# Simultaneous organic nitrogen and sulfate removal in an anaerobic GAC fluidised bed reactor

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**Abstract** A granular activated carbon (GAC) anaerobic fluidised bed reactor treating vinasse from an ethanol distillery of sugar beet molasses was operated for 250 days under three different organic loading rates. The reactor showed good performance in terms of organic matter removal and methane production but an anomalous behaviour in terms of unusual high concentrations of molecular nitrogen and low concentration of hydrogen sulphide in the biogas. The analysis of the different nitrogenous and sulphur compounds and the mass balances of these species in the liquid and gas phases clearly indicated an uncommon evolution of nitrogen and sulphur in the reactor. Up to 55% of the TKN and up to 80% of the sulphur disappear in the liquid phase This is the opposite to any previously reported results in the bibliography. The new postulated anaerobic process of ammonia and sulphate removal seems to follow the mechanism: $SO_4^{=}+2\ NH_4^{+} \rightarrow S + N_2 + 4\ H_2O$ ($\Delta\ G° = -47.8$ kJ/mol).

**Keywords** Anaerobic nitrogen removal; GAC fluidised bed reactor; industrial wastewater; sulphate reduction

## Introduction

The sulphur cycle offers possibilities to integrate nitrogen removal in the treatment process and simultaneous removal of nitrogenous and sulphurous compounds has been already reported in anoxic conditions (Hulshoff Pol *et al*., 1998). In this sense, sulphide can be the electron donor being re-oxidised to S° or sulphate by *Thiobacillus denitrificans* using nitrate as electron acceptor. This type of denitrification reduces the overall requirements for carbon at a nutrient removal plant. Also dissimilatory sulphate reducing bacteria (SRB) can be involved in alternative denitrification routes, as some SRB can use nitrate, instead of sulphate, as the terminal electron acceptor (Widdel, 1998). Also Percheron *et al*. (1999) accept the possibility of anaerobic denitrification from nitrate using reduced compounds of sulphur as electron acceptors. In this process, reduced sulphur compounds are partially oxidised to elemental sulphur, sulphite, thiosulphate, trithionate, tetrathionate and/or completely oxidised to sulphate.

On the other hand it has been reported that ammonia can be oxidised to nitrogen gas in anoxic conditions with nitrite or nitrate as electron acceptor (Mulder *et al*. 1995; Vandegraaf *et al*. 1995, van Dongen *et al*., 2000). However, non sulphurous compounds appeared to play a significant role in the anaerobic oxidation of ammonia or the so-called anammox process.

Thus far, the interactions between the nitrogen and sulphur cycles in microbial ecosystems commonly encountered are in accordance with the observations reported in conventional anaerobic treatments of effluents containing high concentrations of organic matter, nitrogen and sulphates (Lens and Hulshoff Pol, 1998). When a sulphate-rich wastewater is introduced into an anaerobic bioreactor, organic matter is usually removed via sulphate reduction and methanogenesis. The complete removal of organic matter can only

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be achieved if, in addition to sulphate reduction, methanogenesis occurs (Hulshoff Pol *et al.*, 1998). Under anaerobic conditions, dissimilatory sulphate reducing bacteria (SRB) usually use sulphate as a terminal electron acceptor for the degradation of organic compounds and hydrogen. In summary the overall process and the evolution of nitrogen and sulphates can be described as follows:

1. Very low rates of ammonia removal. Although high ammonification rates are commonly observed, the anaerobic oxidation of ammonia is not significant in large-scale reactors, as revealed by the absence of molecular nitrogen in the biogas.
2. Anaerobic treatment always proceeds successfully for COD/sulphate ratios higher than 10, in these conditions almost complete reduction of sulphates to sulphides is expected. These sulphides are usually dissolved into the liquid phase or stripped off to the gas phase as hydrogen sulphide.

This expected or normal behaviour has been confirmed at large scale by numerous practitioners working in the field.

