MARSOL

Demonstrating Managed Aquifer Recharge as a Solution to Water Scarcity and Drought

MAR to Improve Groundwater Quantity and Quality by Infiltration of River Water

- The Llobregat Demonstration Site, Catalonia, Spain -

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EXECUTIVE SUMMARY

This is the fourth and last deliverable in WP 6 aimed at improving groundwater quantity and quality by discharging river water through infiltration basins located in the Lower Valley of the Llobregat River in Catalonia, Spain. Deliverable 6.4 reflects our recent work in MAR field activities at the MARSOL Demo Site of Sant Vicenç dels Horts.

The work is composed by two main contributions. The first one involves a study on enhanced biodenitrification. It is found that the feeding strategy is paramount to characterize (and model) biofilm growth. For nonmature systems, solute transport is properly described by an advection-dispersion equation (ADE), while in mature systems it is best fitted by a non-Fickian (e.g., a dual-domain model). Optimizing the feeding strategy in terms of the concentration of ethanol injected into the system, allows reducing the C:N ratio below the stoichiometric requirements. Nevertheless, this strategy is not sustainable at long term and it only can be properly used whenever biofilm in the subsurface can be considered mature.

Field data implies that the organic layer that was placed at the bottom of the infiltration pond in Sant Vicenç is able to drive denitrification in the underlying groundwater. The process is heavily transient. Denitrification conditions are observed even one month after recharge ceases. It indicates that degradation keeps occurring once MAR activities are discontinued.

Another significant result is the observation that the organic layer, mainly composed by vegetal compost, is able to drive denitrification even three (3) years after its installation. This is somewhat surprising. It was expected to release organic carbon for a few months only, enhancing denitrification during a short period of time. In reality, the layer keeps building up the concentration of labile organic carbon, possibly related to the grow of vegetation at the bottom of the pond.

The second contribution implies the microbiological analysis of the site. It was found that microbial studies prove that the biological component is a very significant issue in the clogging of the Llobregat MAR system.
There is significant evidence that the microbiological system is mostly related to the water content, and thus by the recharge/no recharge activities. For this reason, several water and soil samples were obtained and then analyzed in all the piezometers available in the area, in some cases at different depths.

Both the water and the soil samples show that both the Shannon (entropy index) and the richness index were significantly larger during the recharge periods. On the contrary, under no recharge conditions diversity is significantly reduced.
1 Introduction

1.1 Objective

This is the fourth deliverable in the Work Package entitled DEMO Site 4: Llobregat River Infiltration Basins, Sant Vicenç dels Horts, Catalonia, Spain. The main objective of Deliverable 6.4 is to reflect our recent work in MAR field activities at the MARSOL Demo Site, mainly concentrated in field denitrification potential, and in the description of the microbiological population as a function of the recharge activities.

1.2 Previous work

The site has been extensively monitored for several discontinued periods of time both in terms of infiltration rates and also on geochemical variations of the infiltrated water. Furthermore, a vegetal compost-made reactive organic layer was placed at the bottom of the pond (Figure 1.1).

The objective of the pond was to provide a large concentration of labile organic carbon dissolved in the infiltrating water in order to promote biogeochemical reactions that could degrade a large number of organic molecules, here including emerging compounds (such as personal care products or pharmaceuticals). During the first months after the organic layer was installed, an improvement in the elimination of some pollutants present in the recharge water was observed, leading to a positive impact on the quality of the recharged water. The impact of the organic layer at large times had not yet been assessed.

The work included in Deliverable 6.4 involved the development of biogeochemical models to account for the processes taking place within the soil and the aquifer in an integrated way. Emphasis was placed on the description of processes occurring right below the pond, such as denitrification and reduction of organic matter, boosted by the microbial activity in the non-saturated zone. Furthermore, additional site characterization was carried out indicating how important are in situ parameters to understand the influence of recharge water
into the groundwater system. Lastly, an initial characterization of the microbial communities that can be found in the MAR system under wetting and drying conditions was described.
2 Denitrification modeling

2.1 Introduction

Nitrate is a priority environmental pollutant in many countries due to the combination of high toxicity and widespread presence (European Environment Agency, 2007). Furthermore, nitrate is one of the main pollutants involved in Managed Aquifer Recharge because it can be present in the recharged water creating pollution risk to the aquifer.

A technical solution to diminish the risk of nitrate pollution in MAR facilities is the installation of an organic carbon layer at the bottom of the infiltration pond. This solution is based on the release of organic matter into the system to facilitate the reduction of nitrate to dinitrogen gas by anaerobic heterotrophic facultative bacteria that use nitrate as electron acceptor. Such bacteria are ubiquitous in soil and groundwater (Beauchamp et al., 1989). The injection of organic carbon creates a bioactive zone, characterized by the growth of denitrifier biomass, heterogeneously distributed throughout the porous media depending on nutrient availability. Biomass can be found either as suspended matter or as biofilms attached to the solid matrix. Biofilms occur as micro-colonies or aggregates composed by denitrifier microorganisms, extracellular polymeric or proteinic substances (EPS), and potentially trapped dinitrogen gas formed during denitrification (Dupin and McCarty, 2000; Hand et al., 2008).

