

Combining the biological nitrogen and sulfur cycles in anaerobic conditions

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Abstract The biochemical processes involved in the anaerobic degradation of carbon, nitrogen and sulfur compounds can be represented by an oxidation-reduction or electron donor-acceptor scheme. The theoretic values of Gibbs free energy (ΔG^0) calculated from thermodynamic data indicate the feasibility of the reactions. The interactions C-S and C-N are well known but there is a lack of information about the interaction N-S. The anaerobic transformation of nitrates using reduced sulfur compounds can be explained considering that nitrate acts as electron acceptor while reduced sulfur compounds are the electron donors.

A new N-S interaction in anaerobic conditions (ORP = -425 mV) has been experimentally observed when treating industrial wastewater rich in organic nitrogen and sulfate. The mass balances of the different nitrogenous and sulfur compounds in the liquid and gas phases clearly indicated an uncommon evolution. An important percentage of the nitrogen entering the reactor as TKN was removed from the liquid phase appearing as N_2 in the gas phase. Simultaneously, only part of the sulfate initially present in the influent appeared as sulfide in the effluent or as hydrogen sulfide in the gas. These experimental observations may suggest a new anaerobic N-S biological interaction involving simultaneous anaerobic ammonium oxidation and sulfate reduction, ammonium being the electron donor and sulfate the electron acceptor.

Keywords Anaerobic nitrogen removal; anaerobic sulfur removal; nitrogen/sulfur interaction

Introduction

The biological cycles of carbonaceous organic matter, sulfurous compounds and nitrogenous compounds are well established. Anaerobic treatment of industrial wastewater under conventional conditions generates as end products of the anaerobic degradation: methane and carbon dioxide for the organic matter, sulfide for the oxidised sulfur compounds and ammonia for the organic nitrogen compounds (Lettinga, 1995).

All the biochemical processes involved can be represented by an oxidation-reduction or electron donor-acceptor scheme. The value of the standard Gibbs free energy (ΔG^0) of a chemical reaction can be calculated following the electron transfer scheme and using the values of ΔG^0 of the half-reactions. The ΔG^0 of the half-reactions can be calculated by simple arithmetic as the difference between standard Gibbs free energies of formation (ΔG_f^0) of products and reactants.

Thermodynamic data are compiled as properties of the substances, not reactions, and are quoted as G_f^0 of compounds from their elements at 25°C and in their "standard states" which are generally pure solids, pure liquids or pure gases at one atmosphere partial pressure. Because biological reactions occur almost exclusively in aqueous solution, values for ΔG_f^0 are also quoted at a standard concentration of 1 M in aqueous solution. Values for some compounds of biological interest found in anaerobic treatment are listed in Table 1 (Mosey, 1985).

As mentioned before, using the data of Table 1 it is possible to estimate ΔG^0 of the half-reactions. The standard redox potential (E^0) is calculated from ΔG^0 of the half reaction as $E^0 = (\Delta G^0)/(n.F)$, where n is the number of electrons in the half-reaction and F is the Faraday constant expressed as mV-equivalents (96.42 J/mV). In order to evaluate the redox potential the convention is to write the half-reaction with the electrons on the left, as a

Table 1 Standard Gibbs free energies of formation of compounds found in anaerobic treatment (Mosey, 1985 and CRC, 1971)

Substance	State	ΔG_f° (kJ/mol)	Substance	State	ΔG_f° (kJ/mol)
CH ₄	g	-50.7	SO ₄ ⁼	aq	-743.9
CO ₂	g	-358.9	H ₂ S	aq	-27.3
HCO ₃ ⁻	aq	-586.5	HS ⁻	aq	+12.6
CO ₃ ⁼	aq	-527.6	S ⁼	aq	+83.6
CH ₃ COOH	aq	-399.1	H ₂ O	l	-237.0
CH ₃ COO ⁻	aq	-372.0	H ⁺	aq	0
NO ₃ ⁻	aq	-110.4	OH ⁻	aq	-157.2
NO ₂ ⁻	aq	-34.5	O ₂ , H ₂ , N ₂ ...	g	0
NH ₄ ⁺	aq	-79.4			

reduction process. If the reaction is written the other way round the calculation will yield an “oxidation potential” which is numerically the same but with opposite sign. To calculate the redox potential over a range of concentrations and pressures it is necessary to write the redox equation. The activity of water in aqueous systems is constant at 55 M and it is usual to remove [H₂O] from the equation including its value in E^0 . It is also convenient to use the relationship $\text{pH} = -\log [\text{H}^+]$.

