Autotrophic and heterotrophic denitrification for simultaneous removal of nitrogen, sulfur and organic matter

LORNA GUERRERO[1], JUAN P. AGUIRRE[1], MARIA A. MUÑOZ[1], ANDREA BARAHONA[1], CESAR HUILIÑIR[2], SILVIO MONTALVO[2] and RAFAEL BORJA[3]*

[1] Department of Chemical and Environmental Engineering, Federico Santa Maria Technical University, Ave. España 1680, Valparaiso, Chile

[2] Department of Chemical Engineering, Santiago of Chile University, Ave. Lib. Bernardo O'Higgins 3363, Santiago de Chile, Chile

[3] Instituto de la Grasa (CSIC), Campus Universitario Pablo de Olavide, Edificio 46, Ctra. de Utrera km 1, 41013-Sevilla, Spain.

*Address correspondence to Rafael Borja (Instituto de la Grasa (CSIC), Campus Universitario Pablo de Olavide, Edificio 46, Ctra. de Utrera km 1, 41013-Sevilla, Spain. Phone: +34 954 611550; Fax: +34 954 616790;

E-mail: rborja@cica.es

Abstract

The aim of this investigation was to assess the start-up and operation of a laboratory-scale hybrid UASB-Anaerobic Filter Reactor (UASFB) of 1 L volume, kept at 30°C, in order to carry out a simultaneous autotrophic and heterotrophic denitrification process. First, the heterotrophic and autotrophic populations were separately enriched, with specific cultures and subsequently the UASFB was inoculated with 2 g/L of VSS, with a ratio of 1.5:1 (autotrophs : heterotrophs). The influent or synthetic wastewater used was composed of: $Na_2S_2O_3 \cdot 5H_2O$, CH_3COOK, $NaNO_3$, $NaHCO_3$, K_2HPO_4, NH_4Cl and saline solution. The concentrations varied depending on the organic loading rate (OLR), nitrogen loading rate (NLR) and sulfur loading rate (SLR) applied. In the UASFB reactor two experimental conditions were tested and assessed i) COD/N ratio of 3.6 and SLR of 0.75 kg S/m^3/d; ii) COD/N ratio of 5.8 and SLR of 0.25 kg S/m^3/d. The results obtained demonstrated that an inoculum coming from an anaerobic reactor was able to carry out the process, obtaining a maximum nitrate removal of 85.3% in the 1st stage of operation and 99.5% in the 2st stage. The recovery of sulfur in form of sulfate in the effluent did not present a tendency to stabilize during the measured time, with a maximum thiosulfate removal of 32.5%, when the SLR was lowered to 0.25 kg S/m^3/d. The maximum organic matter elimination, measured as COD, was 75.8%, which indicates the relative good performance and behavior of the heterotrophic microorganisms.

Keywords

Autotrophic denitrification; heterotrophic denitrification; hybrid UASB and filter reactor.

Introduction

The wastewaters generated for the food processing, cellulose, paper, textile industries, etc. are

rich in organic compounds, nitrogen compounds and sulfur. Due to their high organic content, they are generally treated in anaerobic systems, generating high concentrations of reduced sulfur such as sulfide, and ammonium. Additionally, the effluents from the anaerobic processes, in many cases, must be treated aerobically with the objective of reducing the organic load to levels permissible by legislation or to transform ammonium into nitrate and nitrite. In these processes, sulfide has a toxic effect over the nitrifying activity at concentrations superior to 0.5 mg/L. This, in turn, favors the growth of filamentous bacteria and the rupture of microbial floccules, incrementing operational costs due to the higher oxygen demand. [1]

In addition, the effluents from the aerobic systems must be subjected to denitrification in order to eliminate the produced nitrogen compounds (NO_2^-, NO_3^-). The heterotrophic denitrification is the conventional process for the elimination of mentioned compounds, and this process also reduces organic matter due to the high C/N ratio required for carrying out it. [2, 3] On the other hand, in the autotrophic denitrification not only the previously mentioned nitrogen compounds are eliminated, but also sulfur, since it is used in its reduced forms ($S_2O_3^{2-}$, S^0, H_2S) as an electron donor. [4-7] Therefore, it should be interesting to study the possibility of the coexistence of heterotrophic and autotrophic microorganisms in the same reactor for the joint elimination of organic matter, nitrogen and sulfur.

Biological removal of organic matter, nitrogen and sulfur is drawing research interest in search for an efficient and cost-effective wastewater treatment. While extensive work on separate removal of nitrogen and sulfur is well reported, research on simultaneous removal of nitrogen, sulfur and carbon has been scarcely documented. [8] Maintaining stable process control and reactor performance is another major challenge that hinders practical application of simultaneous nitrogen, sulfur and carbon removal.

Therefore, the main objective of this work was to develop an integrated technology, with a high efficiency and low cost, for the simultaneous removal of nitrogen and sulfur compounds in presence of organic matter. The specific objectives were firstly the enrichment of microbial populations of denitrifying heterotrophs and autotrophs separately in independent reactors, and,

secondly, the evaluation of the start-up and operation of a laboratory-scale hybrid UASB-filter bed (UASFB) reactor operated with synthetic wastewater and inoculated with both microbial populations enriched previously and operating simultaneously all together in the mentioned UASFB reactor.

Materials and methods

Assay of enrichment of denitrifying heterotrophic microorganisms

The experiment of enrichment of denitrifying heterotrophic microorganims was carried out in a reactor with a volume of 2.2 L, operated at 30±1°C. The reactor base used in this case was a UASB reactor whose upper section was filled with rasching rings, material that substituted the hood or internal settler used to separate solid, liquid and gas phases, transforming it into a hybrid UASB-filter bed (UASFB) reactor. The inoculum used came from a UASB reactor that was used to treat tobacco industry wastewater.

