Potential of hybrid processes for nitrogen removal from swine wastewater

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Abstract: A comprehensive investigation was carried out using a bench-scale plant (70l) with the aim of evaluating the potential of two hybrid processes that use plastic moving carriers for nitrification and denitrification, given the restriction that low and stable values of ammonium and nitrate had to be met. In one process, the nitrification aerobic reactor operated with the biomass in biofilm exclusively and achieved a nitrification rate of 0.8 gN/m\(^2\)-d. In the other process, nitrification operated as a hybrid process and a similar nitrification rate in the biofilm was found. Batch tests revealed that suspended solids in the hybrid reactor provided an additional 20% nitrification rate. This resulted in a potential volumetric nitrification rate of 380 gN/m\(^3\)-d at 20ºC, which is 2.5 times the expected rate in activated sludge plants under the same conditions. A high anoxic fraction (55%) was used in both processes with hybrid denitrification, since it minimized inhibition of nitrification in the biofilm due to the biodegradable COD applied load. Batch tests undertaken with high suspended solids (4 g/l) revealed that 60% of the denitrification took place in the biomass in suspension, and 40% in biofilm, providing a high level and robust performance. The dosage of methanol was crucial in regulating effluent nitrate. It is concluded that both processes offer great potential regarding the design of more compact new plants and the upgrading of existing facilities.

Keywords: Hybrid denitrification and nitrification; moving carriers; nitrogen removal; swine wastewater

INTRODUCTION

Swine wastewater includes the urine, faeces and cleaning water from pig farms. This wastewater includes a high content of organic matter (30 – 70 gCOD/l) and nitrogen (2 – 6 gTKN/l) within a wide range of concentrations depending on the installation. Since soil application is the preferred means of disposal in Spain, swine wastewater treatment requires nitrogen removal to a greater or lesser extent depending on the specific soil requirements so that nitrate restrictions are met.

Biological predenitrification-nitrification in activated sludge processes is the most widespread technology option. The literature describes activated sludge processes that function under a wide range of operational conditions (Choi and Eum, 2002; Magrí et al., 2007). Among these, plants operated at moderate temperatures (about 20ºC) and at low nitrogen applied loads of 110 – 130 gN/m\(^3\)-d (Bicudo et al., 1999; Tilche et al., 1999) show low effluent ammonium and nitrite nitrogen concentrations, with estimated nitrification rates per aerobic cubic metre of 140 – 170 gN/m\(^3\)aerobic·d. Nevertheless, instabilities are frequently found. It should be taken into account that activated sludge processes present poor clarification characteristics, which reduce nitrifying biomass retention, thus favouring instabilities in nitrification. Advanced biofilm technologies have the capacity of retaining high concentrations of nitrifiers and can overcome this limitation. Different variants of the fluidized-bed processes have been investigated over many years in order to nitrify industrial wastewater (Sutton and Mishra, 1991). However its hydraulic complexity and associated costs, together with the difficulties found in achieving effective nitrification due to poor suspended solids clarification (Oyanedel et al., 2003), have limited its full-scale application. Recently in the urban wastewater field, a simple process, such as the moving-bed biofilm reactor that uses cylindrical plastic carriers, has shown a successful application for nitrogen removal at full-scale. New plants processes that use predenitrificacion and nitrification in exclusive biofilm reactors (Rusten et al., 2000) without sludge recycle (D-N process, Figure 1) have been successfully implemented, demonstrating nitrification rates clearly higher than those found in activated sludge plants. For upgrading existing plants, the addition of carriers to aerobic reactors (where the sludge recycle is maintained) has allowed the nitrification rate to be increased considerably (Christensson and Welander, 2004; Falletti and Conte, 2007) in the aerobic reactor. Thus, biomass in suspension...
and in biofilm coexist in, what is referred to as, hybrid nitrification reactor (D-HN, Figure 1). Denitrification takes place via heterotrophic biomass in suspension.