As biofilm develops and the pore space is occupied, partial bioclogging might take place, affecting a number of hydraulic properties. In addition to bioclogging, a reduction of hydraulic conductivity can be associated with the presence of trapped N2 gas (Amos and Mayer, 2006). While the word clogging is traditionally defined in terms of the overall reduction in hydraulic conductivity (Vandevivere and Baveye, 1992), the decrease in effective pore volume caused by biofilm growth also changes porosity. Due to the variation of these two hydraulic parameters, changes in groundwater velocity might be recorded (Pavelic et al., 2007), changing residence time between injection and extraction wells, thus influencing the overall capacity for biodenitrification. Furthermore, the spatial heterogeneity of hydraulic properties caused by the inhomogeneous
distribution of biofilm throughout the porous media also promotes changes in dispersivity (Seifert and Engesgaard, 2007).

The amount of biomass and the way it grows significantly affect the performance of denitrification. Biomass growth is driven among other things by the feeding strategy, i.e., the frequency of injection, the total carbon supplied, and the resulting carbon-nitrogen (C:N) ratio. With the objective of limiting the biomass growth, some authors suggested injecting the electron donor in discrete pulses rather than as a continuous supply (Gierczak et al., 2007; Peyton, 1996). Nevertheless, little is known about how the frequency of injection pulses affects biomass growth and nitrate degradation. Regarding the C:N ratio, Vidal-Gavilan et al. (2014) observed that even working with low C:N ratios (C:N=1; well below the stoichiometric one: C:N = 2.5), high denitrification rates were achieved after biofilm development. The authors attributed this to the occurrence of endogenous bacterial decay.

Thus, the aim of this work is developing a model capable of reproducing different feeding injection frequencies (from weekly to daily) with different C:N ratios in a long term column experiment, lasting 342 days (Vidal-Gavilan et al., 2014). This modeling study focusses on the denitrification performance in response to the frequency of organic substrate addition as well as the changes in hydraulic and transport properties promoted by the growth of biofilm.

### 2.2 Materials and Methods. Description of the experiment and data set

A full description of the experiment is provided in Vidal-Gavilan et al. (2014), and sketched here in Figure 2.1 for completeness. It consisted of a glass cylindrical column (70 cm length, 8 cm inner diameter) filled with unconsolidated sediment from the topsoil layer in the Sant Vicenç dels Horts MARSOL Demo Site. Water, resembling the chemistry of the Llobregat River, was forced to flow from the bottom to the top of the column with a pump-controlled average flow-rate of 180 mL/d resulting in a residence time in the column of about 6.4 days. A total of eight sampling ports were installed: one at the inflow reservoir, six along
the column (at 6, 16, 26, 36, 46 and 56 cm from inlet), and one at the outflow, allowing the delineation of aqueous compounds and suspended biomass profiles at different predefined times.

The data set provided in Vidal-Gavilan et al. (2014) and used in the modeling effort includes aqueous concentrations of ethanol, nitrate, and biomass at selected times at the sampling ports placed within the column. The experiment ran for 342 days at aquifer temperature (15°C). Ethanol was added as an external organic carbon source by means of four injectors located 16 cm from the inlet (see Figure 2.1). It was added by mixing it with the input water previous to injection (Table 2.1). Different feeding strategies were tested during the experiments, characterized by different injection frequencies (weekly versus daily) and carbon to nitrogen molar ratios (from 2.5 to 1).

Figure 2.1. Experimental flow-through system and location of the sampling ports.
Table 2.1. Average concentration of different species in the input water. Nitrate concentration (*) varied during the experiment due to seasonal nitrate oscillations within the aquifer.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Column solution</th>
<th>Injected solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td></td>
<td>7.2 ± 0.1</td>
<td>7.2 ± 0.1</td>
</tr>
<tr>
<td>Temperature</td>
<td>°C</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Nitrate</td>
<td>mM</td>
<td>1.2-1.6 (*)</td>
<td>1.2-1.6 (*)</td>
</tr>
<tr>
<td>DIC</td>
<td>mM</td>
<td>7.2 ± 1.0</td>
<td>7.2 ± 1.0</td>
</tr>
<tr>
<td>Chloride</td>
<td>mM</td>
<td>0.10 ± 0.04</td>
<td>0.10 ± 0.04</td>
</tr>
<tr>
<td>Sulfate</td>
<td>mM</td>
<td>1.2 ± 0.1</td>
<td>1.2 ± 0.1</td>
</tr>
<tr>
<td>Calcium</td>
<td>mM</td>
<td>3.40 ± 0.07</td>
<td>3.40 ± 0.07</td>
</tr>
<tr>
<td>Sodium</td>
<td>mM</td>
<td>2.20 ± 0.04</td>
<td>2.20 ± 0.04</td>
</tr>
<tr>
<td>Magnesium</td>
<td>mM</td>
<td>1.60 ± 0.03</td>
<td>1.60 ± 0.03</td>
</tr>
<tr>
<td>Potassium</td>
<td>mM</td>
<td>0.100 ± 0.001</td>
<td>0.100 ± 0.001</td>
</tr>
<tr>
<td>Ethanol</td>
<td>mM</td>
<td>-</td>
<td>14-292</td>
</tr>
<tr>
<td>Biomass</td>
<td>mM</td>
<td>2.3 x 10⁻⁷</td>
<td></td>
</tr>
</tbody>
</table>

In the C:N ratio the numerator is computed from the concentration of C in ethanol multiplied by the duration of the injection period (0.5 min). Feeding was twice discontinued, first between days 150 and 175 due to pump failure (no water was supplied during that time), and then between days 286 and 311, this time to evaluate the resilience of the system to the absence of feeding (water with no ethanol was supplied during that period). Two conservative tracer tests were performed (with Bromide), one previous to the start of the experiment, before any feeding took place, and a second one at day 342. The tests were conducted under continuous flow with constant concentration of bromide (1.45 and 2.23 mM, respectively). During the two tracer tests the flow rate was 835 mL/d. The bromide breakthrough curves were monitored at the outflow point.