Table 2 shows the main half-reactions involved in the biological oxidation and reduction of C, N and S compounds and the values of their standard Gibbs free energy and standard redox potentials:

Discussion

In a complex matrix such as an industrial wastewater rich in organic matter, nitrogenous and sulfurous compounds, there may exist manifold interactions between the different species. From a thermodynamic point of view, the predominant reactions depend on the pH, temperature and concentration of the different chemical compounds and the concentration of the different microbial species. Obviously this environment will determine the type

Table 2 Half-reaction expressions, standard Gibbs free energy (ΔG^0) and redox potential (E^0) values of some biological reactions involved in anaerobic treatment. ⁽¹⁾ kJ/reaction, ⁽²⁾ standard redox potential calculated from equation $E^0 = (\Delta G^0)/(n.F)$, ⁽³⁾ standard redox potential including the activity of water (55 M)

	Half-reaction	ΔG^0 ⁽¹⁾ (kJ)	E^0 ⁽²⁾ (mV)	E^0 ⁽³⁾ (mV)	E^0 pH 7 (mV)
	hydrogen				
R₁	2H ⁺ + 2 e ⁻ ⇌ H ₂	0	0	0	-414
	organic matter				
R₂	CH ₃ COO ⁻ + 9 H ⁺ + 8 e ⁻ ⇌ 2 CH ₄ + 2 H ₂ O	-203	264	238	-227
R₃	2 HCO ₃ ⁻ + 9 H ⁺ + 8 e ⁻ ⇌ CH ₃ COO ⁻ + 4 H ₂ O	-147	190	139	-327
R₄	HCO ₃ ⁻ + 9 H ⁺ + 8 e ⁻ ⇌ CH ₄ + 3 H ₂ O	-175	248	197	-269
	nitrogen				
R₅	NO ₃ ⁻ + 2 H ⁺ + 2 e ⁻ ⇌ NO ₂ ⁻ + H ₂ O	-161	835	784	+370
R₆	NO ₃ ⁻ + 6 H ⁺ + 5 e ⁻ ⇌ 1/2 N ₂ + 3 H ₂ O	-600	1246	1215	+967
R₇	NO ₃ ⁻ + 10 H ⁺ + 8 e ⁻ ⇌ NH ₄ ⁺ + 3 H ₂ O	-680	882	843	+326
R₈	NO ₂ ⁻ + 4 H ⁺ + 3 e ⁻ ⇌ 1/2 N ₂ + 2 H ₂ O	-440	1519	1451	+899
R₉	NO ₂ ⁻ + 8 H ⁺ + 6 e ⁻ ⇌ NH ₄ ⁺ + 2 H ₂ O	-519	897	863	+311
R₁₀	1/2 N ₂ + 4 H ⁺ + 3 e ⁻ ⇌ NH ₄ ⁺	-79	275	275	-277
	sulfur				
R₁₁	SO ₄ ⁼ + 8 H ⁺ + 6 e ⁻ ⇌ S ⁰ + 4 H ₂ O	-204	353	284	-267
R₁₂	SO ₄ ⁼ + 9 H ⁺ + 8 e ⁻ ⇌ HS ⁻ + 4 H ₂ O	-191	248	197	-269
R₁₃	SO ₄ ⁼ + 10 H ⁺ + 8 e ⁻ ⇌ H ₂ S + 4 H ₂ O	-231	300	249	-269
R₁₄	SO ₄ ⁼ + 8 H ⁺ + 8 e ⁻ ⇌ S ⁼ + 4 H ₂ O	-120	156	104	-309
R₁₅	S ⁰ + H ⁺ + 2 e ⁻ ⇌ HS ⁻	-13	131	131	-283

and concentration of microorganisms present in such a complex growth media, which will control at the end the biochemical process evolution.