Nevertheless, in the present work an "anomalous behaviour" of an anaerobic reactor treating high strength wastewater containing high concentrations of total Kjeldhal nitrogen (NKT) and sulphates is reported. This so-called anomalous behaviour is such because, as will be further discussed later in the paper, an important removal of TKN (up to 50 percent), appearing in the gas phase as molecular nitrogen was observed. There was no evidence of the anammox process since nitrite was absent in the reactor and the apparent oxidation of the ammonia was accompanied by the reduction of sulphates to elemental sulphur. Results obtained in a smaller anaerobic reactor operating in similar conditions to the reactor of this study reported the existence of molecular nitrogen in the biogas (N. Fernández, pers. comm.). Also the concentration of hydrogen sulphide in the biogas was much smaller than the expected value according to the amount of sulphate being removed in the reactor, which indicated that some unknown process was taking place. Based on these previous results a more systematic analysis of the different forms of organic matter, nitrogen compounds and sulphur compounds in both, liquid and gas phases, was conducted. The experimental results collected are reported and although no certain explanations about the biochemistry involved in the process can be drawn, some valuable observations are presented.

## Material and methods

An anaerobic fluidised bed reactor of 1.5 l of working volume (Figure 1) was operated during an experimental period of 250 days. The reactor was inoculated with sludge from an industrial anaerobic contact reactor treating wastewater from a yeast factory. The reactor was filled with granular activated carbon (GAC) with an average particle diameter between 0.42 and 0.85 mm. During operation the GAC bed was expanded 30%. Temperature was maintained constant at 33±2°C. In order to avoid any possible solution of air in the recirculation loop the liquid level at the top of the reactor was always maintained 10 cm over the recirculation exit. Also the reactor had a lid covering the top. Moreover, the biogas exit was set under 10 cm of water pressure.

The reactor was continuously fed with different organic loading rates (1–6 g COD $l^{-1}.d^{-1}$) and constant COD/$SO_4^=$ and TKN/$SO_4^=$ ratios. The reactor was fed with vinasse from a beet sugar molasses ethanol distillery. The influent composition is shown in Table 1. For this industrial wastewater the ratio COD/S-$SO_4^=$ is equal to 27 and the ratio N-TKN/S-$SO_4^=$ is equal to 2.3.

The biogas produced is collected in a gas collector system placed in the upper part of the reactor and its flow is measured by liquid displacement. The gas composition was analysed daily in a gas chromatograph able to separate and quantify $O_2$, $N_2$, $CH_4$, $CO_2$ and $H_2S$. Dräger tubes were used for eventual ammonia analysis.

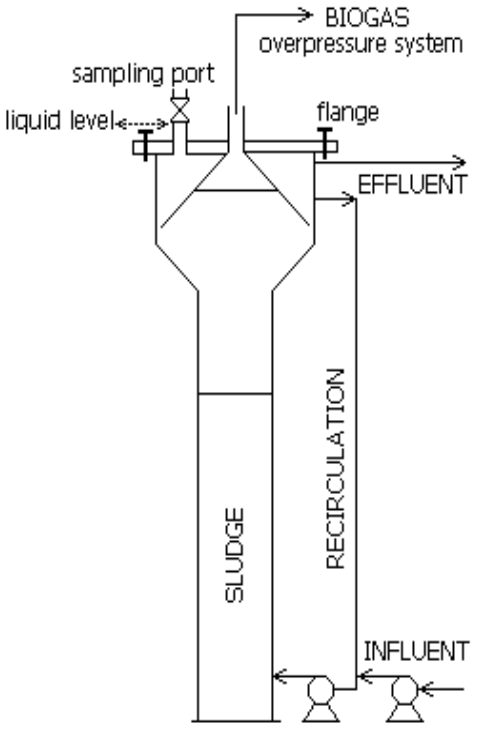


**Figure 1** Schematic diagram of the GAC fluidised bed reactor

**Table 1** Average influent composition

| COD ($mg\ O_2/l$) | Carbon ($mg\ C/l$) | | Nitrogen ($mg\ N/l$) | | | Sulphur ($mg\ S/l$) | | Fatty acids ($mg\ VFA/l$) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Org. | Inorg. | TKN | $NH_4^+$ | $NO_3^-$ | $SO_4^{-2}$ | $S^{-2}$ | Acetate | Propionate | Butyrate |
| 27000 | 21000 | 500 | 2300 | <10 | 50 | 1000 | <1 | 1200 | 160 | 40 |

