor, Table 2).

duction in the control reactors of the previous experiment (Table 1) and this experiment (Table 2). In both cases, two phases can be observed like in a diauxic growth. The first methane production of about 80 h in both cases is probably due to an hydrogenotrophic methanogenesis. The methane production rate is slightly higher for the cold-stored inoculum. This first stage would be sensitive to sulfide. The second phase, presumably attributed to an acetoclastic methanogenesis, is much slower for the cold-stored inoculum. The methanogenic microorganisms involved in this second stage would have been affected by the cold storage.

3.3. Evolution of the sulfide content and the redox potential during denitrification

Sulfate and dissolved sulfide were measured and the redox potential was monitored in culture 7 described above and in the control culture (Fig. 4).

In the control reactor, sulfate reduction began after 40 h (not shown), whereas this activity was completely inhibited in the presence of nitrate (Fig. 4b). This inhibition has been reported before [24].

Fig. 4b shows that the redox potential increased drastically until -300 mV (Ag/AgCl reference) when denitrification started. Conversely, it fell down to -500 mV in the control. Such an increase occurred

in all cultures in which denitrification was demonstrated. Concurrently, sulfide was consumed without any increase of the sulfate content (Fig. 4b). The color of denitrifying reactors turned from black to brown and yellow particles were detected after centrifugation, suggesting the sulfide oxidation to elemental sulfur.

When the nitrate reduction stopped, the redox po-

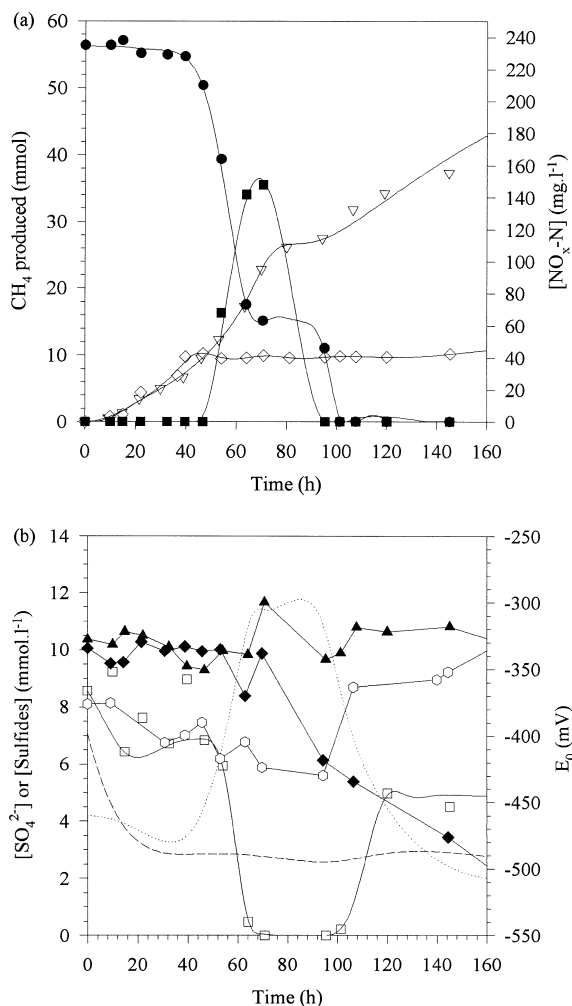


Fig. 4. Comparison of the methane production in the presence of nitrate (culture 7, NO₃-N = 235 mg l⁻¹) and without nitrogen oxide (control). (a) ● NO₃-N (mg l⁻¹), ■ NO₂-N (mg l⁻¹), CH₄ produced in culture 7 (◇) and in the control (▽); (b) SO₄²⁻ (mmol l⁻¹) in culture 7 (▲) and in the control (◆), sulfide (mmol l⁻¹) in culture 7 (□) and control (○), redox potential in culture 7 (.....) and control (—).

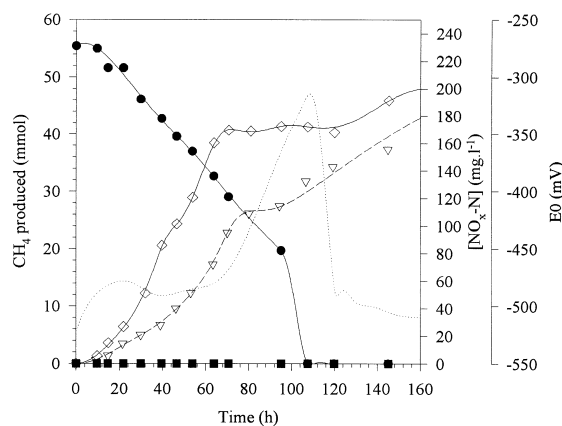


Fig. 5. Methane production and nitrate reduction in the presence of iron (culture 8), ● NO₃-N (mg l⁻¹), ■ NO₂-N (mg l⁻¹), ▽ CH₄ (mmol) produced in the control culture, ◇ CH₄ (mmol) produced and (.....) redox potential (mV) in the presence of 231 mg NO₃-N l⁻¹.

tential went down to -500 mV and the sulfide concentration increased up to a level slightly below its initial content. Both parameters seem to be directly connected.