In order to be able to analyse the possible interactions C-N-S, the thermodynamic study of the couples C-S, C-N and N-S is firstly presented in this discussion. Taking into account the standard Gibbs free energy (ΔG^0) and redox potential (E^0) of the half-reactions shown in Table 2, the values of these thermodynamic properties of all the reactions involving electron transfer in anaerobic biological systems can be calculated. Even limiting the number of species to hydrogen, acetate, methane and bicarbonate for the carbonaceous species, to nitrate, nitrite, ammonia and nitrogen gas for the nitrogenous compounds and to sulfate, sulfide, hydrogen sulfide and sulfur for sulfurous compounds, the number of reactions is enormous (105 combinations are possible). For the present discussion only the more common biological reactions are considered.

Interaction C-S

A simplified approach to explain the competition between sulfate reducing bacteria (SRB) and methane producing bacteria (MPB) can be done taking into account the values of ΔG^0 calculated for the degradation of acetate and hydrogen in the presence and absence of sulfate.

Volatile fatty acids (VFA) are degraded faster by SRB than by the syntrophic consortia. The microbial competition for the organic carbon increases when decreasing the COD/SO₄⁼ ratio. All the papers revised by Lens *et al.* (2000) reported that sulfide is the end product of sulfate reduction in anaerobic conditions. In aerobic or microaerophilic conditions this sulfide can be biologically oxidised to elemental sulfur.

In a medium with no limitation of sulfate, hydrogen is completely consumed by SRB (Colleran *et al.*, 1995). Hydrogenotrophic SRB (HSRB) may out-compete MPB utilising hydrogen (HMPB). This may explain the process fundamentals, i.e. since HSRB gain more energy from the consumption of molecular hydrogen than HMPB due to their higher substrate affinity, the hydrogen concentration may fall below a certain value needed to sustain the growth of HMPB. This also explains the activity decay of HMPB when sulfate is fed to an anaerobic bioreactor.

Interaction C-N

In a conventional anaerobic environment hydrolysis and degradation of organic nitrogen compounds leads to ammonia build-up as end product of the "ammonification" process. Consequently, ammonium is always present in the effluent of anaerobic reactors treating wastewater rich in organic nitrogen compounds.

Another interesting possibility of the anaerobic processes coupled with the nitrogen cycle is related with the denitrification process and implies the use of oxidised forms of nitrogen. The denitrification of nitrates and nitrites in an anaerobic fluidised bed reactor

Table 3 Competition between SRB and MPB for acetate and hydrogen. ⁽¹⁾ kJ/reaction

	From Table 2	Reactions	ΔG^0 (kJ) ⁽¹⁾
	Acetate		
R₁₆	0.5×R ₂ -0.5×R ₃ =R ₂ -R ₄ =R ₄ -R ₃	CH ₃ COO ⁻ + H ₂ O ⇌ CH ₄ + HCO ₃ ⁻	- 28.3
R₁₇	R ₁₂ -R ₃	SO ₄ ⁼ + CH ₃ COO ⁻ ⇌ HS ⁻ + 2 HCO ₃ ⁻	- 44.5
R₁₈	R ₁₃ -R ₃	SO ₄ ⁼ + CH ₃ COO ⁻ + H ⁺ ⇌ H ₂ S + 2 HCO ₃ ⁻	- 84.4
	Hydrogen		
R₁₉	R ₄ -4×R ₁	HCO ₃ ⁻ + 4 H ₂ + H ⁺ ⇌ CH ₄ + 3 H ₂ O	- 175.1
R₂₀	R ₁₂ -4×R ₁	SO ₄ ⁼ + 4 H ₂ + 1 H ⁺ ⇌ HS ⁻ + 4 H ₂ O	- 191.4
R₂₁	R ₁₃ -4×R ₁	SO ₄ ⁼ + 4 H ₂ + 2 H ⁺ ⇌ H ₂ S + 4 H ₂ O	- 231.3

was reported by Acitores *et al.* (1991) using an anaerobic/aerobic double phase system wherein the nitrified effluent of the aerobic phase was recirculated to the anaerobic part of the reactor. The interpretation of the simultaneous process of denitrification and methanogenesis was suggested by Akunna *et al.* (1992) and according to the data shown in Table 4 it is thermodynamically feasible.