Effluent liquid samples were analysed three days per week. COD, $BOD_5$, N-TKN were determined according to Standard Methods (APHA, 1992); TOC was analysed with an organic carbon analyser Shimadzu 5050 equipped with an automatic injector Shimadzu 5000A; $NO_2^-$, $NO_3^-$ and $SO_4^=$ by HPLC; N-$NH_4^+$ and $S^=$ using selective electrodes and VFA by GC with a Hewlett Packard 5890 chromatograph.

## Results and discussion

Three different experimentation periods were carried out, each of them characterised by operating the reactor with different influent flow rates. Three pseudo-steady states were reached for different organic loading rates (ORL) varying between 1.7 and 5.3 g COD $l^{-1}$ $d^{-1}$.

The reactor showed high stability after accidental changes in the organic loading rate and/or after short periods without feeding. The pH varied between 7.8 and 8.3 and the oxidation-reduction potential (ORP) between –425 and –435 mV.

### Organic matter evolution

Throughout the entire experimentation very high removal rates of organic matter (as COD) from the system were observed (Table 2), nevertheless the COD removal efficiency

**Table 2** Organic matter evolution: COD removal in the liquid and biogas production and composition

| Period | OLR g COD/l.d | Effluent mg COD/l | Effluent %$COD_{rem}$ | Biogas l / l.d | Biogas ml/g $COD_{rem}$ | Biogas % $CH_4$ | Biogas 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 |



decreased from 93 to 87 percent when increasing the organic loading rate from 1.7 to 5.3 g COD $l^{-1}$ $d^{-1}$. For this moderate range of organic loading rate the loss of efficiency can be related to the simultaneous appearance of other different biological processes. From an applied and technological point of view of COD removal this behaviour is not relevant because of the good quality of the effluent.

The percentage of methane in the biogas varied from 81% to 62% as a function of the organic loading rate (Table 2), The specific production rate of methane is almost constant when operating the system at low organic loading rates (360 ml $CH_4$/g $COD_{rem}$), but clearly decreases to 310 ml $CH_4$/g $COD_{rem}$ for the higher level of organic loading rates.

#### Nitrogen evolution

Figure 2 shows the evolution an average values of TKN in the effluent and $N_2$ in the biogas indicating the different behaviour for each experimental period.

The main experimental observations obtained following the evolution of nitrogenous species in the liquid and gas phases are compiled in Table 3. For the liquid phase the most meaningful result is that the total nitrogen in the effluent is clearly lower than expected, taking into account that ammonification is the only expected transformation process involving nitrogenous species in anaerobic reactors treating high strength wastewaters. In our system TKN was removed from the reactor with removing percentages ranging between 30 and 55% of the TKN entering the reactor removed. The efficiency of this unusual nitrogen removal process was a function of the organic load entering the reactor. Initially we looked for some anaerobic oxidation of ammonia into molecular nitrogen as it had being previously reported by other authors, in the so-called anammox process. However, the nitrate initially present in the influent was only eventually detected at very low concentration and nitrite was never detected in the reactor, invalidating such hypothesis or demonstrating that the nitrite reduction is not a limiting step. The removal rate of nitrogen under this anomalous process was evaluated reaching values of 0.13 g N $l^{-1}$ $d^{-1}$ for organic loading rates ranging between 3 and 5.3 g COD $l^{-1}$ $d^{-1}$.

