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## RAPID COMMUNICATION

# NEW PROCESS FOR SIMULTANEOUS REMOVAL OF NITROGEN AND SULPHUR UNDER ANAEROBIC CONDITIONS

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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 90 days, the first 40 days of start-up followed by 50 days of operation at constant organic loading rate of 1.7 g COD/Ld. The reactor showed good performance in terms of organic matter removal but an anomalous behaviour in terms of unusual high concentrations of molecular nitrogen in the biogas. The analysis of the different nitrogenous and sulphur compounds and the mass balances of these compounds in the liquid and gas phases clearly indicated an uncommon evolution of nitrogen and sulphur 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<sub>2</sub> in the gas phase. Simultaneously, only 20% of the S-SO<sub>4</sub><sup>2-</sup> initially present in the influent appears as S-S<sup>2-</sup> in the effluent or S-H<sub>2</sub>S in the biogas, indicating that 80% of the sulphur is removed. This behaviour has not been reported previously in the literature. These observations may suggest a new anaerobic removal process of ammonia and sulphate according to an uncommon mechanism involving simultaneous anaerobic ammonium oxidation and sulphate reduction. © 2001 Elsevier Science Ltd. All rights reserved

**Key words**—anaerobic treatment, anaerobic nitrogen removal, anaerobic sulphur removal

## INTRODUCTION

Under anaerobic conditions, dissimilatory sulphate reducing bacteria (SRB) use sulphate as a terminal electron acceptor for the degradation of organic compounds and hydrogen. When a sulphate-rich wastewater is introduced into an anaerobic bioreactor, organic matter will be removed via sulphate reduction and methanogenesis. In the presence of sulphate, acidogenic, acetogenic and methanogenic bacteria compete with SRB for available substrates (Hulshoff Pol *et al.*, 1998).

The degradation pathways of nitrogen and sulphur may interact at various levels. SRB can compete with denitrifying microorganisms for the organic carbon and readily utilise most of the carbon sources added in denitrification process of wastewater. Also, sulphide can be the electron donor being re-oxidised to S<sup>0</sup> or sulphate by *Thiobacillus denitrificans* using nitrate as electron acceptor. Also SRB can be involved in alternative denitrification routes, as some SRB can use nitrate, instead of sulphate, as terminal electron acceptor. However, SRB convert nitrate to

ammonium, and thus other treatment steps are needed if a complete nitrogen removal is required. Percheron *et al.* (1999) accepts the possibility of denitrification of nitrate using reduced compounds of sulphur as electron donors.

In conventional anaerobic reactors treating high-strength wastewater containing high concentration of TKN and sulphates, the “normal behaviour” (Lens and Hulshoff Pol, 1998) is high ammonification with negligible N<sub>2</sub> formation and almost complete reduction of sulphates to sulphides.

Under anoxic conditions nitrite is converted to nitrogen gas with ammonium as electron donor (NO<sub>2</sub><sup>-</sup> + NH<sub>4</sub><sup>+</sup> → N<sub>2</sub> + 2H<sub>2</sub>O), as demonstrated in the Anammox process (van Dongen *et al.*, 2000). No references about the role of sulphate as acceptor of the electrons produced in the ammonium oxidation to nitrogen or nitrite have been found.

## MATERIALS AND METHODS

As experimental set-up an anaerobic fluidised-bed reactor of 1.5 L of reaction volume (Fig. 1) was operated during an experimental period of 90 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

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averaged particle diameter between 0.42 and 0.85 mm. During operation, the GAC bed was expanded 30% by controlling the effluent recycling into the reactor. Temperature was maintained constant at  $33 \pm 2^\circ\text{C}$ .

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 fed with diluted vinasse coming from an ethanol distillery plant processing beet sugar molasses. The influent was kept at  $4^\circ\text{C}$ , and the peristaltic pump suction from the bottom of the feeding tank, in order to minimise the entrapping of air. In any case, considering that only 100 mL/d of liquid are being fed into the reactor, it is impossible that significant amounts of entrapped air enter the reactor.

The influent had a COD/S-SO<sub>4</sub><sup>2-</sup> ratio of 27 and a TKN/S-SO<sub>4</sub><sup>2-</sup> of 2.3, and its composition is shown in Table 1. The average organic load entering the GAC reactor was 1.7 g COD/Ld. Biogas was collected in a home-made gas flow-meter device placed in the upper part of the reactor and its flow is measured by liquid displacement. The gas composition was daily analysed for O<sub>2</sub>, N<sub>2</sub>, CH<sub>4</sub>, CO<sub>2</sub> and H<sub>2</sub>S in a gas chromatograph. Effluent liquid samples were analysed 3 days per week. Analyses of carbonaceous, nitrogenous and sulphurous compounds were conducted according to standard methods (APHA, 1995).

