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# Combination of methanogenesis and denitrification in a UASB reactor for water reclamation applied to small agglomerations

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## ABSTRACT

A two-step system combining an anaerobic/anoxic UASB reactor followed by a low energy consuming rotating biological contactor might be a sustainable option for wastewater treatment and reuse in small agglomerations. This article focuses on the UASB stage. The performance of a lab-scale UASB fed with synthetic wastewater and set aside for simultaneous methanogenesis and denitrification is analysed. The results showed that denitrification began immediately after starting feeding the UASB with nitrate. Methanogenesis was negatively affected for two days after starting adding nitrate to the feed but later on good methanogenic performance was achieved again. Very high average removal rates of both nitrate (97.5%) and COD (91%) were finally reached in the methanogenic/denitrifying UASB at the tested operational conditions (27°C, OLR of 3.3 kg COD/m<sup>3</sup>/d, NLR of 0.122 kgN/m<sup>3</sup>/d and COD/NO<sub>3</sub><sup>-</sup>-N = 26). Therefore there might be a great potential for applying the proposed technology in small agglomerations where low cost but effective technologies are needed.

Keywords: Methanogenesis; Denitrification; UASB

# 1. Introduction

Adequate sewage water treatment from small agglomerations is a task still to be tackled in most of the regions in the world. Spain is no exception and should carry out this job in compliance with the European Directive 91/271/CEE. On the other hand many regions in Spain face chronic water shortages that cause important economical impacts. To contribute to overcome together these challenges an effective wastewater treatment-effluent use system must be designed. Using an upflow anaerobic sludge blanket (UASB) reactor as the core of the treatment process might be interesting because it is low energy demanding, needs low investment costs, requires few space, has low sludge production and high loading capacity, and it can yield biogas of enough quality to be used as a source of energy [1]. Nevertheless effluents from anaerobic reactors cannot be discharged to sensitive areas or used for restricted irrigation without proper post-treatment.

A two steps system combining an anaerobic/anoxic UASB reactor followed by a low energy consuming rotating biological contactor (RBC) might reduce costs and become a sustainable option. Thus, in the crops non

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growing season, simultaneous COD removal (through methanogenesis) and nitrogen removal (through denitrification) might be achieved in the UASB with previous nitrification in the RBC so that an effluent free of N and COD would be achieved. Outside the crop- growing season N removal would not be necessary since crops would behave as N sink (Fig. 1).

Nevertheless, denitrification and methanogenesis are mediated by different microbial populations, requiring distinct environmental conditions and, consequently, an integration of the process might be problematic. First of all reduction of nitrate and nitrite is a far more energy yielding process than methanogenesis, and carbon me-



#### GROWING SEASON

Fig. 1. Set up of the UASB–RBC treatment system in the growing season and in the non-growing season.

Table 1

Carbon and nitrate removal efficiencies reached in anaerobic/anoxic reactors

tabolism via denitrification will consequently be expected to dominate in a given environment when nitrate or nitrite are present and carbon source is limited.

Moreover, since denitrification proceeds at a higher redox potential than methanogenesis, methane production might be directly inhibited [2]. Even under conditions of low redox potential the sole presence of nitrate or the intermediate compounds of denitrification has been shown to inhibit methanogenesis indicating that these compounds are toxic to methanogens [3,4]. Furthermore, in anaerobic reactors, the added nitrate might be mainly reduced to ammonia depending on the C source and COD/NO<sub>3</sub><sup>-</sup>-N ratio [5,6].

Despite of the drawbacks previously exposed, combined methanogenesis and denitrification in a single reactor has been proven to be possible with surplus of Csource when denitrification and methanogenesis are separated either spatially (due to NO<sub>x</sub> gradients) or temporally. Some cases are summarized in Table 1.

This article focuses on the UASB stage. The performance of a lab-scale UASB fed with synthetic wastewater and set aside for simultaneous methanogenesis and denitrification is analysed.

# 2. Methods

A 12.06 l lab scale UASB reactor was started up and its performance was followed before and after addition of nitrate to the feed. In the first stage the reactor was started up and operated under anaerobic digestion conditions to get a reactor with good anaerobic performance and especially with good methanogenic activity. Once a good and stable methanogenic performance of the reactor was achieved, the second stage started. Nitrate was added to the feed to stimulate the denitrification process so that the performance under anoxic/anaerobic (denitrifying/methanogenic) conditions could be monitored and combined sludge could be obtained.