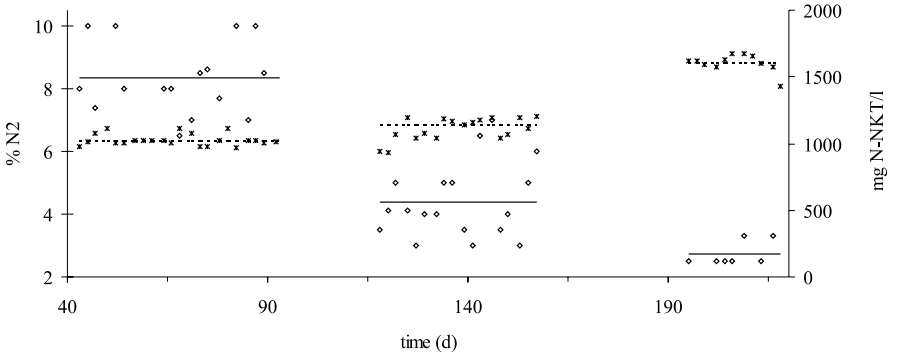


**Figure 2** Concentration of (◇) $N_2$ in biogas and (x) TKN in the effluent. (Theoretical values: %$N_2$=0 and TKN=2150 mg N-TKN/l). Period 1 day 43–93, period 2 day 118–168, period 3 day 195–218

**Table 3** Nitrogen evolution in liquid and gas phases (reactor volume = 1.5 l, TKN influent = 2300 mg N-TKN/l).(1) Experimental value, (2) calculated from mass balance, (3) calculated from the proposed reaction mechanism

| Period | 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 |



On the other hand an important ammonification process took place in the reactor since 80% of all nitrogen detected in the liquid effluent was ammonium and no ammonia was detected in the influent. The rest of the TKN in the liquid effluent was organic nitrogen.

The removal process of nitrogen in the liquid phase was confirmed by measuring the amount of dinitrogen collected with the biogas. Data shown in Table 3 indicate that the process under which molecular nitrogen is produced in the biogas is a function of the organic loading rate entering the reactor. The percentage of dinitrogen in the biogas decreased when increasing the ORL entering the reactor. The disappearance of TKN from the liquid also decreased as the ORL increased which connects the removal process of TKN and the formation of dinitrogen in the biogas. This suggested that a catabolic process in which reduced forms of nitrogen (ammonia and organic) are being oxidised to molecular nitrogen. According to Table 3 the concentration of molecular nitrogen in the biogas was as high as 8% (v/v) for an organic load of 1.7 g COD $l^{-1}$ $d^{-1}$ decreasing to 2.5% (v/v) when the organic load entering the reactor was 5.3 g COD $l^{-1}$ $d^{-1}$. Considering a conventional denitrification process of the nitrate initially present in the reactor influent the amount of molecular nitrogen formed would not exceed 0.3%. This also indicates that an unusual process forming molecular nitrogen is occurring in the reactor.

Under these considerations and supposing that all TKN entering the reactor is: 1) stripped as ammonia, 2) assimilated for biomass growth, 3) transformed into molecular nitrogen, the theoretical concentrations of molecular nitrogen expected in the biogas, obtained by mass balance calculations, are shown in Table 3. The ammonia stripping was calculated assuming 900 mg N-($NH_4^+$ +$NH_3$)/l; pH = 8.2; T = 35 ºC; Henry constant = 4 atm/mol fraction; $pK_B$ = 4.73. The calculated loss of nitrogen from the liquid phase is only 1 mg N/l. Furthermore the experimental concentration of ammonia in the biogas was always under 0.005%, clearly indicating that the stripping mechanism is negligible. Considering a biomass growth of 0.05 g VSS/g $COD_{rem}$ the amount of nitrogen that disappears from the liquid phase is about 150 mg N/l. The difference between these theoretical concentrations of molecular nitrogen and the measured values are higher for increasing organic loading rates entering the reactor. This might indicate significant assimilation processes that could not be quantified in our system due to small reactor size and the difficulties associated with measuring the biomass attached on granular activated carbon particles and the suspended biomass washed out from the reactor.