The synthetic water composition used in this assay was shown in Table 1. The following control parameters were tested three times a week: total and volatile suspended solids (TSS and VSS), nitrate, nitrite and COD. The operating conditions used, maintaining the C/N ratio of 5.5, [9] were:

- First stage: OLR of 1 kg COD/m^3/d and NLR of 0.045 kg N-NO_2^-/m^3/d.
- Second stage: OLR of 3 kg COD/m^3/d and NLR of 0.134 kg N-NO_2^-/m^3/d.

Assay of enrichment of denitrifying autotrophic microorganisms

This experiment was carried out in two SBRs (Sequencing Batch Reactors), with an effective volume of 1.5 L, which were stirred at 150 rpm and maintained at 30±1°C. One was inoculated

with sludge from an anaerobic lagoon that treats swine manure and the other one with sludge from a SBR used in the heterotrophic nitrification-denitrification from a yeast industry wastewater. The synthetic water composition used in this assay is shown in Table 2. The reactors were fed with nitrate and thiosulfate at concentrations of 198 mg NO_3^--N/L and 646 mg $S_2O_3^{2-}$ - S/L, respectively. The stoichiometry ratio of $S_2O_3^{2-}$- S/NO_3^--N was 2.5.

Assay of simultaneous autotrophic and heterotrophic denitrification

Once obtained the autotrophic and heterotrophic inocula in the above-mentioned separate reactors, it was carried out the start-up of the UASFB reactor for the assays of simultaneous autotrophic and heterotrophic denitrification. This UASFB reactor had a volume of 1 L, and operated at 30 ± 1°C. The following loading rates were defined and studied: organic loading rate (OLR), nitrogen loading rate (NLR) and sulfur loading rate (SLR). These loading rates were modified based on the results observed. The initial loading rates used were: OLR: 0.62 kg COD/m^3/d, NLR: 0.28 kg N/m^3/d and SLR: 0.75 kg S/m^3/d.

The inoculum used in this assay corresponded to the mixture of the two inocula previously obtained from both enrichments. The final concentration of the biomass inoculated was 2 g VSS/L, with a autotrophs: heterotrophs ratio of 1.5:1.0 (v/v). This ratio was based on the fact that the autotrophic microorganisms have lower growth rates than heterotrophic ones. For the same reason, the final concentration of the inoculum used was lower than usual due to the low generation of autotrophic sludge during its enrichment period.

A synthetic solution was used as substrate in this experiment. Its composition is shown in Table 3. The COD/N ratios used in the 1st and 2nd stages were 3.6 and 5.8, respectively, while the SLRs were 0.75 and 0.25 kg S/m^3/d, respectively.

Chemical analyses

Chemical oxygen demand (COD), volatile suspended solids and sulfate were analysed according to the closed digestion and colorimetric 5220D, 2540B and 4500-SO_4^{2-} methods, respectively of the Standard Methods for the Examination of Waters and Wastewaters [10]. pH was determined using a pH-meter model Crison 20 Basic. Nitrate, nitrite and ammonium nitrogen were determined by the 4500-NO_3^-, 4500-NO_2^- and 4500-ammonium standard methods respectively, using a Orbeco Hellige MC 500 colorimeter. Thiosulfate was analysed according to the method described by Harris. [11]

Results and discussion

Assay of enrichment of heterotrophic microorganisms

A stable denitrification activity was successfully achieved because the monitored parameters were maintained within the expected range. As an example of this is the pH, which value was in the range from 7.4 to 7.6. The average degradation percentage of organic matter during this experiment was 99.3%.

In relation to the nitrogen compounds (Figure 1), a high concentration of nitrite was observed at the beginning of the first stage, which decreased throughout the process. This suggests that a fraction of the nitrite content in the influent was transformed to nitrate. The ammonia in the effluent may have been generated by the own inoculum, as a product of the degradation of the dead microorganisms, from which NH_4^+ is produced. The conversions of nitrite and nitrate were 100% and 40.4%, respectively. When the OLR and NLR increased by 3 times in the second stage of this study, the nitrite removal efficiency was reduced to 38.5%, value lower than that obtained in the first stage, while the nitrate removal efficiency achieved a value of 38.8%, very similar to that reached in the first stage.

Nitrate reduction was consistently observed during the operation of a fluidized sand biofilter treating aquaculture effluents with average values of 26.9±0.9% removal efficiencies. [12] As can

be seen, these nitrate removal efficiencies were lower than those achieved in the present work. Heterotrophic denitrifying bacteria activities in the range of 0.9-1.5 mg NO_3^-/g VSS/h were also recently reported from granular sludge. [13]

A biomass concentration of 830 mg VSS/L was achieved in the reactor enriched with heterotrophic microorganisms at the end of the operation period, while the initial concentration added to the reactor was 3650 mg VSS/L. Therefore, heterotrophic denitrification due to endogenous metabolism could also have been occurred. This biomass was used as inoculum for the reactor with simultaneous heterotrophic-autotrophic denitrification.