**Figure 1**: Processes using moving bed biofilm concept

Recently, research group CEIT (Larrea *et al.*, 2007) have proposed the configuration HD-N process (Figure 1) in which the secondary settler (including sludge recycle) is located between the anoxic and the aerobic reactor. Nitrification and denitrification rates are enhanced since hybrid denitrification (HD) takes place in the anoxic reactor while biofilm nitrification (N) takes place exclusively in the aerobic reactor. No application of these processes to swine wastewater has been found in the literature, so the study of their potential was considered very innovative. In the case of the D-HN process, the addition of carriers to the anoxic reactor was proposed in order to increase the denitrification rate (HD-HN process, Figure 1).

In summary, the objective of the research work was, first of all, to determine the nitrification and denitrification rates that HD-N and HD-HN processes are able to achieve, given the restriction that low and stable values of ammonium and nitrate are met; and secondly, to focus on assessing the effect of operational conditions on effluent ammonium and nitrites instabilities and the distribution of nitrifiers and heterotrophic biomass between biofilm and suspension.

**MATERIALS AND METHODS**

**Materials**
The swine wastewater used in the experimental tests was collected every 15 – 20 days from a tank feeding the influent to a SBR full-scale plant located in a nearby facility, Egiluze farm. The wastewater was stored in refrigerated tanks and showed average values around 1100 mgN/l in ammonium nitrogen (NH$_4$-N), 3500 mg/l in filtered COD and 4000 mg/l in TSS.

The bench-scale plant was made of transparent PVC and set up so that it could be adapted to both processes under study. In the first process (HD-N process) the anoxic and aerobic reactors had a volume of 44 and 38 litres, respectively, resulting in a high anoxic fraction, intending to minimize the effluent filtered COD from the anoxic reactor. Both reactors were filled with AMB biomedia carriers (Figure 2) with a specific surface (ss) of 500 m$^2$/m$^3$, a diameter (d) of 14 mm and a length (l) of 9 mm. The carriers occupied 50% of the reactors volume, thereby providing a biofilm surface of 250 m$^2$/m$^3$. A methanol dosage to the anoxic reactor was incorporated to reach the desired effluent N-NO$_3$.

To study the HD-HN process the plant was modified by installing the settler after the aerobic reactor. This adaptation reduced the volume of the anoxic and aerobic reactors to 36 and 30 litres, respectively. In addition, the AMB carriers were replaced by those from AnoxKaldnes.
In the anoxic reactor the K3 carrier (Figure 2; ss: 500 m$^2$/m$^3$, d: 25 mm, l: 10 mm) was introduced, occupying 50% of the reactor volume, which resulted in the same biofilm surface as above. In the aerobic reactor, the carrier BiofilmChipP (Figure 2; ss: 900 m$^2$/m$^3$, d: 45 mm, l: 3 mm) was added, resulting in 450 m$^2$/m$^3$ biofilm surface.

**Operational procedure**

The experimental studies for each process extended over several months covering temperatures from 15 to 25ºC. When nitrification was concerned, the dissolved oxygen (DO) in the aerobic reactor was always maintained at a level of 4-6mg/l. The first experimental objective was to determine the maximum ammonium nitrogen applied load to the aerobic reactor while producing low and stable values of ammonium and nitrite nitrogen (range 10-50mg/l). For this purpose, the influent flow rate was altered according to the ammonium concentration in the wastewater, until effluent ammonium or nitrite peaks were observed. This procedure provided a higher sensitivity of nitrification to the operational conditions and allowed the evaluation of their effect.

In the case of denitrification, the aim was to determine the denitrification rate of the anoxic reactor and to evaluate the effect of operational and performance conditions such as filtered COD removal, methanol dosage and mixed liquor suspended solids (MLSS) concentration. The target effluent N-NO$_3$ was fixed in the range of 150-200 mgN/l, and to achieve it the internal recycle ratio was estimated to be maintained at around 800 and 400% of the influent flow rate in HD-N and HD-HN processes, respectively. Sludge recycling from the settler was maintained at around 100%. Mixed liquor suspended solids levels were pre-fixed at around 1000 and 4000 mgTSS/l for the HD-N and HN-HN processes, respectively. This was carried out through regulation of sludge wastage.