3.4. DNRA and methanogenesis

To check the influence of sulfide on nitrate reduction, sulfide was precipitated as FeS, using 12 mM of FeCl₂ (Table 2, culture 8). Then, nitrate reduction began without any lag phase (Fig. 5). However, the rate of nitrate reduction was lower than in previous cultures and nitrite accumulation did not take place. After 90 h, this rate reached 9.7 mg NO₃-N l⁻¹ h⁻¹. The same value was measured in Fig. 4 where denitrification took place. A slight N₂ release occurred only after 90 h.

Moreover, between 0 and 107 h, TKN and NH₄-N concentration increases occurred in culture 8. These increases were respectively 191 and 279 mg l⁻¹ compared to culture 7 achieving only denitrification (Table 2). The redox potential remained low and quite stable for 70 h. Then, it reached quickly -300 mV (Ag/AgCl reference), as previously observed under denitrifying conditions (Fig. 4b).

Finally, Table 2 shows that considering only denitrification, the nitrogen balance was not closed.

Therefore, according to the afore-mentioned ob-

Table 2
Interaction between methanogenesis and denitrification: initial conditions and final nitrogen balances

	Control	Culture 4	Culture 5	Culture 6	Culture 7	Culture 8
<i>Initial</i>						
^S COD (mg)	16510	15130	14760	14880	16760	15150
TOC (mg)	6700	6140	5990	6040	6760	6150
NO ₃ -N (mg)	0	161	281	550	235	231
pH	7.0	7.3	7.3	7.2	7.0	7.2
E _h (mV)	-400	-	-	-	-460	-520
Treatment	-	-	-	-	-	FeCl ₂ 12 mM
<i>Nitrogen balance</i>						
Reduced NO ₃ -N ^a (mg)	-	153	266	514	220	216
Produced N ₂ O-N (mg)	-	1	1	0	0	0
Produced N ₂ -N (mg)	-	45	112	178	36	49
ΔN-gas in the headspace ^b (mg)	-	105	160	330	181	41
Total recovered N (mg)	-	150	272	508	217	90
% of NO ₃ -N de-nitrified	-	98.5	102.4	98.9	98.7	41.5
NH ₄ -N variation (mg)	+247				+96	+375
TKN variation (mg)	+17				-168	+23

Reduced NO_x-N was evaluated considering the amount of nitrogen oxides removed from the flasks at each sampling and thus not available for reduction. In each 1-l culture, the VSS concentration was 2 g l⁻¹. The inoculum was used without any storage.

^aReduced NO_x-N was evaluated considering the amount of nitrogen oxides removed from the broth at each sampling and thus not available for reduction.

^bΔN-gas in the headspace is the total of N-N₂O and N-N₂ in the headspace of the reactors.

servations, it can be suggested that nitrate was reduced through DNRA during the first 90 h and then de-nitrified.

4. Discussion

4.1. Influence of the COD/NO_x-N ratio on denitrification

Denitrification was the only nitrate reduction way in the experiments, whatever the COD/NO_x-N ratio. A concomitant organic nitrogen consumption was observed. It might be suggested that the TKN removal observed in the denitrifying reactors was due to anaerobic ammonia oxidation. However, this process described by van de Graaf et al. [33] seems rather unlikely. A higher ammonia consumption should have been observed and the nitrogen gas production should have been higher according to the amount of nitrate reduced. Furthermore, the Anamox reaction leads to a pH decrease, since it produces H⁺. In the cultures examined here, the pH values rose from 7.3 to 7.5, 8.2 and 8.5 in the reactors at 500, 973 and 1625 mg NO₃-N l⁻¹, respectively, while

in the control, it decreased slightly from 7.3 to 7.2. This increase of the pH values during nitrate reduction is characteristic for denitrification.

Therefore, this experiment showed that, at a high NO₃ concentration and using an industrial wastewater, the COD/NO_x-N ratio seems to have no effect on the shift towards denitrification or dissimilatory nitrate reduction to NH₄⁺.

4.2. Inhibition of the methanogenesis by denitrification

In most of the studies reported in the literature [16,17,19–24], in which denitrification began before methanogenesis, it was concluded that nitrate was a direct inhibitor of methanogens. However, according to Belay et al. [34], methanogenic bacteria would be affected very differently by nitrate. They found that some species could grow at a nitrate content as high as 2800 mg l⁻¹.