#### Sulphur evolution

The anomalous evolution of the nitrogenous species in our system suggested that we also follow the evolution of the sulphurous species. In this sense we looked for all types of oxidised and reduced forms of sulphur in the liquid phase. We used chromatographic methods to measure sulphate, sulphite and tiosulphate as main oxidised forms and selective electrodes for measuring sulphides. Among all possible oxidised forms we never detected species other than sulphates. The reduced forms were quantified as total sulphides. We also

**Table 4** Sulphur evolution in liquid and gas phases. (Influent $S\text{-}SO_4^=$ = 1000 mg/l). (1) mg S = mg $S\text{-}SO_4^=$ + mg $S\text{-}S^=$

| Period | Effluent (mg/l) | | Biogas | Sulphur balance | | | | |
|---|---|---|---|---|---|---|---|---|
| | $S\text{-}SO_4^=$ | $S\text{-}S^=$ | % v/v $H_2S$ | mg $S_{inf}$/d(1) | mg $S\text{-}H_2S$/d | mg $S_{eff}$/d(1) | 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 |

measured the concentration of hydrogen sulphide in the gas. We could not measure the amount of elemental sulphur that might be formed in the reactor. This determination was impossible in practice due to manifold reasons mainly the reactor and substratum characteristics.

According to Table 4 the analysis indicates the almost complete removal of sulphates in the reactor which is the common behaviour in anaerobic reactors operating with sufficient $COD/SO_4^=$ ratio. However the amount of total sulphides in the liquid and hydrogen sulphide in the gas phase were much lower than expected according to previous results obtained treating the same wastewater in an UASB reactor (N. Fernández, pers. comm.). Also it can be observed in Table 4 that the concentration of sulphide and hydrogen sulphide in the liquid and gas phases increased as the organic loading into the reactor increased. We did some calculations considering the solubility of the hydrogen sulphide in the liquid and the equilibrium $S^=/HS^-/H_2S$ for pH and temperature conditions of 7.8 and 35°C, respectively. The calculated values for the concentration of sulphide in the liquid and of hydrogen sulphide in the gas should be around 500 mg/l and 2.9% (v/v) respectively. These expected concentrations are much higher than the experimental values (Table 4).

Figure 3 shows the evolution and average values of $H_2S$ in the biogas and $S^=$ in the effluent for the different experimental periods.

Considering the experimentally determined amounts of sulphur that enter and leave the reactor in the liquid and gas phases we observed that we could not close the mass balance. The amount of "sulphur lost", i.e. undermined experimentally, is shown in Table 4. It can be observed that the amount of sulphur lost decreased as the organic loading increased. For the lower organic loading rate (Period 1) almost 80% of the sulphur entering the reactor is "lost", and for the highest load (Period 3) the amount of sulphur lost decreased to 50%.

#### The overall process

According to the aforementioned observations we can postulate the existence of an overall degradation process wherein TKN/ammonia and sulphur are involved. The main

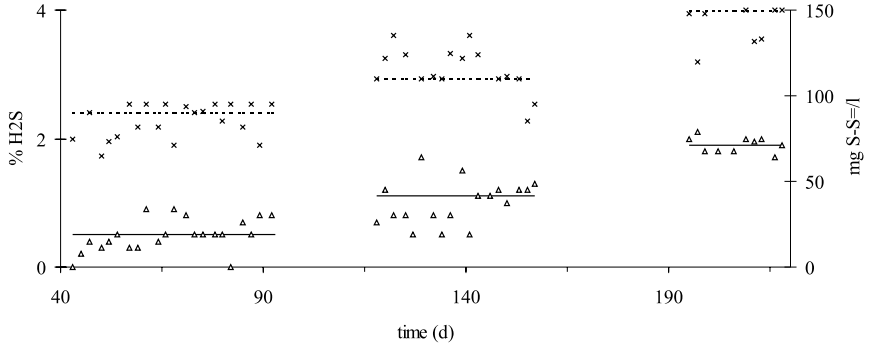


**Figure 3** Concentration of (D) $H_2S$ in the biogas and (x) $S^=$ in the effluent. (Theoretical values: $\%H_2S$=2.9 and $S^=$=500 mg $S^=$/l.). Period 1 day 43–93, period 2 day 118–168, period 3 day 195–218

experimental results are the significant transformation into gaseous dinitrogen of soluble nitrogen compounds initially present in the liquid phase and the simultaneous removal of sulphur from the liquid phases. The most conventional and thermodynamically favourable evolution of sulphate and TKN in anaerobic conditions is to give $S^{=}$ and $NH_4^+$. However, this conventional mechanism is insufficient to explain our observations. Based on the experimental observations the overall process may involve a new oxidation-reduction mechanism wherein ammonia is oxidised to dinitrogen and sulphate is reduced to elemental sulphur. The overall mechanism that may explain the anomalous process taking place in our system where simultaneous removal of TKN and sulphate occur, can be postulated as follows:



$$SO_4^{=} + 2\,NH_4^+ \rightarrow S + N_2 + 4\,H_2O\ (\Delta G° = -47.8\ kJ/mol) \quad (1)$$