The reactor was inoculated with 8 l sludge coming from a pilot scale UASB reactor treating domestic wastewater. This sludge had low methanogenic activity

Reactor	Type of wastewater	Organic C removal	Nitrogen removal	Reference
UAF*	Methanol + nitrate	95–98% of COD	100% of nitrate added	[7]
UAF	Synthetic WW + nitrate	99% of COD	100% of nitrate	[6]
UASB**	Synthetic WW + nitrate	99% of COD	99% of nitrate added	[8]
SBR***	Piggery WW + nitrate	81–91% of TOC	85–91% of TKN	[9]
USBF****	Industrial anaerobic. effl. + NO3	80% of COD	100% of nitrate added	[10]
UASB	Rice wastewater	Unknown	80% 0f TKN	[11]

\*UAF (upflow anaerobic filter)

\*\* UASB (upflow anaerobic sludge blanket reactor)

\*\*\*SBR (sequential batch reactor)

\*\*\*\*USBF (upflow sludge bed filter)

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(0.006 g  $CH_4$ -COD/gVS/d) and relatively high density (84.5 VS/l)

In the first stage (methanogenic) the reactor was fed with synthetic complex wastewater stoichiometrically calculated to be 1.5 g COD/l according the typical COD of low water use sanitation systems. The synthetic wastewater consisted of a solution of organic substrate, macronutrients, micronutrients, phosphate-buffer and yeast extract. The substrate was made of acetate, propionate and glucose (2:1:1, based on COD). This substrate was selected to stimulate the rich amalgam of processes involved in the anaerobic digestion of complex soluble substrates (e.g. acidogenesis, acetogenesis and methanogenesis). Once the reactor reached stable performance under the anaerobic conditions about 57 mg NO<sub>3</sub>-N were added to each litre of feed, giving a COD/ NO<sub>3</sub>-N of 26. Added nitrate was calculated based on the hypothesis that 20% of the COD given in the feed will be consumed by denitrifiers and the extra COD by methanogens assuming a denitrifyers yield  $(Y_{\rm D})$  of 0.5.

The operational conditions of the UASB in the stable anaerobic stage (methanogenic UASB) and in the anaerobic/anoxic stage (methanogenic/denitrifying UASB) are summarised in Table 2.

In order to make C and N mass balances and removal efficiencies calculations for the reactor the following analyses were frequently performed: [COD] influent, [COD] effluent, gas production and composition, [acetate] and [volatile fatty acids] influent and effluent and VSS effluent. Measurements of pH (pH meter WTW inolab) and temperature (thermometer Jenway) were also carried out. UASB sludge analysis such as methanogenic activity tests, denitrification activity tests and TS and VS of the sludge in the reactor at different heights (sludge profile) were also performed.

## 3. Results and discussions

Measurements on the methanogenic USAB from day 220 on showed that the reactor was stable and good

#### Table 2

Operating conditions of the UASB reactor

Period	Methanogenic UASB	Meth/Den. UASB
Temperature, °C	27.2	27.2
Flow rate, l/d	40	40
Vup, m/h	0.21	0.21
HRT*, h	10.8	10.8
OLR**, kg	3.3	3.3
COD <sub>sol</sub> /m <sup>3</sup> /d		
NLR***, kg N/m³/d	0	0.122

\*Hydraulic retention time

\*\*Organic loading rate

\*\*\* Nitrogen loading rate

methanogenic performance was achieved. Removal efficiencies up to 92, 97 and 96% were reached for soluble COD, acetate and total VFA respectively. Analyses of samples taken from the bottom of the reactor showed high methanogenic activity of the sludge (1.4 gCH<sub>4</sub>-COD/g VS/d). Surprisingly the sludge had also denitrifying capacity (0.05 g NO<sub>3</sub><sup>-</sup>-N/gVS/d).

Presence of denitrifying activity in anaerobic sludge might look surprising at the first sight. Nevertheless, this fact has been observed previously by other researchers [3,5,8].Pulses of oxygen might occur in the "anaerobic" reactors giving facultative aerobes like denitrifiers an advantage. In addition, denitrifying activity has been observed in anaerobic nitrate-free sediments [12]. The long-term survival of these denitrifying organisms in such conditions has been explained by their ability to perform low levels of anaerobic fermentation for their maintenance [12]. Moreover, there is a great number of organisms with ability to denitrify that have not been yet cultured and identified as shown in the bacteriological community analysis of a denitrifying reactor performed by [13]. The sludge he analyzed presented high



Fig. 2. Nitrate removal efficiencies and nitrate influent and effluent concentrations in the methanogenic/denitrifying reactor.