Interaction N-N

Recently, the anaerobic oxidation of ammonium by nitrate or nitrite has been reported and several industrial applications are in course following the Anammox (anaerobic ammonium oxidation) process (Mulder *et al.*, 1995 and van Dongen *et al.*, 2000). The thermodynamic feasibility of the Anammox process is clearly indicated in Table 5 showing the redox reactions between ammonium and nitrate or nitrite.

Interaction N-S

The sulfur and nitrogen cycles can also interact in the denitrification process. The coupling of both cycles implicates the oxidation of reduced forms of sulfur and the reduction of nitrates or nitrites. The reactions involved in the process are showed in Table 6:

The values of the standard Gibbs free energy clearly demonstrate the feasibility of the process wherein nitrates, nitrites and oxygen accept electrons coming from reduced sulfurous compounds. In the presence of oxygen, sulfur oxidation is preferentially coupled with oxygen reduction, as reported by Sublette *et al.* (1998) indicating that denitrification using sulfide as electron donor only occurs for dissolved oxygen levels below 1 mg/l. The effect of nitrate addition on the anaerobic digestion of an industrial sulfate rich wastewater was investigated by Percheron *et al.* (1999). At high COD/NO₃⁻-N ratios denitrification to nitrogen gas was the main nitrate reduction pathway instead of dissimilatory nitrate reduction to ammonia found in anaerobic denitrification in a sulfide free environment. Sulfide was consumed during denitrification suggesting that denitrification using this electron donor occurred in the system.

Table 4 Interactions C-N in a denitrification process

	From Table 2	Reactions	ΔG° (kJ)
R₂₂	R ₅ - 1/4.R ₃	NO ₃ ⁻ + 1/4 CH ₃ COO ⁻ ⇌ NO ₂ ⁻ + 1/2 HCO ₃ ⁻ + 1/4H ⁺	-124
R₂₃	R ₆ - 5/8.R ₃	NO ₃ ⁻ + 5/8 CH ₃ COO ⁻ + 3/8 H ⁺ ⇌ 1/2 N ₂ + 5/4 HCO ₃ ⁻ + 1/2 H ₂ O	-509
R₂₄	R ₇ - R ₃	NO ₃ ⁻ + CH ₃ COO ⁻ + H ⁺ + H ₂ O ⇌ NH ₄ ⁺ + 2 HCO ₃ ⁻	-533
R₂₅	R ₈ - 3/8.R ₃	NO ₂ ⁻ + 3/8 CH ₃ COO ⁻ + 5/8 H ⁺ ⇌ 1/2 N ₂ + 3/4 HCO ₃ ⁻ + 1/2 H ₂ O	-384
R₂₆	R ₉ - 3/4.R ₃	NO ₂ ⁻ + 3/4 CH ₃ COO ⁻ + 5/4 H ⁺ + H ₂ O ⇌ NH ₄ ⁺ + 3/2 HCO ₃ ⁻	-409
R₂₇	2.R ₁₀ - 3/4 R ₃	N ₂ + 3/4 CH ₃ COO ⁻ + 5/4 H ⁺ + 3 H ₂ O ⇌ 2 NH ₄ ⁺ + 3/2 HCO ₃ ⁻	-49

Table 5 Anaerobic ammonium oxidation by nitrate and nitrite

	From Table 2	Reactions	ΔG° (kJ)
R₂₈	3/2.R ₅ -R ₁₀	NH ₄ ⁺ + 3/2 NO ₃ ⁻ ⇌ 1/2 N ₂ + 3/2 NO ₂ ⁻ + H ⁺ + 3/2 H ₂ O	-162
R₂₉	8/5.R ₆ -R ₇	NH ₄ ⁺ + 3/5 NO ₃ ⁻ ⇌ 4/5 N ₂ + 2/5 H ⁺ + 9/5 H ₂ O	-281
	3/5.R ₆ -R ₁₀		
	3/5.R ₇ -8/5.R ₁₀		
R₃₀	6/5.R ₆ -R ₉	NH ₄ ⁺ + 6/5 NO ₃ ⁻ ⇌ NO ₂ ⁻ + 3/5 N ₂ + 4/5 H ⁺ + 8/5 H ₂ O	-202
R₃₁	2.R ₈ -R ₉	NH ₄ ⁺ + NO ₂ ⁻ ⇌ N ₂ + 2 H ₂ O	-360
	R ₈ -R ₁₀		
	R ₉ -2.R ₁₀		