Table 2.2. Summary of the different feeding strategies (I to IV) tested during the experiment, in terms of feeding frequency, ethanol concentration supplied, ratio of C (external organic carbon source concentration) to N (nitrate concentration), and duration.

<table>
<thead>
<tr>
<th>Feeding strategy</th>
<th>Feeding frequency</th>
<th>Average C:N</th>
<th>Ethanol injected (mM ethanol)</th>
<th>Days of experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Weekly</td>
<td>2.5</td>
<td>261-292</td>
<td>1-98</td>
</tr>
<tr>
<td>II</td>
<td>Daily</td>
<td>2.5</td>
<td>26-35</td>
<td>99-205*</td>
</tr>
<tr>
<td>III</td>
<td>Daily</td>
<td>1.5</td>
<td>17</td>
<td>206-287</td>
</tr>
<tr>
<td>IV</td>
<td>Daily</td>
<td>1</td>
<td>14</td>
<td>287-342**</td>
</tr>
</tbody>
</table>

*Supply of water was discontinued between days 150 and 175
**Supply of organic carbon was discontinued between days 286 and 311
Table 2.2 provides a summary of the different feeding strategies (from I to IV) tested during the experiment, in terms of engineered parameters in terms of feeding frequency, ethanol concentration supplied, ratio of C (external organic carbon source concentration) to N (nitrate concentration), and duration of feeding.

2.3 Model construction

2.3.1 Denitrification biogeochemical model

Biodenitrification was modelled considering both nitrate respiration and biomass growth (see e.g., Rodríguez-Escales et al., 2014). The reaction expressions considered are:

\[ r_{\text{ED}} = -k_{\text{max}} \frac{[\text{ED}]}{[\text{ED}]+K_{S,\text{ED}}} \quad \frac{[\text{EA}]}{[\text{EA}]+K_{S,\text{EA}}}[X] \]  

(1)

\[ r_{\text{EA}} = Qr_{\text{ED}} - b[X] \]  

(2)

\[ r_X = -Y_h r_{\text{ED}} - b[X] \]  

(3)

where \([\text{ED}]\) is the concentration of the electron donor (ethanol, \(\text{C}_2\text{H}_5\text{OH}\)); \([\text{EA}]\) that of the electron acceptor (nitrate), and \([X]\) the denitrifier biomass concentration, all expressed in \([\text{ML}^{-3}]\); \(k_{\text{max}} [T^{-1}]\) is the consumption rate of electron donor per unit value of biomass; \(K_{S,\text{ED}} [\text{ML}^{-3}]\) and \(K_{S,\text{EA}} [\text{ML}^{-3}]\) the half saturation constants of electron donor and acceptor, respectively; \(b [T^{-1}]\) a biomass decay constant; \(Y_h\) the microbial yield \([\text{C biomass} / \text{C ethanol}]\), and \(Q\) \([\text{N nitrate} / \text{C ethanol}]\) and \(S\) \([\text{N nitrate} / \text{C endogenous}]\).

Both \(k_{\text{max}} (\mu_{\text{max}}/Y_h)\) and \(K_s\) were fitting parameters, whereas \(S\) and \(Q\) were stoichiometric factors determined by the driving denitrification reaction (4). Biomass was conceptualized as having an average chemical composition of \(\text{C}_5\text{H}_7\text{O}_2\text{N}\) (Porges et al., 1956).

\[ 0.943 \text{C}_2\text{H}_5\text{OH} + 1 \text{NO}_3^- + 0.489 \text{H}^+ = 0.273 \text{C}_5\text{H}_7\text{O}_2\text{N} + 0.364 \text{N}_2 + 0.511 \text{HCO}_3^- + 1.864 \text{H}_2\text{O} \]  

(4)
Equation (4) was determined following the instructions of Rittmann and McCarty (2001) and it applies to the following determined parameter values: (i) the portion of substrate (ethanol) used for cell synthesis during denitrification ($Y_h$) was 0.724 C-biomass/C-ethanol (in agreement with Rodríguez-Escales et al. 2014); and (ii) the portion of nitrate consumed by substrate oxidation (Q) was 0.53 mol nitrate-mol C-ethanol. The stoichiometric relationship between nitrate and endogenous carbon (S) was 0.92 mol nitrate-mol C endogenous, following Rodríguez-Escales et al. (2014). The initial biomass concentration was estimated in $6.5 \times 10^{-8}$ mmol/kg, considering a most probable number for denitrifying cells equal to 37.5 cell/ml (Vidal-Gavilan et al. 2014) and converted to moles using a denitrifier cell weight of $10^{-9}$ mg (Alvarez et al., 1994). The initial value used in PHT3D was normalized by liter of water.