Moreover, Clarens et al. [35] recently showed that the acetoclastic *Methanosarcina mazei* was inhibited by denitrification products (NO₂⁻ and N₂O), rather than by nitrate itself. Moreover, methane production by this strain in the presence of nitrate was stopped when a denitrifying strain of *Pseudomonas stutzeri*

was added to the culture. This led to an immediate nitrate reduction to nitrite. Similar results were obtained by Klüber and Conrad with *Methanosarcina barkeri* and *Methanobacterium bryantii* and in anoxic rice field soils [36,37]. These authors also suggested a competition for H₂ between methanogens and denitrifying bacteria.

In salt marsh sediment, Balderston and Payne [20] found that nitrate and nitrite both inhibited methanogens but nitrite had a stronger effect.

These results agree with our experiments where an accumulation of nitrite, which could inhibit strongly the methanogenic activity, began at 45 h. In addition, the important increase of the redox potential (Fig. 4b) could be involved in that inhibition. Since methane production began before nitrate reduction, except in cultures inoculated with cold-stored consortium, nitrate cannot be considered as a strong inhibitor of the methanogenesis. Competition for hydrogen and/or organic carbon may be another explanation for the transient inhibition of methanogens.

In the case of cultures inoculated with cold-stored consortium, methanogenesis did not start before complete NO_x reduction. As it was suggested above, the difference in the sulfide concentrations between the two series of experiments would be the major factor to explain these results.

4.3. Nitrate-dependent sulfide oxidation

The above-mentioned observations suggest that sulfide could be used as an electron donor for denitrification. Nitrate reduction using sulfide, elemental sulfur and thiosulfate has already been reported by others [38–41]. In addition, acetate was also consumed during denitrification since its concentration decreased from 1660 to 1440 mg l⁻¹, in spite of the acidogenesis which produced volatile fatty acids from the molasses wastewater (not shown). Such a simultaneous utilization of acetate and sulfide as electron donors for denitrification has been reported [40]. We recently showed, using cultures performed without any carbon source, that denitrifiers present in our system were able to use sulfide as electron donors [42]. Furthermore, this previous study comparing nitrate and nitrite reduction in the same wastewater revealed that, while a lag phase preceded

nitrate denitrification, nitrite was consumed immediately. A chemical reduction of nitrite by ferrous iron (Fe²⁺) was considered responsible for this difference. Evidence of such a chemodenitrification has been presented by using a sterilized sludge which kept its ability to reduce nitrite while it lost its capacity to use nitrate. Methanogenesis was completely inhibited in the presence of nitrite, which is in agreement with previous studies carried out with pure methanogenic strains [35,37].

Of course, the redox potential increase could by itself induce elemental sulfur formation from sulfide. However, the origin of this increase remains unclear. The methanogenesis inhibition, the dissolved sulfide consumption or unknown oxidation induced by denitrifiers could be responsible for this phenomenon. Furthermore, although the redox potential was quite low when methane production stopped, it could also explain inhibition of methanogens.

4.4. DNRA and methanogenesis

Iron promoted DNRA but its exact role is still unknown. Sulfide inhibition might be stronger on ammonifiers than on denitrifiers. Thus, in a sulfide free environment, DNRA organisms could transiently outcompete denitrifying bacteria for electron donors. During our experiments, the consortium used originated from a 20-l reactor fed with a sulfate rich effluent. Therefore, it contained a high number of sulfate reducing organisms. Some of these bacteria have been shown to be strongly sensitive to sulfide [43] and to respire nitrate [22,44–47]. Thus, sulfate reducing bacteria could be responsible for the ammonia production.

Moreover, iron may have contributed to maintain the redox potential to a level hindering denitrification for at least 90 h.

Finally, iron promoted the methanogenesis since the methane production rate in the presence of FeCl₂ was higher than in the control culture (Fig. 5). H₂S precipitation using FeCl₂ is often recommended during anaerobic digestion of sulfate rich wastewater, in order to reduce its toxicity [48,49].

Furthermore, methane production was observed concurrently to nitrate dissimilation to ammonia, which means that both activities are compatible. The methanogenesis stopped when the redox poten-

tial increased and when denitrification took place as previously observed.

If this phenomenon is good for the anaerobic digestion process since methanogenesis was not inhibited by DNRA, it is of course unsuitable for the nitrogen removal treatment. Indeed, if DNRA should be the major nitrate reduction pathway, nitrate produced in a nitrifying reactor from ammonia would then be reduced to ammonia, resulting in a non-transformation of nitrogen in the overall process.

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