## RESULTS AND DISCUSSION

After a start-up period of 40 days a constant organic loading rate (OLR) of 1.7 g COD/Ld was

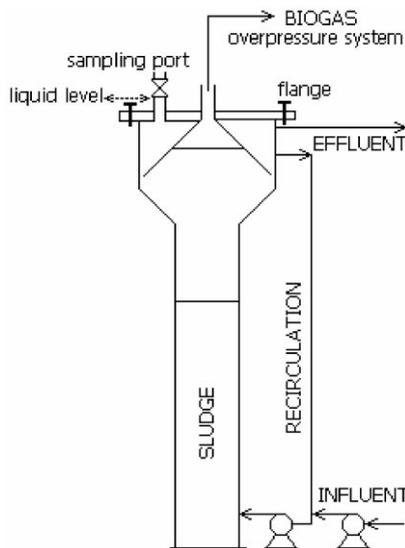


Fig. 1. Schematic diagram of the GAC fluidised-bed reactor.

applied to the reactor. In these conditions, high COD removal rates were attained with an averaged efficiency of 93% and an average concentration of CH<sub>4</sub> in the biogas of 80% (v/v). The specific production of CH<sub>4</sub> was fairly constant with a value of 360 mL CH<sub>4</sub>/g COD<sub>removed</sub>. This relatively high value seems to suggest that the removal of organic matter and the production of methane was not influenced by the presence of high concentrations of ammonia and sulphate in the media. Also the reactor showed very good stability overcoming accidental changes in the organic loading rate and/or short shutdown periods of few hours without feeding. The pH within the reactor varied between 7.8 and 8.3 and the oxidation reduction potential (ORP) between -425 and -435 mV.

The evolution of nitrogenous compounds in the liquid and gas phases is shown in Fig. 2. The average values are compiled in Table 1. The most relevant observation was that the total nitrogen in the effluent was clearly lower than expected, according to the up-to-date reported processes involving TKN in conventional anaerobic process wherein solely ammonification is expected. Even considering a biomass growth of 0.05 g VSS/g COD<sub>rem</sub>, the amount of nitrogen devoted to assimilation is only 150 mg N/L and this mechanism cannot explain the disappearance of nitrogen observed in all the experiments. This apparent nitrogen removal process was significant being 50% of the TKN entering the reactor. The observed removal rate of nitrogen under this anomalous process was as high as 0.15 g N/Ld.

Furthermore, an important ammonification process took place in the reactor. Eighty percent of all nitrogen detected in the liquid effluent was ammonium. Also the small amount of nitrates entering the reactor was removed by some simultaneous denitrification processes taking place into the reactor.

The removal process of nitrogen in the liquid phase was confirmed by measuring the amount of nitrogen collected with the biogas. According to Table 1 the concentration of molecular nitrogen in the biogas was as high as 8% (v/v). Considering a conventional denitrification process of the oxidised forms of nitrogen present in the reactor influent the amount of molecular nitrogen formed would not exceed 0.3%. Considering that 2300–1025 mg N-TKN/L are removed from the liquid phase and that 150 mg N/L are assimilated due to microbial growth and assuming that all this nitrogen is converted into N<sub>2</sub>,

Table 1. Average composition of influent, effluent and biogas for the experimental period of 50 days. Organic loading rate (OLR)=1.7 g COD/Ld

	COD	C <sub>org</sub>	CO <sub>2</sub>	CH <sub>4</sub>	TKN	NH <sub>4</sub> <sup>+</sup>	NO <sub>2</sub> <sup>-</sup>	NO <sub>3</sub> <sup>-</sup>	N <sub>2</sub>	NH <sub>3</sub>	SO <sub>4</sub> <sup>2-</sup>	S <sup>2-</sup>	H <sub>2</sub> S
Influent	27000	21000			2300	<10	n.d.	50			1000	<1	
Effluent	1950	570			1025	825	n.d.	<10			5	90	
Biogas			10.5	81					8	0.005			0.5
Units	mg/L	mg/L	% v/v	% v/v	mg N/L	mg N/L	mg N/L	mg N/L	% (v/v)	% (v/v)	mg S/L	mg S/L	% v/v