Assay of enrichment of autotrophic microorganisms

The microbial growth (measured as VSS) obtained from the reactor whose inoculum was sludge from an anaerobic lagoon that treats swine manure is shown in Figure 2. When contrasting these results with those obtained by Fajardo, [14] it was observed that even though the initial inoculum concentration was higher in the present study, the variation of the VSS with time was very similar in both studies, obtaining a decrease in the VSS content in the same period of time. However, this decrease was more evident in the present study, because in the other experiment carried out by Fajardo [14] there was a decrease that finally ended in approximately 40% of the initial biomass, while in the present study a value even lower than 25% of the initial biomass was achieved. Finally, the concentration of VSS varied between 300 and 1400 mg/L.

The observed behavior makes sense, since the inoculum contained a certain variety of microbial species adapted to the environment from which it came from (an anaerobic biodigester, where the processes are heterotrophic), conditions very different to which they are exposed in the autotrophic enrichment reactor. However, a higher enrichment was obtained with this inoculum than with the other reactor inoculated with sludge from SBR in which the final decrease was less than 15% the initial VSS. In addition, the denitrification activity observed with this inoculum was also higher. For these reasons, only the sludge from the anaerobic reactor was used in the start-up of the hybrid UASFB reactor for simultaneous autotrophic and heterotrophic

denitrification.

Since the application of autotrophic denitrification to wastewater could be limited by the low biomass growth rate, reactor systems with good biomass retention like those based on membrane separation [15] or biofilm technologies [16, 17] have been also used. Low sludge production was recently reported in an autotrophic denitrification process using thiosulfate as an electron donor at concentrations up to 800 mg N/L. [18] In this case, denitrification required a S/N ratio of between 2.5-5.1 for complete nitrate removal.

Due to the above-mentioned reasons, the following results correspond only to the reactor inoculated with the sludge from the anaerobic digester. With regards to the elimination of nitrogen compounds, Figure 3 shows the variations of the effluent concentrations of these compounds with time. As can be observed, all the nitrite was eliminated (the final amounts were so low, that they were not detected by the analytical method used) as well as nitrate, except at the beginning of the assay where there exists accumulation, but afterwards the removal was stable and of 100%. This was to be expected, because it was the limiting compound.

The reaction that is carried out in this process utilizes thiosulfate as an electron donor, therefore, for the reduction of nitrate to occur, thiosulfate must be consumed and sulfate produced, which can be observed in Figure 4. This autotrophic denitrification reaction can be described as follows: [19]

$$5\ S_2O_3^{-2} + 8\ NO_3^- + H_2O \rightarrow 10\ SO_4^{-2} + 4\ N_2 + 2\ H^+$$

In this case, and given the presented results in Figure 3, a higher decrease of thiosulfate concentration was expected. For the first days the average thiosulfate removal achieved was 66.8% and after day 30 a 53.3% removal was reached, a low value considering that starting at day 43 a total elimination of nitrate was obtained. However, it must be taken into account that in this case thiosulfate was added in an excess of 12%. Even so, a low thiosulfate concentration decrease was still observed. The sulfate production was in accordance with what was expected by stoichiometry of the process.

Assay of simultaneous heterotrophic and autotrophic denitrification

This experiment was divided in two stages. The SLRs, OLRs and NLRs tested in each stage were shown in Table 4. The degradation of the organic matter reached a maximum of 75.8% for the operating conditions of the second stage. The COD of the effluent was relatively constant and with the stabilization of the system, the removal of the organic matter increased.

With regards to the degradation of nitrate, the average removal was 80.4%, reaching a maximum of 99.5% in the second stage (Figure 5A). Hence, with sufficiently incubated symbiotic heterotrophs and autotrophs and unlimited nitrate supply, it is possible to achieve simultaneous removal of nitrogen ($NO_3^- \rightarrow N_2$), sulfide ($S_2^- \rightarrow S_0$), and carbon (acetate $\rightarrow CO_2$) in the same reactor. [20, 21]

Sulfur compounds present the issue that they are the substrates of autotrophic microorganisms, which do not have the same capacity of adaptation as their heterotrophic peers. This was also evidenced during the enrichment of these organisms, when the thiosulfate consumption was less than expected. With regards to this last compound, it can be observed in Figure 5B that there are a difference between the experimental values and the corresponding theoretical ones, and did not present a significant variation with varying conditions (when SLR decreased from 0.75 to 0.25 kg $S/m^3/d$). It was observed that the decrease in thiosulfate concentration reaches a maximum value of 32.5% during the second stage, a lower value than that was obtained during the autotrophic microorganism enrichment stage (53.3%). It has been previously reported that the presence of sulfides inhibits denitrification at concentrations of 0.5 mg S^{2-}/L, [20] compound that may have been present in the environment. This, in turn, would favor the growth of filamentous bacteria and the rupture of microbial floccules, hindering thiosulfate removal. In addition, it is well known that sulfide reacts with the iron from cytochromes inhibiting the respiration. [21, 22] Moreover, it is corrosive and possesses a high chemical oxygen demand. [22]

However, higher thiosulfate consumptions were reported in the research works of Chen et al. [21] and Reyes-Ávila et al. [22], who worked with an EGSB and CSTR, respectively. As can be observed in Table 5, these researchers reached maximum removals close to the maximum value,