**Experimental procedure**

To characterize the behaviour in every experimental period, grab samples were taken from the influent and reactors, and the following parameters were measured: total suspended solids (TSS), total and filtered COD, TKN, NH$_4$-N, NO$_2$-N, NO$_3$-N and alkalinity. Daily measurements of pH, temperature and dissolved oxygen in the aerobic reactor were taken.

Batch test were carried out during the HD-HN experimental study, in order to evaluate the activity of nitrifiers separately in both biofilm and in suspension, whilst maintaining DO at 8 mg/l, and pH at 8 through addition of alkalinity (AUR batch test). First of all, 40mg/l of NO$_2$-N (as NaNO$_2$) was added to determine the maximum removal rate of NO$_2$-N (over 30 minutes) caused by the concentration of Nitrite-Oxidizing Bacteria ($X_{NOB}$) present. An ammonium concentration (NH$_4$-N) of 35mgN/l (as NH$_4$Cl) was then added to determine both the maximum removal rate of NH$_4$-N by Ammonium-Oxidizing Bacteria ($X_{AOB}$) and the net rate of NO$_2$-N by $X_{NOB}$ calculated from NO$_2$-N variation.

Batch tests were also carried out to evaluate the activity of heterotrophic biomass ($X_H$) in both the aerobic and the anoxic reactors, and in both the biofilm and suspension. For this purpose, acetate or methanol in excess was added, respectively, in order to determine either the maximum
oxygen uptake rate (OUR batch test) or the denitrification rate (NUR batch test). Settling tests were undertaken to determine the sludge volume index (SVI) and the TSS in the supernatant after 30 minutes settling time (TSS$_{30}$). A comparison was made with the sludge taken from the Egiluze farm SBR process.

RESULTS AND DISCUSSION

Tables 1, 2 and 3 provide the average values of performance conditions in specific periods extracted from experimental studies carried out for both HD-N and HD-HN processes. Table 1, of wastewater characteristics, reveals first that alkalinity was not high enough to maintain the pH in the aerobic reactor above 7 due to the alkalinity consumption associated with nitrification. Consequently, NaHCO$_3$ was added to the wastewater tank in order to increase alkalinity by 700 mgCaCO$_3$/l. Because of the low COD/TKN ratio, methanol was added so as to meet the objective nitrate level (150 – 200 mgN/l).

Table 1: Characteristics of the influent swine wastewater (average ± standard deviation)

<table>
<thead>
<tr>
<th>Process</th>
<th>Total COD (mg/l)</th>
<th>Alkalinity* (mgCaCO$_3$/l)</th>
<th>Total TKN (mgN/l)</th>
<th>Filtered COD (mg/l)</th>
<th>NH$_4^+$-N (mgN/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HD-N (20ºC)</td>
<td>10650±1598</td>
<td>4292±711</td>
<td>1455±377</td>
<td>3962±1047</td>
<td>1011±145</td>
</tr>
<tr>
<td>HD-HN (17ºC)</td>
<td>7784±2079</td>
<td>4363±390</td>
<td>1439±114</td>
<td>3542±631</td>
<td>1088±85</td>
</tr>
<tr>
<td>HD-HN (23ºC)</td>
<td>8172±2756</td>
<td>5298±759</td>
<td>1457±189</td>
<td>3065±1027</td>
<td>1072±88</td>
</tr>
</tbody>
</table>

*Without NaHCO$_3$ addition

Table 2: Operational conditions (average ± standard deviation)

<table>
<thead>
<tr>
<th>Process</th>
<th>Q$_{IN}$ (l/d)</th>
<th>Q$_{IR}$ (%)</th>
<th>Total HRT (day)</th>
<th>K$_{NH4}$ applied load (gN/m$^3$ aero·d)</th>
<th>K$_{Methanol}$ applied load (gDQO/m$^3$ anox·d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HD-N (20ºC)*</td>
<td>5.2±0.8</td>
<td>862±138</td>
<td>16.0±2.3</td>
<td>149±35</td>
<td>179±132</td>
</tr>
<tr>
<td>HD-HN (17ºC)</td>
<td>7.0±2.2</td>
<td>475±37</td>
<td>8.8±0.7</td>
<td>290±58</td>
<td>154±74</td>
</tr>
<tr>
<td>HD-HN (23ºC)</td>
<td>10.0±3.1</td>
<td>401±57</td>
<td>6.4±1.1</td>
<td>390±70</td>
<td>277±104</td>
</tr>
</tbody>
</table>