Table 6 Redox reactions involving oxidised forms of nitrogen and reduced sulfurous compounds

	From Table 2	Reactions	ΔG° (kJ)
R₃₂	R ₅ -1/3.R ₁₁	$\text{NO}_3^- + 1/3 \text{S}^0 + 1/3 \text{H}_2\text{O} \Leftrightarrow \text{NO}_2^- + 1/3 \text{SO}_4^{2-} + 2/3 \text{H}^+$	-93
R₃₃	R ₆ -5/6.R ₁₁	$\text{NO}_3^- + 5/3 \text{S}^0 + 1/3 \text{H}_2\text{O} \Leftrightarrow 1/2 \text{N}_2 + 5/6 \text{SO}_4^{2-} + 2/3 \text{H}^+$	-431
R₃₄	R ₇ -4/3.R ₁₁	$\text{NO}_3^- + 4/3 \text{S}^0 + 7/3 \text{H}_2\text{O} \Leftrightarrow \text{NH}_4^+ + 4/3 \text{SO}_4^{2-} + 2/3 \text{H}^+$	-408
R₃₅	R ₈ -1/2.R ₁₁	$\text{NO}_2^- + 1/2 \text{S}^0 \Leftrightarrow 1/2 \text{N}_2 + 1/2 \text{SO}_4^{2-}$	-337
R₃₆	R ₉ -R ₁₁	$\text{NO}_2^- + \text{S}^0 + 2 \text{H}_2\text{O} \Leftrightarrow \text{NH}_4^+ + \text{SO}_4^{2-}$	-314
R₃₇	R ₁₀ -1/2.R ₁₁	$1/2 \text{N}_2 + 1/2 \text{S}^0 + 2 \text{H}_2\text{O} \Leftrightarrow \text{NH}_4^+ + 1/2 \text{SO}_4^{2-}$	+23
R₃₈	R ₅ -1/4.R ₁₃	$\text{NO}_3^- + 1/4 \text{HS}^- \Leftrightarrow \text{NO}_2^- + 1/4 \text{SO}_4^{2-} + 1/4 \text{H}^+$	-113
R₃₉	R ₆ -5/8.R ₁₃	$\text{NO}_3^- + 5/8 \text{HS}^- + 3/8 \text{H}^+ \Leftrightarrow 1/2 \text{N}_2 + 5/8 \text{SO}_4^{2-} + 1/2 \text{H}_2\text{O}$	-481
R₄₀	R ₇ -R ₁₃	$\text{NO}_3^- + \text{HS}^- + \text{H}^+ + \text{H}_2\text{O} \Leftrightarrow \text{NH}_4^+ + \text{SO}_4^{2-}$	-489
R₄₁	R ₈ -3/8.R ₁₃	$\text{NO}_2^- + 3/8 \text{HS}^- + 5/8 \text{H}^+ \Leftrightarrow 1/2 \text{N}_2 + 3/8 \text{SO}_4^{2-} + 1/2 \text{H}_2\text{O}$	-368
R₄₂	R ₉ -3/4.R ₁₃	$\text{NO}_2^- + 3/4 \text{HS}^- + 3/4 \text{H}^+ + \text{H}_2\text{O} \Leftrightarrow \text{NH}_4^+ + 3/4 \text{SO}_4^{2-}$	-375
R₄₃	R ₁₀ -3/8.R ₁₃	$1/2 \text{N}_2 + 3/8 \text{HS}^- + 5/8 \text{H}^+ + 3/2 \text{H}_2\text{O} \Leftrightarrow \text{NH}_4^+ + 3/8 \text{SO}_4^{2-}$	-8
R₄₄	R ₅ -2.R ₁₅	$\text{NO}_3^- + 2 \text{HS}^- + 2 \text{H}^+ \Leftrightarrow \text{NO}_2^- + 2 \text{S}^0 + \text{H}_2\text{O}$	-186
R₄₅	R ₆ -5.R ₁₅	$\text{NO}_3^- + 5 \text{HS}^- + 6 \text{H}^+ \Leftrightarrow 1/2 \text{N}_2 + 5 \text{S}^0 + 3 \text{H}_2\text{O}$	-663
R₄₆	R ₇ -8.R ₁₅	$\text{NO}_3^- + 8 \text{HS}^- + 10 \text{H}^+ \Leftrightarrow \text{NH}_4^+ + 8 \text{S}^0 + 3 \text{H}_2\text{O}$	-781
R₄₇	R ₈ -3.R ₁₅	$\text{NO}_2^- + 3 \text{HS}^- + 4 \text{H}^+ \Leftrightarrow 1/2 \text{N}_2 + 3 \text{S}^0 + 2 \text{H}_2\text{O}$	-479
R₄₈	R ₉ -6.R ₁₅	$\text{NO}_2^- + 6 \text{HS}^- + 8 \text{H}^+ \Leftrightarrow \text{NH}_4^+ + 6 \text{S}^0 + 2 \text{H}_2\text{O}$	-595
R₄₉	R ₁₀ -3.R ₁₅	$1/2 \text{N}_2 + 3 \text{HS}^- + 4 \text{H}^+ \Leftrightarrow \text{NH}_4^+ + 3 \text{S}^0$	-117