244 although in the case of the CSTR higher HRT and lower OLR values than those used in the
245 present work were assayed.
246 Total nitrogen removal of up to 68% was reported in a four-stage rotating biological contactor,
247 which was designed and operated to treat synthetic wastewater (COD: 1000 mg/L: 112 mg
248 NH_4^+/L), observing the presence of autotrophic and heterotrophic denitrifiers in the mixed
249 bacterial biomass. [23] This nitrogen removal efficiency was lower than that achieved in the
250 present work (99.5%).
251
252
253 **Conclusion**
254
255 An innovative biotechnological system to eliminate nitrogen and sulfur in the presence of
256 organic matter with low comparable cost has been implemented and set out.
257 The process of inoculum enrichment showed that the sludge coming from an anaerobic reactor
258 obtained the best performance, especially in autotrophic denitrification, being this biomass able
259 to develop the simultaneous autotrophic and heterotrophic process proposed.
260 The simultaneous autotrophic and heterotrophic denitrification in a hybrid UASFB reactor
261 reached a maximum nitrate removal of 85.3% in the 1st stage of operation (COD/N ratio of 3.6
262 and SLR of 0.75 kg S/m3/d). However, in the second stage (COD/N ratio of 5.8 and SLR of
263 0.25 kg S/m^3/d), the efficiency of nitrate removal in the reactor was 99.5%.
264 The recovery of sulfur in form of sulfate in the effluent did not present a tendency to stabilize
265 during the measured time. The maximum thiosulfate removal percentage reached was 32.5%,
266 when the SLR was lowered to 0.25 kg S/m^3/d.
267 The maximum organic matter elimination, measured as COD, was 75.8%, which indicates the
268 relative good performance and behavior of the heterotrophic microorganisms.
269
270

Acknowledgements

The authors wish to express their gratitude to FONDECYT, Project No. 1130108 (Chile), for its financial support.

References

[1] Valdés, F.; Muñoz, E.; Chamy, R.; Ruiz, G.; Vergara, C.; Jeison, D. Effect of sulphate concentration and sulphide desorption on the combined removal of organic matter and sulphate from wastewaters using expanded granular sludge bed (EGSB) reactors. Electron. J. Biotechnol. **2006**, *9*, 370-378.

[2] Kim, E.W.; Bae, J.H. Alkalinity requeriments and the possibility of simultaneous heterotrophic denitrification during sulphur-utilizing autotrophic denitrification. Water Sci. Technol. **2000**, *42*(3-4), 233-238.

[3] De la Rua, A.; Rodelas, B.; González-López, J.; Gómez, M.A. Submerged filter biofilm formation by nitrate-contaminated groundwater microbiota. J. Environ. Sci. Health A **2011**, *46*(10), 1113-1121.

[4] Wang, H.; Qu, J. Comparison of two combined bioelectrochemical and sulfur autotrophic denitrification processes for drinking water treatment. J. Environ. Sci. Health A **2011**, *38*(7), 1269-1284.

[5] Moon, H.S.; Ahn, K.H.; Lee, S.; Nam, K.; Kim, J.Y. Use of autotrophic sulphur-oxidizers to remove nitrate from bank filtrate in a permeable reactive barrier system. Environ. Pollut. **2004**, *129*, 499-507.

[6] Kellermann, C.; Griebler, C. Thiobacillus thiophilus sp. nov., a chemolithotrophic, thiosulphate-oxidizing bacterium isolated from contaminated aquifer sediments. Int. J. Systematic Evolution. Microbiol. **2009**, *59*, 583-588.

[7] Han, G.B.; Park, J.K. Using porous ceramic media in the upflow packed-bed reactor (UPBR) system for nitrogen removal via autotrophic nitrification and denitrification. J. Environ. Sci. Health A **2012**, *47*(5), 786-793.

[8] Show, K.Y.; Lee, D.J.; Pan, X. Simultaneous biological removal of nitrogen-sulfur-carbon: Recent advances and challenges. Biotechnol. Adv. **2013**, *31*, 409-420.

[9] Vásquez, M. Desnitrificación vía nitrito en un reactor UASB utilizando zeolita chilena. Master Thesis of Chemical Engineering, Universidad Técnica Federico Santa María, Chile, 2010.

[10] American Public Health Association (APHA). *Standard Methods for the Examination of Water and Wastewater,* 20*th* ed.; American Public Health Association/American Water Works Association/Water Environment Federation, Washington DC, USA, 1998.

[11] Harris, D.C. Análsis químico cuantitativo, 3a, Reverté Ed., Barcelona, Spain, 2001; ISBN: 10: 8429172246.

[12] Tsukuda, S.; Christianson, L.; Kolb, A.; Saito, K.; Summerfelt, S. Heterotrophic denitrification of aquaculture effluent using fluidized sand biofilters. Aquacul. Eng. **2015**, *64*, 49-59.

[13] Ke, Y.; Azari, M.; Han, P.; Gu, J.D.; Denecke, M. Microbial community of nitrogen-converting bacteria in anammox granular sludge. Int. Biodeterior. Biodegrad. **2015**, *103*, 105-115.

[14] Fajardo, M.,. Autotrophic denitrification for treatment of wastewater with high concentration of sulphur and nitrogen compounds. Ph.D. Thesis, Universidad de Santiago de Compostela, Spain, 2011.

[15] McAdam, E.J.; Judd, S.J. A review of membrane bioreactor potential for nitrate removal from drinking water. Destilation **2006**, *196*, 135-148.