### 2.3.2 Transport model parameters evaluated from the tracer tests

Two tracer tests with a conservative tracer (Bromide) were performed at days 0 and 342 in order to build a conceptual model for conservative transport and to estimate the corresponding hydraulic parameters. We first attempted to fit the breakthrough curves with the simplest available transport model, that of the one-dimensional advection-dispersion equation (ADE). The ADE model could properly reproduce the tracer test performed at time 0, but failed to fit the tail of the experimental breakthrough curve obtained during the second test performed at day 342.

As an alternative model, we selected one involving two sites (dual porosity model, Haggerty and Gorelick, 1995; Seifert and Engesgaard, 2007), representing the porous medium as composed of a mobile and of an immobile region that coexist at any given point in the domain. The first one was an aqueous phase where advection and dispersion were the main transport processes, whereas the second one was a (diffusion zone governed by biofilm dynamics). Both regions exchange mass proportionally to the difference in their
concentrations at any given time. The equation describing the concentration of species $i$ in the mobile zone, $c_{m,i}$, is:

$$\phi_m \frac{\partial c_{m,i}}{\partial t} = -q \frac{\partial c_{m,i}}{\partial x} + \phi_m D \frac{\partial^2 c_{m,i}}{\partial x^2} - \Gamma_i$$  \hspace{1cm} (5)

where $D$ is the dispersion coefficient, $q$ is Darcy’s velocity, $\phi_m$ the porosity corresponding to the mobile zone (aqueous phase with aqueous solution), and $\Gamma_i$ the source-sink term controlling the mass transfer of species $i$ between the mobile ($m$) and the immobile regions ($im$) (biofilm phase with microorganisms attached to the sediment), given by:

$$\Gamma_i = \alpha \phi_{im} (C_{m,i} - C_{im,i})$$  \hspace{1cm} (6)

with $\alpha$ the mass transfer rate $[T^{-1}]$, $\phi_{im}$ [-] the porosity associated with the immobile region (volume fraction occupied by the biofilm), and $C_{im,i}$ the concentration of species $i$ in the immobile region. The actual total porosity is $\phi_t = \phi_m + \phi_{im}$, and remains constant during biofilm formation.

### 2.3.3 Used codes and calibration process

The PHT3D model code (v. 2.17) (Prommer and Post, 2010) was used to simulate the evolution of groundwater hydrochemistry during enhanced biodenitrification in the column. This model couples the transport simulator MT3DMS (Zheng and Wang, 1999) and the geochemical model PHREEQC-2 (Parkhurst and Appelo, 1999), by means of a sequential split-operator technique. Regarding the tracer tests, the interpretation using the traditional ADE and the dual domain model was carried out with the CXTFIT code (Toride et al., 1999). To assist the biodenitrification model calibration process, the model independent parameter estimation program PEST (Doherty, 2005) was coupled to PHT3D and used to estimate the reaction rate parameters ($k_{\text{max}}$, $K_{\text{S,ED}}$, $K_{\text{S,EA}}$, and $b$).
2.4 Results and discussion

2.4.1 Tracer tests interpretation: derivation of transport processes and parameters

The first step is the interpretation of the one dimensional conservative tracer tests. The traditional ADE equation was capable of properly fitting the curve corresponding to the first test, but it failed to provide a good fit of the tail of the BTC corresponding to the second test, with a maximum error of 3% in the estimated concentrations. An analysis of the reasons leading to this mismatch is presented later. On the other hand, the dual domain model was capable to reproduce the tail of the BTC corresponding to the second test indicating a transition from a Fickian description of transport at the start to an anomalous description of transport at the end of the denitrification experiment. The reported BTCs are presented in Figure 2, together with the best fits obtained either with code CTXFIT at day 0 (single porosity) and at day 342 (dual porosity); the fitted parameters are listed in Table 2.3.

Groundwater velocity measured in both tracer tests was very similar (see Table 2.3). The hydraulic gradient could not be measured in the applied experimental setup. Therefore, the expected reduction in hydraulic conductivity due to biofilm growth could not be assessed. Total (single-phase) porosity and dispersivity were estimated from the first test; total porosity, the proportion of immobile and mobile porosity, dispersivity, and the mass transfer rate were estimated from the second one.

Total porosity values estimated from both tests were statistically not different, with best estimates of 0.33±0.03 to 0.34±0.05, and estimation intervals largely overlapping (Table 2.3). However, the dual porosity model estimated an immobile porosity of 0.015±0.009 at day 342. There was a remarkable seven-fold increase in the dispersivity coefficient estimated from the two tests, with the mean value changing from 0.48±0.01 to 3.44±0.25 cm (see Table 2.3). This effect has been reported in the literature previously and it is attributed to the rearrangement of the open pore structure as it is colonized.
Figure 2.2. Model fits using the ADE (black lines) and a dual domain model (red dashed lines). Square symbols (□) correspond to measurements corresponding to the tracer test performed at day 0 (red and black lines run in top of each other), whereas circles (○) correspond to the BTC from the test at day 342. The error bars are related to the bromide analyses.