Several processes for nitrate removal adding a reduced sulfur source have been proposed using *Thiobacillus denitrificans* for the autotrophic denitrification. Driscoll and Bisogni (1978), Batchelor and Lawrence (1986) and Matsui and Yamamoto (1986) treating synthetic wastewater enriched with nitrate and thiosulfate reported high percentages of denitrification. Autotrophic denitrification with reduced sulfur of municipal wastewater was investigated by Kuai and Verstraete (1999). The authors reported that a sulfur lime bed removed 75% of nitrogen at a loading rate of 0.2 g N/l.d with a hydraulic retention time of 3 h. Denitrification of mining wastewater enriched with nitrates was studied by Bouffard *et al.* (1998). Denitrification of drinking water was performed by Van der Hoek *et al.* (1992), Montiel and Welté (1994) and Trouvé and Chazal (1999) producing water with nitrate concentration below the European Guide Level.

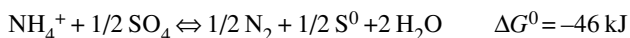
In conclusion the only interaction N-S reported in the technical literature is the reduction of nitrates or nitrites to nitrogen accompanied by the oxidation of sulfide or elemental sulfur. The oxidised form of nitrogen acts as electron donor and sulfur as electron acceptor. This mechanism imposes the obligation of use oxidised nitrogenous species generally not found in anaerobic environments.

A new interaction N-S

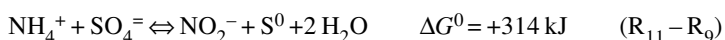
Recently Fdz-Polanco *et al.* (2001) have reported experimental data indicating the possibility of a new pathway that could implicate an ammonium-sulfate interaction. A granular activated carbon (GAC) anaerobic fluidised bed reactor treating vinasse from an ethanol distillery of sugar beet molasses was operated at constant organic loading rate of 1.7 g COD/L.d. The influent had a COD/SO₄²⁻-S ratio of 27 and a TKN-N/SO₄²⁻-S of 2.3, the pH varied between 7.8 and 8.3 and the oxidation reduction potential (ORP) was almost constant with an average value of -430 mV. The reactor showed good performance in terms of organic matter removal but an anomalous behaviour in terms of unusually high concentration of molecular nitrogen in the biogas. The analysis of the different nitrogenous and sulfur species and the mass balances of these compounds in the liquid and gas phases clearly indicated an uncommon evolution of nitrogen and sulfur in the reactor. About 50% of the nitrogen entering the reactor as total Kjeldahl nitrogen (TKN) was removed from the liquid

phase appearing as N_2 in the biogas. Simultaneously, only 20% of the SO_4^{2-} -S initially present in the influent appears as S^{2-} -S in the effluent or H_2S -S in the biogas, indicating that 80% of the sulfur is removed. No other oxidised sulfur forms were detected by HPLC analysis of the liquid phase.