*Values from period 2

Table 3: Performance characteristics (average ± standard deviation)

<table>
<thead>
<tr>
<th>Process</th>
<th>Anoxic Reactor</th>
<th>Aerobic Reactor - Effluent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FCOD (mg/l)</td>
<td>NO$_2^-$N (mgN/l)</td>
</tr>
<tr>
<td>HD-N*</td>
<td>559±90</td>
<td>41±45</td>
</tr>
<tr>
<td>HD-HN (17º)</td>
<td>494±99</td>
<td>40±33</td>
</tr>
<tr>
<td>HD-HN (23º)</td>
<td>601±93</td>
<td>54±42</td>
</tr>
</tbody>
</table>

*Values from period 2 after day 120
**Range
***In HD-N, TSS = 0.8 g/l in the anoxic reactor. In HD-HN, the same as in the aerobic reactor.

It is noted that the different parameters present a significant variability which was associated with the periodic collection of wastewater that took place every 2-3 weeks. Although that variability was partly compensated for by the changes in influent flow rate, the operational conditions in each experimental period also showed moderate variations (Table 2).

Table 3 presents the average results of parameters associated with the behaviour of nitrification and denitrification that is analysed in depth below. Regarding settling characteristics, it is noted that the degree of thickening (SVI ≈ 110 ml/g) and clarification (TSS$_{30}$ = 350 mg/l) of the MLSS from the HD-N process was similar to those results obtained from the SBR full-scale plant in Egiluze farm (SVI = 105 ml/g, TSS$_{30}$ = 310 mg/l). The HD-HN process presented a satisfactory
SVI (90 ml/g) and a better clarification degree (TSS$_{30} = 210$ mg/l), indicating an enhanced entrapment capacity of influent colloids into the flocs and biofilm as a whole.

**Nitrification in the HD-N process**

Two different performance periods can be distinguished (Figure 3) during the experimental study of the HD-N process. In period 1, instabilities in the behaviour of ammonium and nitrite in the effluent were observed, where the temperature was in the range of 16 – 19ºC. Those instabilities are closely associated with variations in ammonium applied load per aerobic cubic metre ($K_{\text{NH}_4}$). It is noted that the nitrite always took higher values (50 – 130 mg/l) than the ammonium (25 – 50 mg/l). In period 2 (average results in Tables 1, 2, 3) the temperature was in the range of 19 – 21ºC and the effluent ammonium and nitrite took, in general, very low values due to the low average ammonium applied load (150 gN/m$^3$·aero-d).

**Figure 3**: Variations of ammonium, nitrite and ammonium applied load in the aerobic reactor during the HD-N process

Only one small peak of nitrite (25 mg/l) was observed when the ammonium applied load increased to 225 gN/m$^3$·d. This seems to indicate that the HD-N process, operated at about 20ºC, is able to provide complete and stable nitrification for ammonium applied loads of about 200 gN/m$^3$·d, implying nitrification rates of the same order of magnitude. Taking into account that the biofilm surface ratio was 250 m$^2$/m$^3$, the nitrification rate expressed per biofilm square metre results in 0.8 gN/m$^2$·d. This is slightly higher than that found for domestic wastewater (Bengtsson et al., 2008), even though in the present case the filtered COD removed in the aerobic reactor amounted to around 100 mg/l (Table 3), causing inhibition of nitrification in the biofilm.