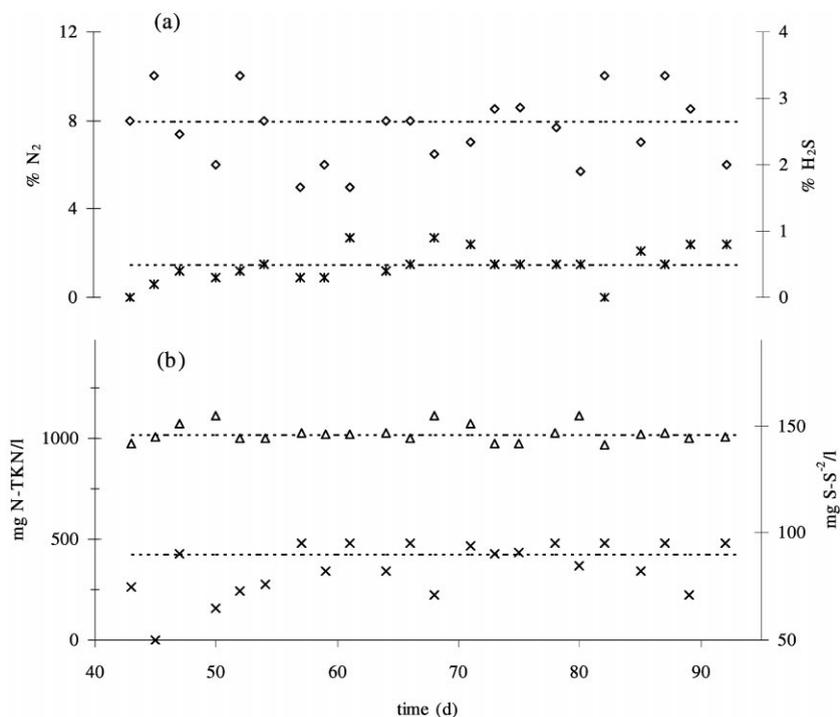


Fig. 2. (a) Evolution of ( $\diamond$ ) N<sub>2</sub> and (\*) H<sub>2</sub>S in the biogas. The expected theoretical concentrations (in % v/v) for N<sub>2</sub> and H<sub>2</sub>S were zero and 2.9, respectively; (b) evolution of ( $\Delta$ )TKN and ( $\times$ ) S<sup>2-</sup> in the liquid phase. The expected theoretical concentrations were 2300 mg N-TKN/L and 510 mg S<sup>2-</sup>/L.

the theoretical percentage of N<sub>2</sub> in the biogas is 8.3% (v/v), which reasonably matches the experimental observations of Table 1.

Finally, we considered the possibility of any ammonia being stripped off the system due to the high ammonification taking place in the reactor. Considering a concentration of 823 mg N/L of ammonia in the liquid (NH<sub>4</sub><sup>+</sup> + NH<sub>3</sub>) at pH 8.2 and 35°C, and a Henry constant of 4 atm/mol fraction and a pK<sub>B</sub> of 4.73, the theoretic concentration of ammonia (NH<sub>3</sub>) in the biogas would be 0.033%. Nevertheless, we experimentally determined the concentration of ammonia in the biogas and in all samples provided the concentration was always under 0.005%. Consequently, based on theoretical calculations and on the experimental evidence we can confirm that the stripping of ammonia cannot justify the disappearance of 1200 mg N/L from the liquid phase and that the stripping of ammonia was insignificant. These observations suggested that an unknown anaerobic removal process of TKN and/or ammonia was taking place in the reactor resulting in significant amounts of molecular nitrogen in the biogas.

According to these observations we also looked for other chemical elements that could be involved in this anomalous process. All the expected forms of oxidised and reduced sulphurous compounds in the liquid and gas phases were experimentally determined. It was observed that almost complete removal of sulphate was taking place into the GAC reactor,

which is a common process in anaerobic media containing a sufficient COD/SO<sub>4</sub><sup>2-</sup> ratio. Since the sulphate removal process usually results in the production of sulphides, we measured the amount of total sulphides in the liquid and gas phases (Table 1). However, the experimental values were much lower than the expected in accordance with the previous results reported when treating the same vinasse wastewater in an UASB reactor (Fernández, 1999).