This global biochemical reaction could be obtained combining [(1) = (2) + (3) + (4)] reactions involving nitrite formation (2) and anammox reaction (4):

$$3\,SO_4^{=} + 4\,NH_4^+ \rightarrow 3\,S^{=} + 4\,NO_2^- + 4\,H_2O + 8\,H^+ \quad (2)$$

$$3\,S^{=} + 2\,NO_2^- + 8\,H^+ \rightarrow N_2 + 3\,S + 4\,H_2O \quad (3)$$

$$2\,NO_2^- + 2\,NH_4^+ \rightarrow 2\,N_2 + 4\,H_2O \quad (4)$$

Considering the thermodynamics of the process according to theoretical data (CRC, 1971) we could evaluate the free standard energy, $\Delta G°$, obtaining a value of –47.8 kJ/mol, which indicates that the reaction is thermodynamically possible. The presence of the GAC in our system must also be taken into account. The adsorption of the nitrogenous and sulphur species over the activated carbon would increase its local concentration near the biofilm, which can favour this unusual biochemical pathway.

To confirm this hypothesis we performed a mass balance considering that all the sulphur lost in our system (i.e. nondetected) is elemental sulphur. Also we supposed that this elemental sulphur is consumed in the oxidation of the ammonia according to the above postulated mechanism. Under these considerations we could evaluated the amount of molecular nitrogen being formed in our reactor (Table 3). Except for low loading rate conditions (Period 1) the theoretical concentrations obtained fit reasonably with the values detected experimentally.

On the other hand the observation of GAC particles under the microscope allowed for the experimental confirmation that elemental sulphur was being formed within the reactor, furthermore elemental sulphur was also detected on the suspended solids washed out of the reactor. Nevertheless it has not been possible to quantify it by already explained reasons.

## Conclusions

The main conclusions that can be drawn from the present work are:

A GAC anaerobic fluidised bed reactor treating diluted vinasse from an ethanol distillery of sugar beet molasses showed an anomalous behaviour in terms of unusual high removal percentages of TKN and sulphates, high concentrations of molecular nitrogen in the biogas, and low concentrations of hydrogen sulphide in the biogas. The analysis of the different nitrogenous and sulphur compounds and the mass balances of these species clearly indicate an uncommon evolution of nitrogen and sulphur in the reactor. This is the opposite to any previous reported results in the bibliography where most references indicate that the expected evolution of organic nitrogen in anaerobic conditions stops in

ammonia nitrogen and a possible sulphate reduction to $S^{=}$ (liquid phase) and $H_2S$ (gas phase).

The nitrogen mass balance indicated the coexistence of a conventional ammonification process and an unusual anaerobic nitrogen removal mechanism, demonstrated by the loss of N in the liquid phase and the presence of $N_2$ in the biogas. Up to 55% of the TKN disappeared by a nonreported anaerobic process.



The concentrations of $S^{=}$ and $H_2S$ were much lower than expected according to the sulphates being removed from the system. The highest amount of S in all its forms detected in the outlet streams (liquid effluent and biogas) is 50% when the reactor operated with the highest loading rate. Elemental S (not quantified) is detected in the solid phase.

The new overall mechanism postulated to explain the experimental observation, i.e. simultaneous removal of TKN/ammonia and sulphate, is the following:

$$SO_4^{=} + 2\,NH_4^{+} \rightarrow S + N_2 + 4\,H_2O\ (\Delta G^{\circ} = -47.8\ kJ/mol) \qquad (5)$$

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