323 [16] Soares, M.I.M. Denitrification of groundwater with elemental sulphur. Water Res. **2002**, *36*,
324 1392-1395.
325 [17] Sierra-Alvarez, R.; Bersitain-Cardoso, R.; Salazar, M.; Gómez, J.; Razo-Flores, E.; Field,
326 J.A. Chemolithotrophic denitrification with elemental sulfur for groundwater treatment.
327 Water Res. **2007**, *41*, 1253-1262.
328 [18] Chung, J.; Amin, K.; Kim, S.; Kwon, K.; Bae, W. Autotrophic denitrification of nitrate and
329 nitrite using thiosulfate as an electron donor. Water Res. **2014**, *58*, 169-178.
330 [19] Tandukar, M.; Pavlostathis, S.; Cervantes, F. Autotrophic denitrification for the removal of
331 nitrogen and sulphur compounds contaminants from wastewaters. Environmental
332 Technologies to Treat Nitrogen Pollution **2009**, *12*, 319-365.
333 [20] Aguirre, J.P. Diseño y puesta en marcha de un reactor UASB híbrido para la
334 desnitrification simultánea autotrofa y heterotrofa. Master Thesis of Chemical Engineering,
335 Universidad Técnica Federico Santa María, Chile, 2014.
336 [21] Chen, C.; Ren, N.; Wang, A.; Yu, Z.; Lee, D. Simultaneous biological removal of sulfur,
337 nitrogen and carbon using EGSB reactor. Appl. Microbiol. Biotechnol. **2008**, *78*, 1057-
338 1063.
339 [22] Reyes-Avila, J.; Razo-Flores, E.; Gomez, J. Simultaneous biological removal of nitrogen,
340 carbon and sulfur by denitrification. Water Res. **2004**, *38*, 3313-3321.
341 [23] Singh, V.; Mittal, A.K. Characterization of biofilm of a rotating biological contactor treating
342 synthetic wastewater. Water Sci. Technol. **2012**, *66*, 429-437.
343
344

FIGURE CAPTIONS

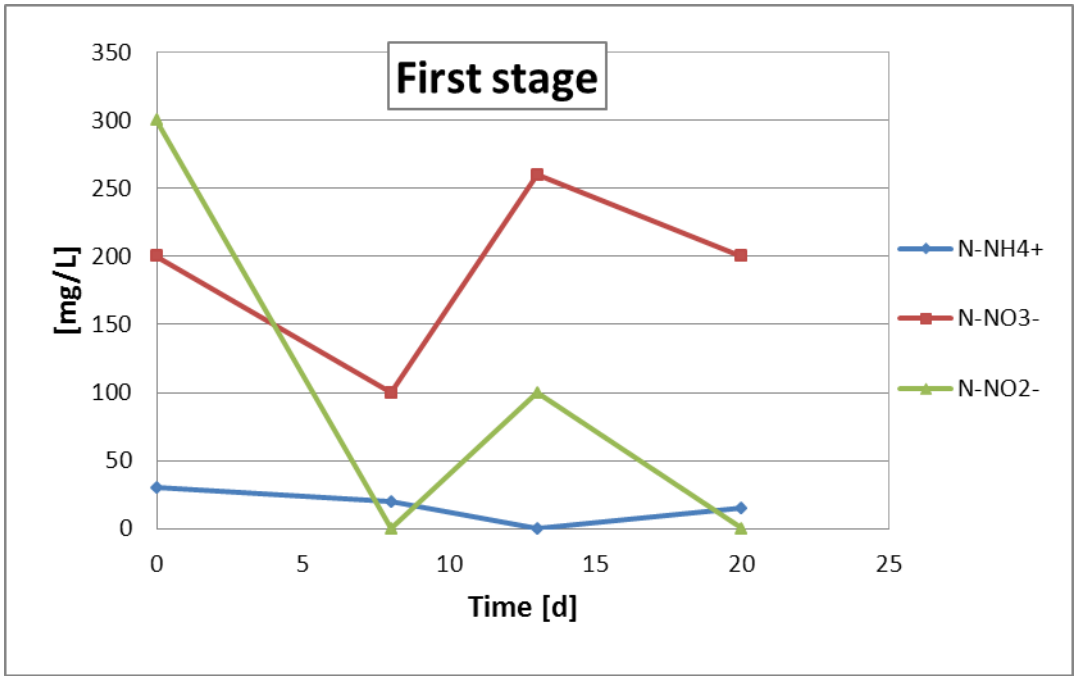
Figure 1. Variation of nitrogen compounds with time in the effluent of the hybrid UASFB reactor during the enrichment of heterotrophic microorganisms.

Figure 2. Variation of the volatile suspended solids (VSS) concentration in the denitrifying autotrophic reactor.

Figure 3. Variation of the effluent nitrate and nitrite concentrations with time in the autotrophic enrichment reactor.

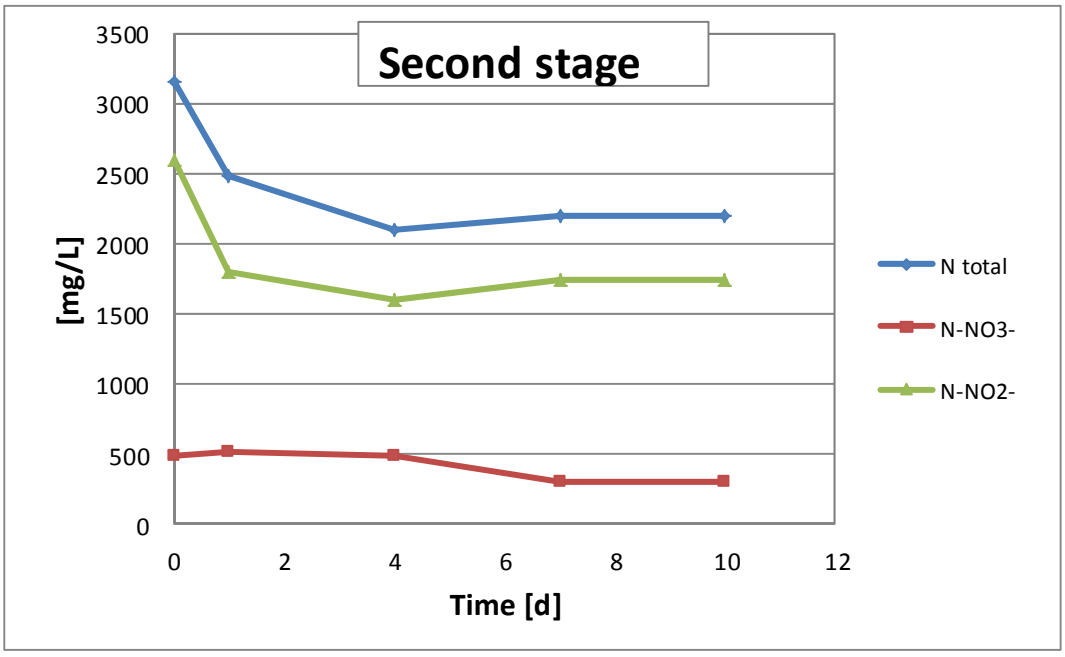
Figure 4. Variation of the effluent sulfate concentration with time in the autotrophic enrichment reactor.

