Topic: 1C. Remediation technologies and approaches

DENITRIFICATION CAPACITY OF LEACHATES FROM WETLAND SOILS AND VEGETAL BIOMASS IN THE LLOBREGAT RIVER BASIN

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1. INTRODUCTION

Biomass from wetlands is a natural source of organic material able to decrease nitrate present in groundwater before the discharge to rivers [1]. The desired predominant mechanism of nitrate removal is heterotrophic denitrification by supplying organic carbon that, additionally, enhances anoxic conditions in subsurface environment. The stoichiometric reaction of a complete heterotrophic denitrification could be described as eq. 1 [2].

$$5CH_2O + 4NO_3^- \rightarrow 2N_2(g) + 4HCO_3^- + 3H_2O + CO_2(g) \qquad \text{Eq. 1}$$

Nevertheless, an excess of organic matter (CH_2O) vs nitrate could activate other mechanisms as Dissimilatory Nitrate Reduction to Ammonia (DNRA), that could be formulated as

$$2CH_2O + NO_3^- + H_2O \rightarrow NH_4^+ + 2HCO_3^- \qquad \text{Eq.2.}$$

DNRA can be significant or even a dominant process in some ecosystems [3]. DNRA is a respiratory or fermentative pathway where nitrate is slowly reduced to ammonium competing with denitrification for the nitrate into riparian zones [3], [4].

Conditions favouring DNRA are less understood than denitrification although It is believed that heterotrophic denitrification supplies more free energy than DNRA. Under nitrate limiting conditions or high organic matter concentrations. However, DNRA could be favoured because more electrons can be transferred per mole of nitrate, [6]. Nitrite could appear an intermediate in both, denitrification and DNRA.

The leaching of organic matter rich soils (peat or Horizon O layer) could be a mechanism to promote denitrification in deeper subsurface layers as provides organic matter avoiding high concentrations of organic matter that could promote DNRA pathways.

In the present work, the study at laboratory scale of the denitrification capacity of leachates of biomass and wetland soils linking with the growing of *Phragmites sp* and *Arundo donax* reeds have been studied. A close understanding of nitrate removal by using these materials ?has to improve knowledge of strengths and weaknesses of Mediterranean wetlands to remediate nitrate contamination.

2. MATERIALS AND METHODS

2.1. Study area and sampling

Samples of soils and degraded reed biomass were collected in two sites in the Llobregat river basin (Barcelona, Spain) in February and May 2014 (figure 1 a) and b)).

a) Reed extension (*Arundo donax*) in Castellbell

b) Wetland la Bòbila (*Phragmites sp.*) in Sant Pedor

c) Sample C2

d) Sample A2

Figure 1. Sampling points and samples used in this work

First site corresponded to a riparian zone of Llobregat river in Castellbell I el Vilar (UTM coordinates 31 T 404973 E 4608625N) and second site is an artificial wetland in Sant Pedor (UTM coordinates 31 T 40375 E 4625635 N). Both sites were chosen because were zones where reeds species grew.

In the first site three types of samples were taken. These samples consisted in degraded reed on the soil in the middle of the reed field (February 2014) (C1) and degraded reed close to the riparian zone (C2) (figure 1 c), both sampled in February. In May a sample of green leaves of *Arundo donax* was taken (CV). In the second site a sample of degraded leaves of *Phragmites sp.* on the soil (A2, figure 1d) and degraded stem of *Phragmites sp.*(B1) were taken.

2.2. Leachates

Leachates were obtained by using different amounts of the described materials, deionised water and different leaching methods that are summarized in table 1. The three leaching procedures were: percolation in a column filled with material, shaking with water and lixiviation by using a vertical stirrer leaching test were tested to extract the maximum of Dissolved Organic Carbon (DOC) at room temperature from the biomass samples.