These experimental observations may suggest an uncommon mechanism involving simultaneous anaerobic ammonium oxidation and sulfate reduction that can be represented by the reaction:



This global redox reaction could be obtained combining two anaerobic ammonium oxidation reactions, one thermodynamically not favourable using sulfate as electron acceptor wherein nitrite and elemental sulfur are produced, and the second thermodynamically favourable wherein nitrite acts as electron acceptor (reaction known as Anammox).



In standard conditions the ammonium oxidation by sulfate is not thermodynamically possible as indicated by the positive value of its standard Gibbs free energy. Two effects can contribute to facilitate the viability of this reaction. Initially the selective adsorption of the ammonium and sulfate on the activated carbon produce a local high concentration of reactants near the biofilm. Furthermore the nitrite produced can be rapidly reduced by the second reaction, which is thermodynamically favourable.

Thus far more experiments were conducted using the same fluidised bed reactor and wastewater but increasing the loading rate and similar results were obtained (Fdz-Polanco *et al.* 2000). The main results for three experimental periods are compiled in Table 7.

The main conclusion drawn from this experimentation that lasted more than two thousand days is the experimental evidence of an uncommon evolution of nitrogenous and sulfurous compounds within the reactor. The nitrogen mass balance indicates the co-existence of a conventional ammonification process and an unusual anaerobic nitrogen removal mechanism, demonstrated by the removal of nitrogen in the liquid phase and the

Table 7 A) Organic matter evolution. B) Nitrogen evolution (reactor volume 1.5 L, TKN influent = 2300 mg N/L) ⁽¹⁾ experimental value, ⁽²⁾ calculated from mass balance, ⁽³⁾ calculated from the proposed reaction. C) Sulfur evolution (SO_4^{2-} influent = 1000 mg S/L), ⁽⁴⁾ mg S = mg S- SO_4^{2-} + mg S- S^{2-}

A)	OLR g COD/L.d	Effluent		Biogas			
		mg COD/L	% COD _{rem}	L/L.d	mL/g COD _{rem}	% CH ₄	mL CH ₄ /g COD _{rem}
1	1.7	1950	93	0.7	443	81	358
2	3.0	2925	89	1.5	561	65	364
3	5.3	3615	87	2.3	498	62	308

B)	Effluent (mg N/L)		TKN removal		Biogas			
	TKN	NH ₄ ⁺	mg N/L.d	% TKN	L/L.d	% N ₂ ⁽¹⁾	% N ₂ ⁽²⁾	% N ₂ ⁽³⁾
1	1025	823	80	55	0.7	8.0	8.1	5.0
2	1140	868	129	50	1.5	4.1	5.9	3.5
3	1613	1230	135	30	2.3	2.5	3.7	3.1

C)	Effluent		Biogas % H ₂ S	Sulfur balance				
	SO ₄ ⁼ -S	S ⁼ -S		mg S _{in} /d	mg S-H ₂ S/d	mg S _{eff} /d ⁽⁴⁾	mg S _{lost} /d	mg S _{lost} /L
1	5	90	0.5	94	8	9	78	826
2	8	110	1.1	167	35	20	112	677
3	14	150	1.9	294	94	48	152	529

presence of N_2 in the biogas. According to the average values of Table 7, the maximum TKN removal of 55% was reached in period 1, while the higher nitrogen removal rate of 0.13 g N/L.d corresponds to periods 2 and 3. Simultaneously with this loss of nitrogen the concentrations of sulfide and hydrogen sulfide were much lower than expected according to the sulfate being removed from the influent. The highest amount of S in all forms detected in the outlet streams (effluent and biogas) is 50% of the total sulfur entering the reactor as sulfate and was obtained in period 3 when the reactor operated with the highest loading rate. Elemental S (not quantified) is detected in the solid phase. The chemical analysis of the solids retained in the fluidised bed indicated that 5% was elemental sulfur. Also the solids washed-out from the reactor had a variable content of sulfur.