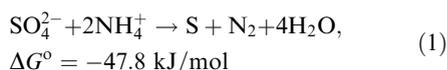
Some calculations were done considering the solubility of the hydrogen sulphide in the liquid and the equilibrium S<sup>2-</sup>/HS<sup>-</sup>/H<sub>2</sub>S for pH and temperature conditions of 7.8 and 35°C, respectively. The theoretical values resulting from these calculations are 500 mg S<sup>2-</sup>/L and 2.9% (v/v) for the dissolved sulphides and hydrogen sulphide in the gas, respectively. These theoretical values are much higher than the experimental values (Table 1) which suggested that an uncommon anaerobic process removing sulphate was taking place in the reactor.

The observation of GAC particles under the microscope allowed the experimental confirmation that significant amounts of elemental sulphur were being formed within the reactor, nevertheless, its quantification has not been possible due to experimental limitations mainly linked to the reactor and substratum characteristics. However, the experimental evidence of a significant formation of elemental sulphur together with the fact that almost 80% of the sulphate entering the reactor was removed suggest that there might be a

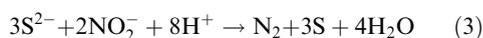
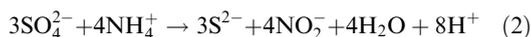
connection between the two observations. Since the mass balance must close, we can conclude that the "sulphur lost", i.e. that was undetermined experimentally, accounting for more than 880 mg S/L, resulted from the reduction of sulphate.

#### *A new process*

The most conventional and thermodynamically favourable mechanisms involving sulphate and TKN compounds in anaerobic processes that result in the formation of  $S^{2-}$  and  $NH_4^+$  fail when trying to explain our observations. The significant production of molecular nitrogen resulting from the oxidation of TKN/ammonia and the simultaneous reduction of sulphates resulting in significant formation of  $S^0$ , suggests that there might be a new degradation process wherein TKN/ammonia and sulphate are involved. Considering that most organic nitrogen is being transformed into ammonia by a conventional ammonification process we will further consider ammonia as the only nitrogenous compound being oxidised to molecular nitrogen in this uncommon process. Thus far, the new global oxidation–reduction mechanism postulated here as a first approximation to explain the anomalous behaviour encountered in our system is



This global biochemical reaction could be obtained combining reactions involving nitrite formation and Anammox reaction:



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.

#### CONCLUDING REMARKS

1. 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 concentra-

tions of molecular nitrogen 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.

2. The nitrogen mass balance indicated the coexistence of a conventional ammonification process and an unusual anaerobic nitrogen removal mechanism, demonstrated by the disappearance of organic nitrogen from the liquid phase and the appearance of  $N_2$  in the biogas. Up to 50% of the ammonia disappeared by an unknown anaerobic process.
3. The concentrations of  $S^{2-}$  and  $H_2S$  were much lower than expected according to the stoichiometry of the reduction of  $SO_4^{2-}$ . The amount of S in all its forms detected in the outlet streams (liquid effluent and biogas) is only 20%. Elemental S (not quantified) was detected in the solid phase.
4. More sophisticated experiments with labelled substrates must be planned and performed in order to demonstrate the fate of the nitrogenous and sulphurous compounds in the process.

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#### REFERENCES

- APHA-AWWA-WEF (1995) *Standard Methods for the Examination of Water and Wastewater*, 19th Edition.
- Fernández N. (1999) Anaerobic treatment of vinasse wastewater. Internal Report.
- Hulshoff Pol L. W., Lens P. N. L., Stams A. J. M. and Lettinga G. (1998) Anaerobic treatment of sulphate-rich wastewaters. *Biodegradation* **9**, 213–224.
- Lens P. N. L. and Hulshoff Pol L. W. (1998) (Editors). Biological sulfur cycle: environmental science and technology. *Special issue of biodegradation. Biodegradation* **9**, 157–318.
- Percheron G., Bernet N. and Moletta R. (1999) Interactions between methanogenic and nitrate reducing bacteria during the anaerobic digestion of an industrial sulphate rich wastewater. *FEMS Microbiol. Ecol.* **29**, 341–350.
- van Dongen, U., Strous, M., van de Pas-Schoonen, K., van Loosdrecht M., Kuenen J. G. and Jetten M. (2000) Combination of partial nitrification (Sharon) and anaerobic ammonium oxidation (Anammox) for the removal of ammonia from concentrated wastewater. *Proceedings of the Fourth International Symposium on Environmental Technology, Noordwijkerhout, The Netherlands*, pp. 42–45.