Fig. 3. Acetate, total VFA and  $\text{COD}_{sol}$  measured in the influent and effluent of the UASB and their corresponding removal efficiencies % R (expressed as a percentage of the influent concentration). The separation line indicates the start of addition of nitrate to the feed.

denitrifying activity but relatively low number of known denitrifying bacteria.

Whatever the reason, it seems that, in practice, the presence of organisms capable to denitrify in anaerobic sludge is the rule rather than the exception. Therefore, the start up of a methanogenic/denitrifying reactor could be done with any methanogenic sludge with no need of inoculation with denitrifying sludge.

Denitrification started immediately after starting adding nitrate to the feed on day 231 and was almost complete from the very beginning (Fig. 2). First two days after  $NO_3^-$  addition nitrate removal efficiency was 100% and averaged 97.5% onwards. Denitrification activity of the biomass developed was enough to cope with the nitrogen loading rate applied (0.122 kg N/m<sup>3</sup>/d)

Fig. 3 shows the acetate, total volatile fatty acids and  $\text{COD}_{\text{soluble}}$  removal efficiencies of the UASB before and after the addition of nitrate. The day  $\text{NO}_3^-$  was added to the reactor there was a temporal drop in the removal of  $\text{COD}_{\text{sol}}$  (up to 76.3%), VFA (up to 69.8%) and acetate (up to 66.7%) indicating that the biodegradation of these compounds was affected by the addition of nitrate. None-theless, from 2 days onwards after nitrate addition removal efficiencies were completely recovered reaching values for  $\text{COD}_{\text{sol}}$  and acetate of 91.9% and 90.6% respectively.

The limited amount of nitrate added to the reactor should have consumed just a small amount of COD through denitrification. Extra COD should have been completely consumed through methanogenesis. This was not the case during two days after starting adding nitrate indicating a toxic effect on methanogenesis. The toxicity shock on methanogenesis is further suggested by the temporal drop in the methane percentage of the biogas produced when nitrate was added to the feed (Fig. 4)

Inhibition of methanogenesis due to eventual peaks of nitrite and other denitrification intermediates could play an important role in the drop of the methanogenesis activity. Temporal accumulation of denitrifiaction intermediates (NO<sub>2</sub>, NO and N<sub>2</sub>O) has been identified as the main inhibitory mechanism of denitrification on methanogenesis [4,14,15]. In addition, the expected increase in the redox potential could also have an impact on the methanogenesis.

Two days after denitrification start up the reactor reached rapidly again stable performance with very high removals of nitrate, acetate and COD<sub>sol</sub>. That means a very fast adaptation of the biomass and/or the reactor to the new conditions. The reactor was likely performing as a plug flow reactor. Then, within two days after nitrate addition most of the nitrate and other N-oxides were removed in the bottom of the sludge bed while the top would be almost free of N-oxides. In addition the development of small denitrifying biofilms around the sludge flocks and the appearance of micro-niches may have favor the rapid adaptation of the reactor to the new conditions.

The high and stable removals reached of both  $\text{COD}_{sol}$  (91%) and nitrate (97.5%) and the high methane percentage in the biogas (73%) demonstrated that the integration of methanogenesis and denitrification in the UASB was successively achieved.

Regarding the proposed (anaerobic/anoxic)-(aerobic) sewage treatment system for water reclamation, a rapid denitrification start up is to be expected when nitrate is recycled to the UASB from the RBC in the non-growing season. The high nitrate and COD removal efficiencies obtained in the lab scale UASB at 27°C offer a promising future for the implementation of this technology in real conditions. Nevertheless, one should bear in mind that nitrate will be recycled to the UASB in the non-growing season, which is likely to be winter depending on the climate and crop. Therefore the plant should be designed for winter conditions. In addition, in the non-growing season, recirculation of nitrified wastewater from the RBC to the UASB reactor will modify the hydraulic behaviour of the UASB reactor and probably also the COD removal efficiency.