Fernández *et al.* 2000 have reported similar results operating anaerobic fluidised reactors filled with activated carbon and natural zeolite and treating vinasse with organic loading rates up to 10 g COD/L.d. Their results are especially surprising due to the very low concentration of sulfide detected in the effluent and in the biogas. At the operation temperature the chemical reaction between GAC or zeolite and sulphur and nitrogen compounds of the vinasse was not detected.

Figure 1 shows the calculated values of redox potential for the half reactions of reduction of nitrogen to ammonium and sulfate to elemental sulfur. For both of them the ORP at pH = 8 ranges between -330 and -360 mV. These values are close to the ORP typical of the methanogenesis in anaerobic processes, indicating that the three processes, methanogenesis, sulfate reduction and ammonium oxidation can coexist together in an anaerobic environment. The concentration interval of the species presented in Figure 1 indicates that the concentration of total N, i.e. $N-NH_4^+ + N-N_2$ is constant, and the concentration of total S, i.e. $S-SO_4^- + S^0$ is also constant. The initial concentration of $N-NH_4^+$ and $S-SO_4^-$ was arbitrarily set equal to 1.

Conclusions

The oxidation-reduction reactions or electron donor-acceptor mechanisms can be used to explain the interactions among the different chemical species that are present in an anaerobic environment. For industrial wastewater rich in nitrogen and sulfur compounds in addition to the C-N and C-S interactions the coupling of nitrogen and sulfur cycles is also

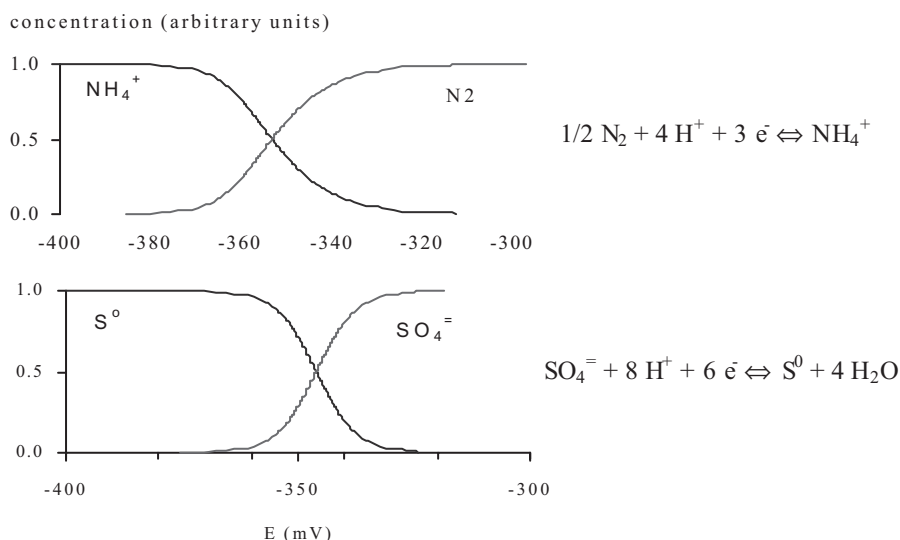


Figure 1 Redox potential for the half reactions of reduction of nitrogen to ammonium and sulfate to elemental sulfur at pH = 8

possible. The interaction N-S can be interpreted as an autotrophic denitrification process using reduced sulfur compounds. The oxidised forms of nitrogen (nitrates or nitrites) are reduced acting as electron acceptors while reduced sulfur compounds (sulfide or elemental sulfur) are oxidised acting as the electron donor.

Experimental data obtained in a granular activated carbon anaerobic fluidised bed reactor treating vinasse indicated uncommon behaviour. Up to 50% of the TKN disappeared from the liquid phase while up to 8% of the biogas was N₂. At the same time, the concentrations of sulfide in the effluent and hydrogen sulfide in the biogas were much lower than expected according to the concentration of sulfate in the influent. These experimental results can be explained by the biochemical interaction of ammonium and sulfate. In the overall process ammonium (electron donor) is oxidised to nitrogen gas and sulfate (electron acceptor) is reduced to elemental sulfur.

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