The change in the conceptual model of transport was associated with the growth of biofilm during the duration of the experiment. Thus, the fitted parameters of the dual domain model have a clear physical explanation; for example, the calibrated $\alpha$ parameter ($\alpha = 0.019 \pm 0.018$ d$^{-1}$) can be interpreted as the inverse of the characteristic diffusive time of bromide transport through the immobile phase (thus being equal to 45 days). Moreover, the $\beta$ value ($\beta = 0.046 \pm 0.030$) represented the proportion of the void volume occupied by the biofilm (4.65±2.96 %).

Table 2.3. Hydraulic parameters estimated for the two bromide tracer tests. The $R^2$ of two fitted curves were 0.999 and 0.998, respectively.

<table>
<thead>
<tr>
<th>Groundwater velocity (m d$^{-1}$)</th>
<th>MODEL TYPE</th>
<th>Mobile Porosity</th>
<th>Dispersivity (cm)</th>
<th>Mass transfer parameter ($\alpha$, d$^{-1}$)</th>
<th>Immobile porosity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>ADE</td>
<td>0.331 ± 0.033</td>
<td>0.485 ± 0.006</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>End</td>
<td>Dual domain</td>
<td>0.326 ± 0.044</td>
<td>3.440 ± 0.246</td>
<td>0.019 ± 0.018</td>
<td>0.015 ± 0.009</td>
</tr>
</tbody>
</table>
2.4.2 Long-term modeling of denitrification. Impact of organic carbon injection strategies

Based on tracer tests results the column experiment was first interpreted using a Fickian representation of transport, i.e., based on the ADE. Emphasis was placed on the performance of the daily and weekly feeding strategies upon the observed temporal evolution of the concentrations of nitrate, ethanol, and biomass. Since Table 2.3 displays two dispersivity values corresponding to days 0 and 342, but no intermediate values were obtained, the 342-day column experiment was modeled using both dispersivity values, by assuming that they lasted the full duration of the experiment, thus providing the two limiting cases of the real behavior.

The column was discretized into 70 elements of 1 cm length. The time discretization was refined until it satisfied the Peclet and Courant criteria. Dispersive transport was computed by the third-order Total Variation Diminishing (TVD) solution, a feature directly available in PHT3D. The actual data and the fittings with the two dispersivity values are shown in Figure 2.3. Neither porosity (obtained from the tracer test, 0.33), nor the geochemical parameters of reactions in equilibria (selected from the PHREEQC2 database) were calibrated. The only calibrated parameters were the microbiological ones (Table 2.4) and, all were in range compared to values reported in the literature. The lowest dispersivity value (0.48 cm) resulted in a good fitting of the experimental data during the weekly feeding strategy (Figure 2.3), lasting the first 98 days, indicating that during this period dispersivity did not change significantly.
Table 2.4. Biogeochemical constants used in the denitrification model, compared with values compiled from the literature. Both the median and the standard deviation were determined by automatic calibration using PEST.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>This work</th>
<th>Literature values</th>
<th>Reference(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\mu_{\text{max}})</td>
<td>([d^{-1}])</td>
<td>3.01x10(^1) ± 1.82x10(^1)</td>
<td>1 x 10(^1); 1.1 x 10(^1); 2x10(^1); 1.08x10(^2)</td>
<td>1,2,3,4</td>
</tr>
<tr>
<td>(K_{S,\text{EA}}) (nitrate)</td>
<td>([M])</td>
<td>8.18x10(^{-6}) ± 4.84x10(^{-6})</td>
<td>1.6 x 10(^{-6}); 3.2 x 10(^{-6}); 1.2 x 10(^{-5}); 1.8 x 10(^{-4})</td>
<td>1,3,2,4</td>
</tr>
<tr>
<td>(K_{S,\text{ED}}) (ethanol)</td>
<td>([M])</td>
<td>1.18x10(^{-4}) ± 4.55x10(^{-5})</td>
<td>8.3 x 10(^{-6}); 1.7 x 10(^{-4}); 6.6x10(^{-4}); 7.3 x 10(^{-2})</td>
<td>1,2,3,4</td>
</tr>
<tr>
<td>(b)</td>
<td>([d^{-1}])</td>
<td>1.73x10(^{-1}) ± 4.66x10(^{-2})</td>
<td>6x10(^{-2}); 1.5x10(^{-1}); 2x10(^{-1})</td>
<td>2,4,3</td>
</tr>
</tbody>
</table>

\(^a\) References are 1, Chen and MacQuarrie (2004); 2, Lee et al., (2009); 3, Kinzelbach et al., (1991); 4, Rodríguez-Escales et al., (2014).

We note that Figure 2.4 reports the modeling results assuming a constant representative dispersivity value all throughout the column. We expect, though, that most of the biomass colonization took place around the injection point (see e.g., Kildsgaard and Engesgaard, 2001), associated with the highest EA and ED concentrations and, consequently, the modification of the transport parameters too. Although the general trends were well captured, the limitation of considering only one set of transport parameters could explain the discrepancies between the experimental data and the simulated results.