## 4. Conclusions

- Combination of methanogenesis and denitrification is possible in a UASB with flocculent sludge.
- Presence of denitrification activity in anaerobic sludge is the rule rather than the exception.



Fig. 4. Methane and nitrogen gas percentage in the gas collected from the UASB before and after the addition of nitrate to the feed. The separation line indicates the start of addition of nitrate to the feed.

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- Denitrification started up in the lab scale UASB immediately after starting feeding with nitrate. Complete denitrification was achieved from the first day of nitrate addition. Methanogenesis was negatively affected for two days after starting adding nitrate to the feed.
- Very high average removals of both nitrate (97.5%) and COD (91%) were achieved in the methanogenic/ denitrifying UASB at the tested operational conditions (27°C, OLR of 3.3 kg COD/m<sup>3</sup>/d, NLR of 0.122 kg N/m<sup>3</sup>/d and COD/NO<sub>2</sub><sup>-</sup>-N = 26)
- Therefore, there might be a great potential for applying the proposed technology in small agglomerations if the good performance of the system is proved in a long term pilot plant.

# References

- J.B. Van Lier and G. Lettinga, Appropriated technologies for effective management of industrial and domestic wastewater: the decentralized approach. Water Sci. Technol., 40 (1999) 171– 183.
- [2] A.J.B. Zehnder and W. Stumm, Geochemistry and biochemistry of anaerobic habitats. In A.J.B. Zehnder, ed., Biology of Anaerobic Microorganisms, Wiley-Intersciences, New York, 1998, pp. 1–38.
- [3] J.C. Akkuna, C. Bizeau and R. Moletta, Denitrifcation in anaerobic digesters: possibilities and influence of wastewater COD/ N-NO, ratio. Environ. Technol., 13(9) (1992) 825–836.
- [4] K.C. Chen and Y.F. Lin, The relationship between denitrifying bacteria and methanogenic bacteria in a mixed culture system of acclimated sludges. Water Res., 27(12) (1993) 1749–1759.

- [5] J.C. Akkuna, C. Bizeau and R. Moletta, Nitrate and nitrite reductions with anaerobic sludge using various carbon sources: glucose, glycerol, acetic acid, lactic acid and methanol. Water Res., 27(8) (1993) 1303–1312.
- [6] J.C. Akkuna, C. Bizeau and R. Moletta, Nitrate reduction by anaerobic sludge using glucose at various nitrate concentrations: ammonification, denitrification and methanogenic activities. Environ. Technol., 15(1) (1994) 41–49.
- [7] K. Hanaki and C. Polprasert, Contribution of Methanogenesis to Denitrification with an Upflow Filter. Water Pollution Control Fed, 61, 1989.
- [8] V.H. Hendriksen and K. Ahring, Integrated removal of nitrate and carbon in an upflow anaerobic sludge blanket (UASB) reactor: operating performance. Water Res., 30 (1996) 1451–1458.
- [9] N. Bernet, J.-P. Delganes and R. Moletta, SBR as a relevant technology to combine anaerobic digestion and denitrification in a single reactor. Water Sci. Technol., 43 (2001) 209–214.
- [10] A. Mosquera-Corral, M. Sanchez, J.L. Campos, R. Méndez and J.M. Lema, Simultaneous methanogenesis and denitrification of pretreted effluents from a fish canning industry. Water Res., 35 (2001) 411–418.
- [11] L.F. Lopes, P.R. Koetz and M.S. Santos, Denitrification on the top of UASB reactors of rice wastewaters. Water Sci. Technol., 44 (2001) 79–82.
- [12] K. Jorgensen and J.M. Tiedje, Survival of denitrifiers in nitrate free anaerobic environments. Appl. Environ. Microbiol., 59 (1993) 3297–3305.
- [13] C. Etchebehere, M.I. Errazquin, P. Dabert and L. Muxí, Community analysis of denitrifying reactor treating landfill leachate. Microbiol. Ecol., 40 (2002) 97–106.
- [14] M. Quevedo, E. Guynot and L. Muxí, Denitrifying potential of methanogenic sludge. Biotechnol. Lett., 18 (1996) 1363–1368.
- [15] R. Roy and R. Conrad, Effect of methanogenic precursors (acetate, hydrogen, propionate) on the suppression of methane by nitrate in anoxic rice field soil. Microbiol. Ecol., 28 (1999) 49– 61.

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