Figure 5. Variation of nitrogen compounds (A) and thiosulfate (B) with time in the first (I) and second (II) stage of the simultaneous autotrophic and heterotrophic denitrification carried out in the hybrid UASFB reactor.



372

373



374

375

376 **Fig. 1**

377

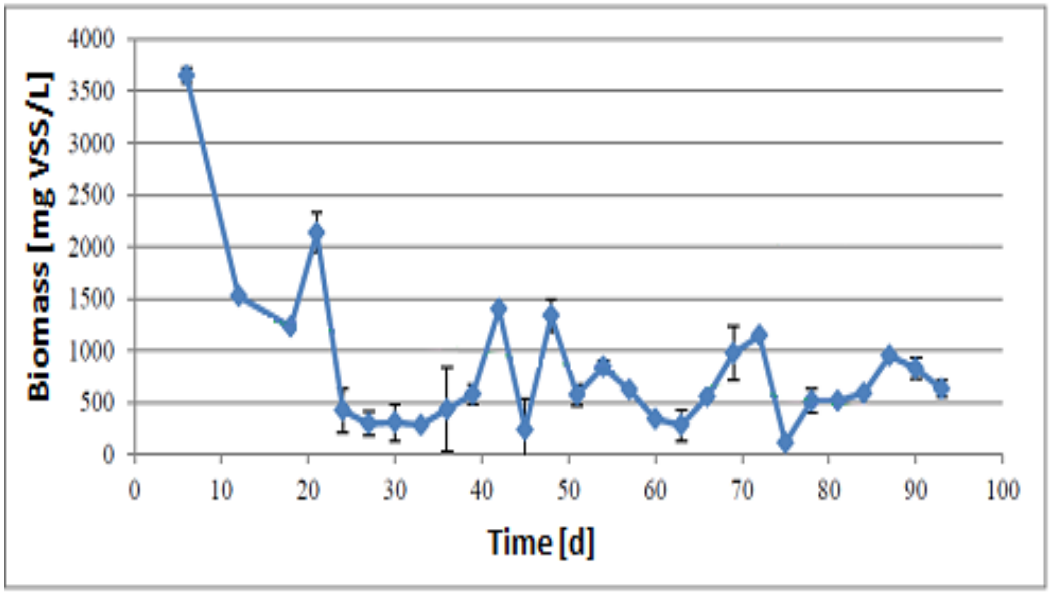
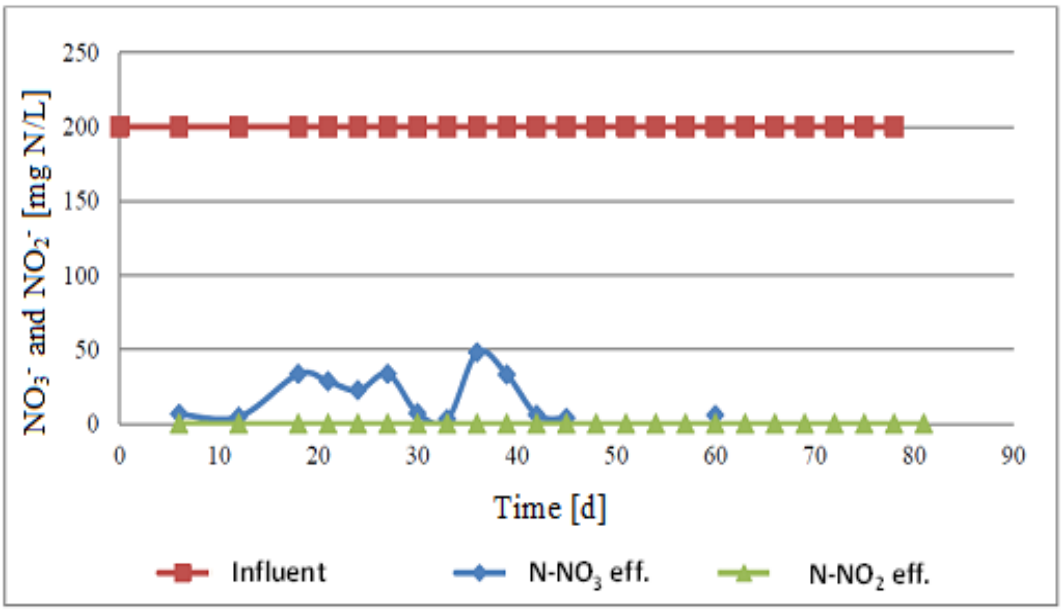