During the daily feeding strategy, starting after day 99, the best overall fit of nitrate concentration was obtained with the final dispersivity value of 3.43 cm. This is visible both for time-series (Figure 2.3) and for spatial profiles (Figure 2.4).
Figure 2.3. Results of the denitrification models considering different injection strategies at the outflow of the column (70 cm). The black and the red solid lines were obtained with dispersivity values of 0.48 and 3.43 cm, respectively, considering an ADE equation. The dashed-dotted blue line corresponds to a model using a dispersivity value of 3.43 cm and a dual model mass transfer. The dashed black line corresponds to ethanol concentration in the injection solution. Grey areas represents the two periods without feeding. The bottom plot shows the simulated biomass concentrations (represented in mM) at the last cell of the model domain (70 cm).
Figure 2.4. Nitrate distance profiles (simulated vs. measured) at 12 different times. In each plot, the first number represents the sampling day, and that in brackets reflects the elapsed time since the last injection period. The black and the red dashed lines were obtained with dispersivity values of 0.48 and 3.43 cm, respectively, considering an ADE equation. The blue dashed dotted line in the last two plots corresponds to a model using a dispersivity value of 3.43 cm and a dual model mass transfer. The grey zone corresponds to the injection point.
2.5 Summary and conclusions

A denitrification experiment, performed in a 70 cm long column under virtually constant flow rate and different feeding strategies was modeled. Injection strategies were defined in terms of periodicity of injection of organic carbon (ethanol), and thus resulting C:N ratio. A long term reactive transport (342 d) model based on the Advection Dispersion Equation (ADE) fitted properly most of the experimental data.

Throughout the experiment, estimated dispersivity varied from the beginning to the end of the experiment. During the weekly supply strategy I (up to day 98), the best fit was obtained using a low dispersivity value (0.48 cm), whereas during the daily strategy, it was best fitted with a larger dispersivity value (3.43 cm). We attributed this increase to the change in injection periodicity, from weekly to daily, after day 98, resulting in biofilm growth. Furthermore, after day 252, with a very mature system, data fitted better using a dual-domain model (i.e., non-Fickian) as compared to one based on the ADE. This change was associated with the presence of a diffusive layer (biofilm) increasing its relevance with time. Although the dynamic conditions of the system, the presented model has been capable of reproducing satisfactorily the experimental observations in all feeding strategies.

On the other hand, reducing the C:N ratio below the stoichiometric requirements allowed the optimization of ethanol injection into the system avoiding its presence at the column outlet. At this point, biomass decay increased and the endogenous carbon acted as partial source of electron donor during the denitrification process. Nevertheless, the decrease of modelled biomass concentration in time showed that this strategy is not sustainable at long term and that it only can be used when a mature biofilm exists in the subsurface.
3 Resilience of the organic layer and in-situ denitrification processes

3.1 Introduction
As mentioned before, a layer mainly composed by a mixture of sand and vegetable compost (with small percentages of clay and iron oxides) was installed in order to create favourable conditions for contaminant biodegradation.

To test the efficiency of this layer with time, different experiments were carried out at the field scale using nitrate isotopes. Those are a useful tool since an increase in δ15N-NO3 and δ18O-NO3 of residual nitrate with decreasing nitrate concentrations is characteristic of kinetic isotope fractionation induced by the breakage of N-O bonds during denitrification. This work was done in cooperation with project WADISMAR (Water harvesting and Agricultural techniques in Dry lands: an Integrated and Sustainable model in Maghreb Regions -ENPI/2011/280-008).

3.2 Batch experiments
In order to evaluate the efficiency of the reactive layer along time, a complete denitrification experiment was performed with new organic compost and material of the organic layer obtained in 2014, three years after its installation in the field.

3.2.1 Experimental set-up
Groundwater used in the experiments was obtained from the Llobregat aquifer. Commercial compost was obtained from a composting plant located in Moià (Catalonia, Spain). Reactive layer samples were obtained directly form the Llobregat site in 2014.
In addition a ‘sterilized control’ (SC) was carried out adding autoclaved material to autoclaved groundwater, and an ‘absence control’ (AC) was carried out only with groundwater. 0.80 mM of NO3⁻ was added. All batch experiments were set up in an anaerobic glove box with an Ar atmosphere to avoid the presence of O2. Bottles were manually shaken once a day and aqueous samples (5 ml) were collected daily using sterile syringes. Samples were collected for anions.

### 3.2.2 Results

The results of batch experiments confirmed that both new and old materials induced denitrification. Figure 3.1 shows that complete nitrate consumption was obtained in less than 12 days for the new commercial compost. An initial NO3⁻ release by the compost of up to 2.58 mM was observed. In the sterilized control and absence control (AC) experiments, nitrate reduction did not occur (Figure 3.1, displaying individually the results for all three replicates, CC1 to CC3).

The isotopic enrichment factors for N (εN) and O (εO) in the new organic compost batch experiments was calculated from the slope of the regression lines that fit the data corresponding to the natural logarithm of nitrate concentration vs. δ¹⁵N or δ¹⁸O-NO₃⁻, respectively. The registered values of εN (-15.49‰), εO (-9.05‰) and the ratio εN/εO (1.71‰) for the compost experiment confirmed denitrification.

When comparing the results with those obtained for the old material, denitrification is also observed, indicating that the reactive layer is still working after three years of installation. However, the initial release of nitrate observed with the new organic compost is reduced by less than a half (Figure 3.2).
Figure 3.1. Variation of nitrate concentration over time in batch experiments: commercial compost triplicates (CC1 to CC3) and absence control (AC) experiments.