**Nitrification in the HD-HN process**

To analyse the effect of operational conditions a period of time was selected in each experimental phase, that corresponded to different temperatures (Figure 4). At 17ºC the process showed low and stable values of effluent ammonium and nitrite until the ammonium applied load ($K_{\text{NH}_4}$) reached 400 gN/m$^3$·d. At that point the effluent ammonium increased to 60 mgN/l, but nitrite did not appear. Operating at 23ºC and $K_{\text{NH}_4}$ of 500 gN/m$^3$·d, the process experienced some instabilities with ammonium peaks between 50 and 120 mg/l and associated effluent nitrite peaks at lower values (25-70 mg/l). This behaviour differs from that observed in the HD-N process, where the nitrite experienced an increase clearly higher than that of the ammonium. From an overall analysis it is deduced that at 20ºC the HD-HN process is able to achieve low and stable values of ammonium and nitrite for ammonium applied loads or nitrification rates of about 380 gN/m$^3$·d which takes place both in a biofilm surface ratio of 450 m$^2$/m$^3$ (compared to 250 in HD-N process) and in suspension. This value is about 2.5 times the rate in activated sludge plants under the same conditions.
Figure 4: Variations of effluent ammonium and nitrites with operational conditions in nitrification during the HD-HN process

Results from batch tests (Table 4 and Figure 5) carried out during the HD-HN process provide insight into the distribution of $X_{\text{AOB}}$ and $X_{\text{NOB}}$ in the biofilm (biof) and in suspension (susp), and the different behaviour observed in both processes regarding the appearance of ammonium and nitrite peaks, as follows.

Table 4: Average results of batch tests corrected at 20ºC

<table>
<thead>
<tr>
<th>Batch test</th>
<th>Rates (gN/m$^3$·d)</th>
<th>Biof</th>
<th>Susp</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUR</td>
<td>N-NO$<em>2$ removal rate by $X</em>{\text{NOB}}$ (a)</td>
<td>1100</td>
<td>220</td>
</tr>
<tr>
<td></td>
<td>N-NH$<em>4$ removal rate by $X</em>{\text{AOB}}$ (b)</td>
<td>480</td>
<td>150</td>
</tr>
<tr>
<td></td>
<td>N-NO$_2$ net balance (c)</td>
<td>90</td>
<td>-60</td>
</tr>
<tr>
<td></td>
<td>N-NO$<em>2$ removal rate by $X</em>{\text{NOB}}$ in presence of NH$_4$ (calculated*)</td>
<td>390</td>
<td>210</td>
</tr>
<tr>
<td>OUR</td>
<td>Oxygen uptake rate by heterotrophic biomass</td>
<td>500</td>
<td>1380</td>
</tr>
<tr>
<td>NUR</td>
<td>Nitrogen uptake rate by denitrifying heterot. biomass</td>
<td>130</td>
<td>210</td>
</tr>
</tbody>
</table>

*Production of N-NO$_2$ by $X_{\text{AOB}}$ – removal of N-NO$_2$ by $X_{\text{NOB}}$ = (b)-(c)

Figure 5: Evolution of NO$_2$-N and NH$_4$-N in AUR batch test using only biofilm

Initially they revealed a high rate of activity (1100 gN/m$^3$·d) of $X_{\text{NOB}}$ in the biofilm in the absence of ammonium and in the presence of NO$_2$-N in excess, which evidences high values of the maximum specific growth rate of $X_{\text{NOB}}$ ($\mu_{\text{NOB}}$) and of $X_{\text{NOB}}$ concentration in the biofilm.
After the addition of NH$_4$-N a lower rate of activity (480 gN/m$^3$·d) of $X_{AOB}$ was found that agrees with the known lower values of $\mu_{AOB}$ at 20ºC. The ammonium removal rate was higher than the value found in the continuous pilot plant that was attributed to the higher DO and ammonium in the bulk liquid and their increased penetration in the biofilm.