Biomass [mg VSS/L]
4000
3500
3000
2500
2000
1500
1000
500
0
0
10
20
30
40
50
60
70
80
90
100
Time [d]

Fig. 2

382



383

384 **Fig. 3**

385

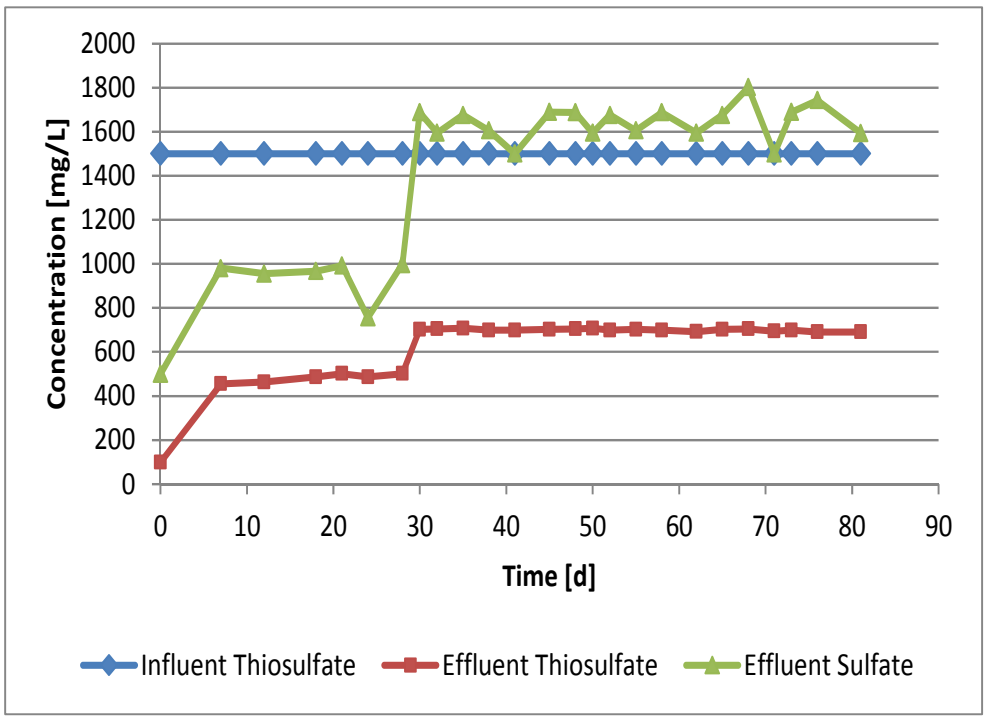
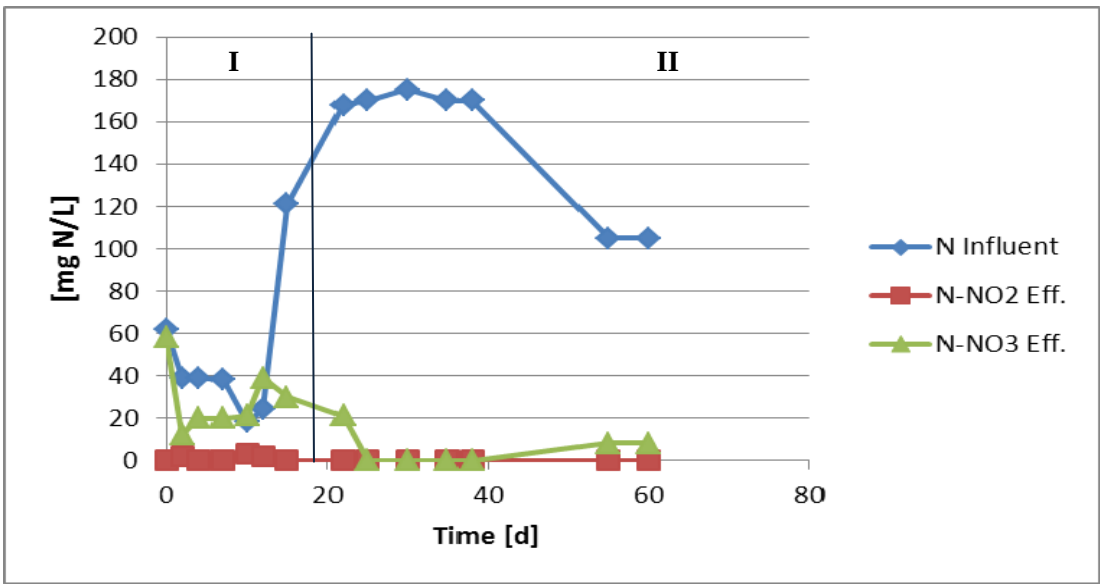
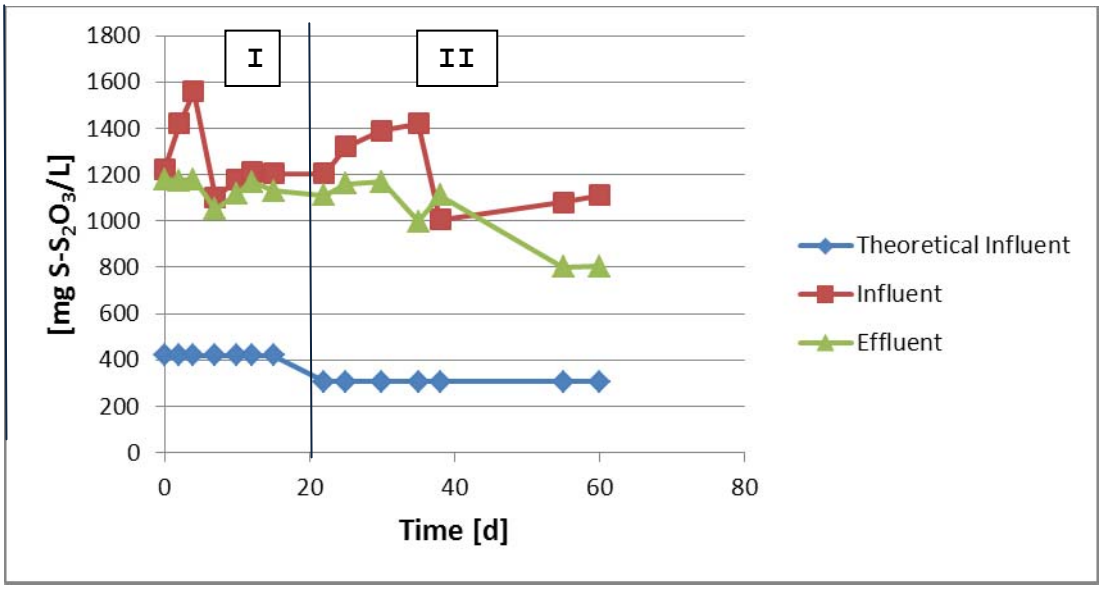