Figure 3.2. Variation of nitrate concentration over time in batch experiments for (new) fresh compost and old compost.
3.3 Nitrate isotopes

In order to evaluate the observed denitrification processes in the laboratory, different field surveys using isotopes were carried out in the MARSOL Llobregat site.

3.3.1 Field surveys

Samples for chemical and isotopic characterization were collected in June 2013, September 2013, and July 2014. The June and July campaigns took place with the infiltration pond full (recharge conditions), this situation representing an infiltration period. The September campaign was carried out with the infiltration pond empty, representing a non-infiltration period. Moreover, samples from the Llobregat river were also collected during each field survey. In some of this surveys, the river was sampled twice, right before and after the groundwater sampling.

3.3.2 Results

According to the isotopic signal, denitrification processes were observed in the different field surveys. However, despite several points show a denitrification along the recharge system, there is not a clear trend indicating how these processes are occurring. One of the reasons for this behaviour could be the change in the initial isotopic signal observed in the river water. The variations observed in the surface water, much more variable than groundwater, could buffer the isotopic signal observed in groundwater. In this way, surface water is recharging the aquifer too.

Despite there is not a clear trend, it is possible to see that the points less (or completely non-) influenced by the recharge pond (BSV-0, BSV-1/P1, BSV-3/P3) show the lowest denitrification (see Figure 3.3). In the same way, the
points more affected by recharged water (Pou, BSV-5 and BSV-2) show the highest denitrification signal.

It is important to highlight that during the non-recharge period some points still show denitrification. This implies that the induced denitrification processes in the Llobregat system are very complex and can respond to a large number of variables apart from the flow paths.

![Figure 3.3](image)

Figure 3.3. Variation of nitrate isotopes during the dry (no recharge) and wet (recharge) periods in the Llobregat site.

### 3.4 Main findings

- Batch experiments indicate that the organic compost is still capable of inducing denitrification after 3 years of its installation.
• Nitrate is released from the organic layer, with rates decreasing with time.
• Denitrification processes occurring at the Llobregat site have been observed by means of nitrate isotopes.
4 Microbial communities in the Llobregat MAR system

4.1 Introduction

MAR processes modify the natural conditions of the aquifer by introducing into the groundwater system water with different physicochemical parameters (such as temperature, dissolved Oxygen, and pH) and in most cases hydrochemical parameters (water composition). These changes modify the natural conditions of the soil-aquifer system affecting the microbial communities available. These changes can affect the biofilm formation (clogging) but also the capacity of the system to degrade some target pollutants.

In order to characterize these microbial community changes two field campaigns were performed in July 2014 and March 2015 as explained in Deliverable 6.2. The first one refers to a recharge (wet) period and the second one, to no recharge (dry, natural conditions). Soil samples were taken at the bottom of the pond, and then sent to the Microbiological Laboratory of the Department of Environmental Microbiology at the Universitat Autònoma de Barcelona (UAB). The sampling points were distributed along the sedimentation pond, reactive layer, vadose zone and groundwater (upstream and downstream at different depths in each piezometer). Analytical methods were already explained in Deliverable 6.2.

4.2 Microbial community description

The initial and basic results of this field survey were already reported in Deliverable 6.2. In this way, based on Shannon and Richness indexes, there is evidence that the microbiology system is characterized by the water content (recharge versus no recharge conditions), and fully controlled by the presence of the reactive layer at the bottom of the pond. In other words, having an organic bed layer placed at the bottom of the pond determines the type of environmental microbial communities.

However, to completely understand the changes produced in the microbial ecosystem, the communities have to be studied considering the flow system.
created under managed artificial conditions. The recharge flow system has been defined based on the flow and transport model and tracer test data presented in Valhondo et al., (2016) (see Figure 4.1). Under MAR conditions, recharged water has different transit times when reaching the different monitoring piezometers which affect the environmental conditions (temperature, conductivity, water composition, etc) and therefore microbial ecosystems.

Figure 4.1. Distribution of advective times along the study area during recharge conditions
A cluster dendrogram showing similarity among different band patterns was elaborated using Dice coefficient and UPGMA algorithm for the samples corresponding to the July 2014 campaign and considering advection time (time needed for the recharged water to reach the sampling point) to each monitoring point (Figure 4.2).

As expected, all soil samples are separated from water samples as they are related with attached biomass, while the water samples are representative of...
suspended biomass. In the same way, surface water and monitoring point P1 (not affected by MAR) stay completely away from the other samples. Once we refer to the points affected by the artificial recharge, it is possible to observe than the advective time and the distance from the recharge pond determine how the microbial communities are.

Looking at the campaign under no recharging condition (Figure 4.3), it is possible to see again that soil and water samples group separately. However, in this case the similarity or difference between the microbial communities is not controlled by the vertical flow induced by MAR, but rather by the natural gradient of the aquifer in the approximate direction P1-P10 (Figure 4.1)

![Dendrogram](image-url)

**Figure 4.3.** Dendrogram corresponding to a non-recharge period (March 2015) generated by Cluster analysis using UPGMA algorithm representing the similarities between samples. The notation is identical to that used in Figure 4.2.
4.3 Microbial ecosystems

Considering both field surveys together, 9 Phyla of 18 bacteria phylotypes had been identified in the different samples, here combining those corresponding to water and soil samples. In some cases, it was possible to discriminate the genus clade belonging to the phylotypes. In other cases, it was only possible to identify the class.