Table 1. Conditions used in the leaching tests

Experiment	Material	Wet mass (g)	Leaching methods
1	C2 B1	38,6 19,2	Leaching in column using 4 ml/min and recycling approx 200 ml water for 3 h. Final volume 250 ml. Filtered 0.45 µm before batch.
2	C2 C1 A2	190,4 195,3 179,6	Direct shaking in glass funnel with 200 ml of water for 30 min. Final volume was 250 ml. Filtered 0.45 µm before batch. Inoculum used here was from A2 soil unlike rest of experiments (C1 soil)
3	C2 CV	39,0 37,0	Stirring in a vertical shaker with 100 ml of water for 30 min. Final volume was 250 ml. Not filtered for batch reaction.

2.3. Inoculum

Samples of soil in C1 and A2 were also taken in both sites and were used for inoculum extraction. The procedure of extraction consisted in using an amber glass bottle sterilized by using acetone and left 24 hours at 100 ºC. After cooling the bottle at ambient temperature, 250 grams of wet soil were mixed with 500 ml of deionised water and were mixed in the bottle for 24 hours by using a vertical stirrer. The mix was left settle down overnight and 250 ml of supernatant solution were separated by decantation and the use of a plastic pipette. This volume was centrifuged (Centronic-BL II, Selecta) at 3500 rpm for 10

minutes. The Supernatant of the centrifuged tubes was discarded and the samples were centrifuged two or three times to get pellets. These pellets were mixed in a tube that was centrifuged.

1 ml of pellet was mixed with 29 ml of culture medium. This culture medium consisted in a dissolution of 100 mg·dm^{-3} NO_3^-, 2,1 g·dm^{-3} K_2HPO_4, 1 mg·dm^{-3} $FeCl_3$•6 H_20 and 200 mg/ dm^{-3} of glucose. This mixture was stirred 48 hours at room temperature (20-25ºC) and the obtained suspension was used for batch experiments.

2.3. Batch tests

Batch tests were performed at room temperature (20-25ºC) in amber bottles previously sterilized with acetone and heating at 100 ºC. 10 ml of inoculum were mixed to a final volume of 125 ml using the leachate and a nitrate solution to reach a concentration of 25 mg NO_3^-·dm^{-3}. Some experiments were performed using a dissolution of 200 mg/l glucose (DOC 80 mg/l) instead the leachates to test the correct setup.

Blanks of inoculum (10 ml inoculum diluted to 125 ml with deionised water) and controls of TOC and TN (mixtures of 200 mg·dm^{-3} glucose and 25 mg·dm^{-3} nitrate without inoculum) were also used. In all these mixtures nitrogen gas was used to purge dissolved oxygen. The bottles were filled completely to avoid head space ad were closed to avoid the air to enter in the experiment. After some days of experiment all the samples were filtered at 0.45 µm before analysis.

2.4. Analytical methods

Nitrate, nitrite and ammonium were analysed by ion chromatography (Dionex ICS-1000/ICS-1100) with ionic suppressor conductivity. The system was equipped with an Ionpac AS19 column (Dionex) and was operated with 10-45 mM KOH mobile phase. Ammonium was analysed by means of the same ion chromatography system equipped with an Ionpac CS16 column (Dionex) and using methanosulphonic acid (30mM) as mobile phase. The detection limit was 0,01 mg·dm^{-3} for NH_4^+ and NO_2^- and 0,02 mg·dm^{-3} for NO_3^-.

DOC content (as Non-Purgeable Organic Carbon, NPOC) was determined by means of a Shimadzu TOC-V CPH equipped with a carrousel ASI-V. Total N was determined by Shimadzu a TNM-1 module analyser connected to TOC equipment. Samples were filtered through 0.45 µm nylon membrane filters and acidified with HCl solution to a pH lower than 2. Before analysis samples were purged during 20 minutes in order to volatilise all the inorganic carbon present. The detection limit of the method was 1 mg DOC·dm^{-3}.

HP 8453 UV-visible Spectrophotometer (Hewlett-Packard) was used to measure the absorbance signal at 254 nm. A Crison pH-meter was used to measure the pH of the dissolution.