A slight increase of NO$_2$-N under the presence of ammonium was observed, as seen in the continuous pilot plant results from the HD-HN process (Figure 4b). This indicated that a relevant reduction of the NO$_2$-N removal rate by $X_{NOB}$ in the presence of ammonium from 1100 to 390 gN/m$^3$·d took place. In contrast, this reduction was not found in batch tests with biomass in suspension (Table 4). Consequently, the reduction found in the biofilm was attributed to inhibition of $X_{NOB}$ activity due to low oxygen concentrations, taking into account that the $X_{NOB}$ biomass (Kim et al., 2006) is assumed to be mainly located in the inner layers of the biofilm, the oxygen saturation constant ($K_O$) for $X_{NOB}$ is higher than for $X_{AOB}$ (Wyffels et al., 2004), and the oxygen consumption by $X_{AOB}$ (located in more external layers) is increased considerably when ammonium is added.

Batch test with biomass in suspension also revealed clear lower $X_{NOB}$ activity (220 gN/m$^3$·d, 17% of total activity), which is attributed to its low detachment from the biofilm surface. The level of $X_{AOB}$ activity in suspension (150 gN/m$^3$·d) resulted in a higher proportion (24%), indicating a higher degree of detachment. This behaviour seems to confirm the different distribution of $X_{AOB}$ and $X_{NOB}$ over the biofilm layers. The higher amount of $X_{AOB}$ and $X_{NOB}$ in the biofilm is consistent with the fact that variations in suspended solids in the aerobic reactor (aerobic TSS) from 5000 to 2200 mg/l (Figure 4a) do not seem to substantially affect the nitrification rate in the continuous plant.

Moreover, at the same time, the OUR batch tests indicated that 73% of heterotrophic ($X_H$) activity took place in the biomass in suspension. Consequently biodegradable COD removal from the aerobic reactor (~100 mg/l in HD-HN process, Table 3) is thought to mainly occur via biomass in suspension, with a corresponding growth in $X_H$. In contrast, in the HD-N process the suspended solids in the aerobic reactor were only 300 mg/l (Table 3) and the growth of $X_H$ (from the same biodegradable COD removal of 100 mg/l) is thought to occur more intensively in the biofilm, leading to a thicker external layer. This behaviour may explain the higher increase of nitrite found in the HD-N process under an increase of applied nitrogen load. The associated increase in COD load would cause an increase in oxygen consumption in the external layers, leading to a greater $X_{NOB}$ inhibition in the inner layers.

**Denitrification**

Table 3 shows that the effluent nitrate objective fixed at 150 – 200 mgN/l was satisfactorily achieved in both processes under the internal recycle ratios established. The mass flow rate of effluent nitrate (expressed in g/d) is the result of the mass balance of nitrification and denitrification. Denitrification rates depend on different operational conditions such as COD removed load in the anoxic reactor, methanol applied load, concentration of heterotrophic biomass $X_H$ and temperature. These correlations are analysed below, taking into consideration both average values (Table 3) and daily variations shown in Figures 6 and 7.

**Effect of filtered and total COD removal in the anoxic reactor.** Effluent filtered COD amounted to about 450 mg/l (Table 3), which is considered as inert COD and corresponds to 12% of influent filtered COD. The rest is considered slowly biodegradable substrate since the small fraction of readily biodegradable substrate (Ss) is assumed to be removed during swine storage.
As the filtered COD removed from the aerobic reactor amounted to only about 100 mg/l in both processes (Table 3), a large amount of filtered COD was removed from the anoxic reactor, due to the high anoxic fraction implemented. This helped to both enhance denitrification and decrease inhibition of biofilm nitrification associated with heterotrophic growth. The influent flow rate in the HD-N process was increased in the HD-HN process. It is thought that filtered and total COD removal rate also increased as a consequence, and thus may have contributed to higher denitrification rates. The opposite may well have occurred in the HD-N process with low influent flow rate.