The functional traits of the identified phylotypes indicate that microbial ecosystem found in the MAR system are capable of doing a very large number of biological functions, according to the prokaryotic functions described in Mardigan et al. (2015). For example, we found bacteria classes capable of performing oxygenic phototrophy, while others are characteristic of dissimilative iron reduction.

When both field surveys are considered separately, it is possible to observe several differences between wetting and dry conditions. This can be observed for example in Figure 4.4 where the relative abundance of the microorganisms detected in each sample is provided. It is found that richness (in terms of detected species) was higher in the samples taken during recharge conditions, as compared to those obtained during the dry one. Moreover, samples taken in a specific piezometer but obtained at different depths show similar distribution of microorganism abundance. Finally, it is shown that samples taken in recharge conditions and in those piezometers with the smallest advection time (i.e., P2, P3) show the highest presence of Dehalococcoidia class; this class belongs to Chloroflexi Phylum, which is composed mainly by species capable to catalyze anaerobic dehalogenation (Krzmarzick et al., 2012).
Changes in each class must be analyzed separately. However, the most notifiable change is the increase of Betaproteobacteria during the recharge season. Looking in detail to this phylum in our samples, it is possible to demonstrate that processes (hydrobiogeochemical) linked to MAR activities modify the bacterial communities in class clade and also in family clade (Figure 4.5). It is clear that recharge increases the diversity of the Betaproteobacteria. In this way, it is necessary to highlight that under recharge conditions, in the monitoring point P1, not affected by the artificial recharge, we only observe the genus Vogesella.
During the dry season, the most abundant genus is again *Vogesella*. This genus is very typical in fresh water bodies (Grimes et al. 1997) and it is capable of doing denitrification in waters with low dissolved oxygen content (as would usually be the case in groundwater). Some strains of this genus might catabolize a few monosaccharides also under low-oxygen conditions (Grimes et al., 1997). During the wet season, the proportion of this bacteria increases if we compare with the dry season (data not shown).

Figure 4.5. Distribution of *Betaproteobacteria* during recharge (A) and non-recharge conditions (B).
4.4 Main findings

- Microbial ecosystems are found in groundwater regardless the depth and the recharge conditions.
- The microbial communities present in groundwater at any given sampling point are characterized by the presence of a large microbial diversity, capable of doing several biogeochemical processes.
- MAR activities and the induced changes in groundwater (quality, temperature, etc) modify the microbial ecosystems.
- The relation between the induced microbial ecosystem during MAR activities and degradation and clogging processes needs to be studied in detail.
5 Conclusions

This section compiles the most relevant conclusions that can be extracted from this work.

Regarding the potential for enhanced biodenitrification based on MAR activities, we found that:

- Column experiments changes in the feeding strategy (going from weekly to daily), regarding the amount of labile carbon supplied, results in significant differences in biofilm growth.

- Even minute differences in biofilm growth might affect significantly the driving equations for transport of conservative (and eventually for reactive) species, so that young systems might be represented by the advection-dispersion equation, while more mature systems need to be modelled using more complex models displaying a non-Fickian behaviour of transport, with enhanced asymmetry in the observed breakthrough curves. A dual porosity model suffices to provide a reasonable representation of the observed breakthrough curves from tests involving a conservative tracer.

- Reducing the Carbon to Nitrogen (C:N) ratio below the stoichiometric requirements in the column experiments reported, allowed the optimization of ethanol injection into the system avoiding its presence at the column outlet (ethanol is fully consumed in the denitrification process). Nevertheless, the decrease of modelled biomass concentration in time showed that this strategy is not sustainable for a long time and that it only can be used when a mature biofilm had already been developed.

- Field experiments show that the organic layer clearly is able to drive denitrification in the groundwater present in the underlying aquifer. However, how these conditions are distributed spatially still needs to be studied in more detail.
Again, under field conditions, the denitrification process could still be observed to take place even one month after recharge is discontinued. This seems to indicate that the degradation processes can be occurring for some time once MAR activities stop.

- A layer capable of supplying labile organic carbon can be a good idea to enhance biofilm growth, potentially having beneficial effects regarding the removal of different (emerging) organic compounds. While in theory this layer should be exhausted after a few months, in reality it is observed that it might last for many years, thus indicating a process of self-feeding that we associated to plant growth.

The second contribution implies the microbiological analysis performed in the area affected by the MARSOL Site facility in Sant Vicenç dels Horts. The main conclusions obtained were:

- Microbial ecosystems are found in groundwater, regardless the depth and the recharge conditions, and are a significant and relevant signature for all water and soil samples, affecting the geochemical reactions that may take place.
- Microbial studies prove that the biological component is a very significant issue influencing the temporal effect of clogging of the Llobregat MAR system.
- The microbial communities present in groundwater at any given sampling point are characterize by the presence of a large microbial diversity, capable of doing several biogeochemical processes.
- The microbiological signature of soil and water samples is very distinct. In the former, attached colonies can be sampled, while in the later only those that are in suspension.
- Microbial systems are dependent heavily on water content conditions, and thus affected by the cycles of recharge/no recharge used in MAR operation.
- Pond soil samples indicate that both the Shannon and the richness indexes values are significantly larger during recharge periods. On the contrary, during dry conditions (biofilms are dehydrated) diversity is significantly reduced.
References