3. RESULTS AND DISCUSSION

3.1. Characterization of inoculum and leachates

Blanks of inoculum showed average values of 2,7 $mg \cdot dm^{-3}$ of nitrate, 0,3 $mg \cdot dm^{-3}$ nitrite, 0,2 $mg \cdot dm^{-3}$ ammonium and 5 mg $DOC \cdot dm^{-3}$. Table 2 summarizes the characterization of leachates

Table 2. Characterization of the leachates

Experiment	Sample	DOC ($mg \cdot dm^{-3}$)	Nitrate ($mg \cdot dm^{-3}$)	Nitrite ($mg/\cdot dm^{-3}$)	Ammonium ($mg \cdot dm^{-3}$)	UV Signal (A.U.)
2	A2	23,91	28,93	nd	nd	0,77
1	B1	12,55	22,32	nd	0,36	0,43
2	C1	18,27	23,45	nd	nd	0,88
1	C2	10,20	1,98	nd	0,34	0,63
2	C2	24,38	0,53	2,79	nd	0,85
3	C2	31,02	na	na	0,22	na
3	CV	523,00	na	na	3,66	na

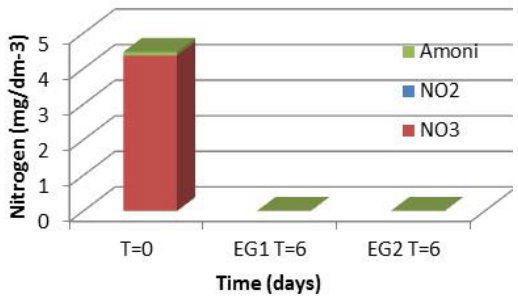
nd: non-detected, na: not analysed

DOC in the materials of experiments 1 and 2 ranged from 10 to 31 $mg \cdot dm^{-3}$. The ratio of the UV signals at 254 nm divided by DOC was also used to assess quantitatively the presence of aromatic compounds in the leachates, which could be important in samples of soils. This ratios showed values from 0,03 to 0,06 $A.U \cdot dm^3$ /mg that are in the range of fulvic acid (0.05 $A.U.dm^3$/mg). [7]

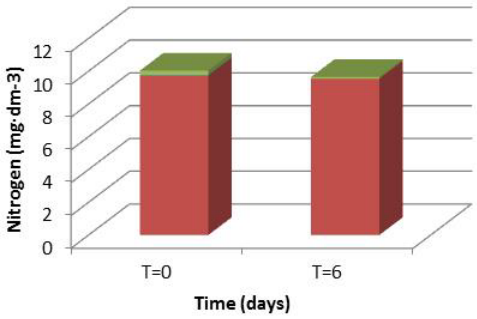
Nitrate in the leachates from all the biomass showed values above 20 $mg \cdot dm^{-3}$, with the exception of degraded *Arundo donax* in soil very close to the river that exhibit values below 2 $mg \cdot dm^{-3}$.

A third leaching experiment was prepared by shredding green Arundo donax leaves sampled in May 2014. DOC of these filtered leachates reached 523 $mg \cdot dm^{-3}$ and total nitrogen 49.8 $mg \cdot dm^{-3}$. These values of nitrogen could correspond to organic forms from chlorophyll.

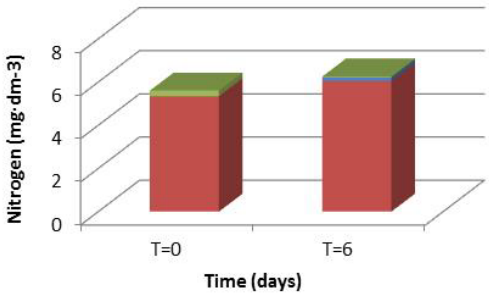
Comparing C2 samples, it was observed that the samples obtained by vertical stirrer obtained more concentration of dissolved organic matter in the lixiviates.