![Figure 6: Variations of effluent and anoxic nitrate and denitrification rate with operational conditions in denitrification during the HD-HN process](https://iwaponline.com/wpt/article-pdf/4/3/wpt2009046/384138/46.pdf)

![Figure 7: Variations of anoxic and effluent nitrate and denitrification rate in anoxic reactor during the HD-N process](https://iwaponline.com/wpt/article-pdf/4/3/wpt2009046/384138/46.pdf)

**Effect of methanol applied load (K_met).** Figure 6 for the HD-HN process shows, first of all, the logical correlation between anoxic NO_3^-N and effluent NO_3^-N, which is associated with nitrification. High levels of anoxic NO_3^-N are not satisfactory since they indicate that the denitrification potential of the anoxic reactor has been exceeded. In order to minimize effluent NO_3^-N, anoxic NO_3^-N should be kept at levels as low as possible, mainly by changing the methanol dose (K_met). Methanol is a readily biodegradable substrate that produces nitrate removal in an approximate ratio of 4 gCODmethanol/gNO_3^-N. It contributes significantly to an increase in the denitrification rate. This is made evident in Figure 6b where changes in the methanol dose entailed observing relevant variations in anoxic and effluent NO_3^-N and in denitrification rates. Maximum methanol doses (200 gCOD/m^3·d at 17ºC and 400 gCOD/m^3·d at 23ºC) and maximum denitrification rates (200 gN/m^3·d at 17ºC and 300 gN/m^3·d at 23ºC, Figure 6) correspond to the maximum ammonium applied loads described above.
Operating the HD-N process (Figure 7), low levels of effluent and anoxic NO$_3$-N were achieved after day 120, but a relatively low denitrification rate (130 gN/m$^3$·d) was observed, in spite of the relatively high methanol dose. It is considered that this is partly due to the low MLSS used (800 mg/l), as explained below.

Effect of temperature and heterotrophic biomass $X_H$ in the anoxic reactor. The concentration of $X_H$ in the anoxic reactor and the temperature determine the denitrification rate due to both the removal of particulate biodegradable COD and anoxic endogenous respiration. During operation of the HD-HN process, denitrification rates obtained in separate batch tests (under methanol in excess) (NUR, Table 4) for carriers and suspended sludge revealed that the distribution of $X_H$ was 62% in suspended sludge and 38% in the biofilm of the carriers. Accordingly, Figure 6a shows that a correlation can be observed between variations in suspended solids in the anoxic reactor (and consequently in $X_H$) and variations in anoxic NO$_3$-N, effluent NO$_3$-N and in denitrification rates.

It is hypothesised that filtered COD biodegradation via $X_H$ partly takes place in the biofilm. However, only a low proportion of particulate biodegradable COD is expected to be removed in the biofilm since it would be mostly entrapped by suspended sludge. It is thought that the addition of carriers contributes to higher denitrification rates due to an increased solids retention time of $X_H$, anoxic endogenous respiration and to a more robust performance under variations in MLSS. Finally, it is noted that the increase in temperature in period 2 operating within the HD-N process contributes to the increase in $X_H$ denitrifying activity via anoxic endogenous respiration.

CONCLUSIONS
Both processes have shown great potential to nitrify within the biofilm. A nitrification rate of about 0.8 gN/m$^2$·d at 20ºC has been found to achieve low and stable values of ammonium and nitrite (less than 50 mgN/l), regardless of the carrier used. The application of a hybrid nitrification process (in the HD-HN process) entails an increase in the nitrification rate of about 20% due to high mixed liquor suspended solids (MLSS). This results in a potential volumetric nitrification rate of 380 gN/m$^3$·d at 20ºC, which is 2.5 times the expected rate in activated sludge plants under the same conditions. Under high loading rates, the HD-HN process leads to more effluent ammonium and less nitrite, while the HD-N process produces the opposite effect. This occurs to a lesser or greater extent because of the Nitrite Oxidizing Biomass ($X_{NOB}$) oxygen inhibition associated with the presence or the absence of MLSS, respectively. This behaviour can be taken into account to promote partial nitrification, if desired, through regulation of dissolved oxygen.

Regarding denitrification, the use of a high anoxic fraction (50%) is recommended in order to minimize inhibition of nitrification in the biofilm due to biodegradable COD applied load. The hybrid denitrification with high MLSS (4g/l) is also recommended since it provides a high level and robust performance. An appropriate regulation of the methanol dose and internal recycle ratio allows the desired effluent nitrate to be obtained. In summary, both processes offer great potential regarding the design of more compact new plants and the upgrading of existing facilities.

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