Concentration [mg/L]
2000
1800
1600
1400
1200
1000
800
600
400
200
0
0
10
20
30
40
50
60
70
80
90
Time [d]
Influent Thiosulfate
Effluent Thiosulfate
Effluent Sulfate

Fig. 4


I
II
[mg N/L]
Time [d]
N Influent
N-NO2 Eff.
N-NO3 Eff.

A


I
II
[mg S-S2O3/L]
Time [d]
Theoretical Influent
Influent
Effluent

B

Fig. 5

422

423 **Table 1.** Synthetic water composition used for the enrichment of heterotrophic microorganisms.

Compound	Unit	First stage	Second stage	Saline solution	
CH_3COOK	g/L	63.8	51.0	Compound	Concentration
$NaNO_2$	g/L	14.0	11.2	EDTA, g/L	0.15
Yeast Extract	g/L	2.0	1.6	HCl, mL/L	1.0
Na_2CO_3	g/L	10.0	8.0	$FeSO_4$, g/L	2.0
K_2HPO_4	g/L	31.6	25.3	HBr, µL/L	70
KH_2PO_4	g/L	25.0	20.0	$ZnCl_2$, g/L	0.05
Saline solution	mL/L	13.5	10.8	$MgCl_2$, g/L	0.05

424

425

426

427 **Table 2.** Culture medium used in the assay of enrichment of denitrifying autotrophic bacteria.

Compound	(g/L)	Micronutrient solution			
$Na_2S_2O_3 \cdot 5H_2O$	5.0	Compound	(g/L)	Compound	(g/L)
$NaNO_3$	1.2	$Na_2MoO_4 \cdot 5H_2O$	0.73	$CuCO_4 \cdot 5H_2O$	0.25
$NaHCO_3$	1.5	$FeSO_4 \cdot 7H_2O$	30.0	$CoCl_2 \cdot 6H_2O$	0.25
Na_2HPO_4	1.5	$ZnCl_2 \cdot 4H_2O$	1.0	$NiCl_2 \cdot 6H_2O$	0.25
KH_2PO_4	0.3	$CaCO_3$	2.0	H_3BO_3	0.50
NH_4Cl	0.1	$MnCl_2 \cdot 4H_2O$	1.5	HCl (32%)	50.0
Micronutrient solution (mL/L)	1.0				

428

429

Table 3. Synthetic water composition used as feed of the reactor with simultaneous autotrophic and heterotrophic inocula.

Compound	Unit	First stage	Second stage	Saline solution (g/L)	
$Na_2S_2O_3 \cdot 5H_2O$	g/L	1.78	1.19	Compound	Concentration
$NaNO_3$	g/L	1.06	1.06	$ZnSO_4$	15.65
CH_3COOK	g/L	0.634	1.02	$CaCl_2$	22.00
$NaHCO_3$	g/L	3.0	3.00	$MnCl_2 \cdot 4H_2O$	20.24
NH_4Cl	g/L	0.056	0.056	$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	4.40
K_2HPO_4	g/L	0.07	0.07	$CuSO_4$	4.00
Saline solution	mL/L	5	5	$CoCl_2 \cdot 6H_2O$	6.44

435

436 **Table 4.** Loading rates tested in the two stages of the autotrophic-heterotrophic denitrifying

437 reactor.

Loading rates	Unit	1st Stage	2nd Stage
SLR	kg $S/m^3/d$	0.75	0.25
OLR	kg $COD/m^3/d$	0.62	1.0
NLR	kg $N\text{-}NO_3^-/m^3/d$	0.28	0.28

438

439

Table 5. Comparison of the results obtained in different reactors with simultaneous autotrophic and heterotrophic denitrification.

Parameter	Reactor		
	UASBF*	EGSB	CSTR
HRT (h)	14.7	11.2	48
NLR (kg N/m^3/d)	0.28	1.45	0.209
SLR (kg S/m^3/d)	0.25	3.00	0.294
OLR (kg COD/m^3/d)	1.00	2.77	0.303
Maximum N removal	99.5	99.0	90.0
Maximum S removal	32.5	100	99.0
Maximum COD removal	76.0	90.0	69.0

*Present work