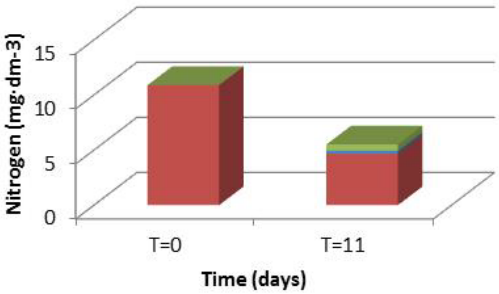
a) Glucose Experiment 1



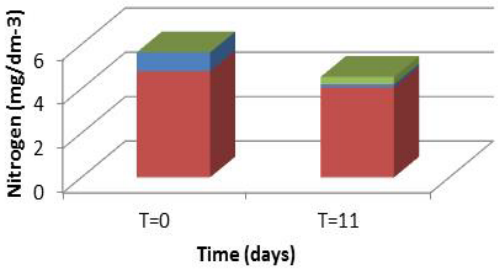
b) B1 Experiment 1



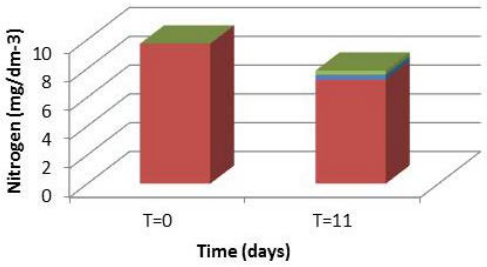
c) C2 Experiment 1



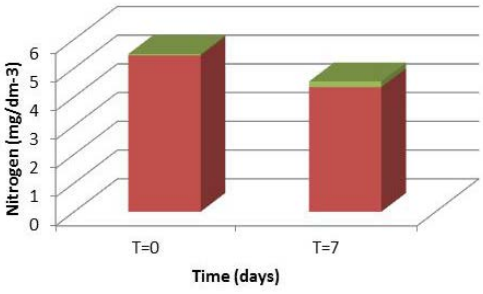
d) A2 Experiment 2



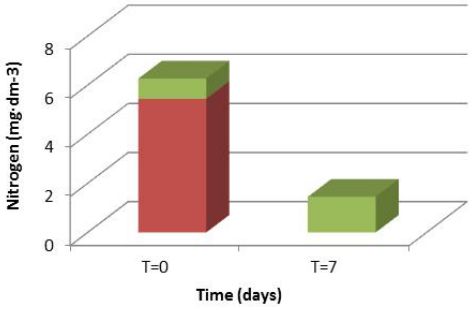
e) C2 Experiment 2



f) C1 Experiment 2



g) C2 Experiment 3



h) CV Experiment 3

Figure 2. Evolution of species in batch experiments. Colours correspond to legends in figure 2 a)

(left side T=0 and right T=final time)

3.2. Evolution of nitrogen species and dissolved organic carbon

All these characterized leachates were used as matrix in batch experiments 1 to 3. The reaction was performed for a period of 6 to 11 days and results were compared with initial N species at T=0 (figure 2).

Parallel experiments, where DOC was replaced by 100 $mg \cdot dm^{-3}$glucose, showed complete denitrification for all experiments after a period of 6 days or more (see figure 2a).

In the case of samples C2 for different experiments the results (figure 2, left samples) showed no nitrate reduction when t=6 days and very slight reduction at 7 and 11 days. Sample B1 in experiment 1 showed no denitrification after 6 days and sample C1 in experiment 2 showed also slight nitrate reduction.

Degraded leaves of *Phragmites sp.* on the soil (A2) and the green *Arundo donax* leaves (CV) showed more than 50% and complete elimination of nitrate respectively. In all the samples (except in CV) final ammonium decreased when compared with initial values (from the lixiviates and inoculum). This means that DNRA is present in the experiment with CV, probably because samples were not filtered during the batch experiment and level of DOC was very high, conditions that impose a higher reduction potential able to promote ammonium instead nitrogen gases. [6]

4. CONCLUSIONS

Most of the leachates of biomass materials and soils sampled in February in Llobregat river basin sites were not able to leach important amounts of DOC and could not denitrify or reduce nitrate. Soil samples showed the presence of UV-absorbing compounds that could be associated to complex organic matter as fulvic acids. These types of compounds are formed by complex organic matter that cannot participate in the reduction of nitrates. Degraded leaves of *Phragmites sp.* of the soil (A2) were able to eliminate 50% of the initial nitrate.

Arundo donax green leaves sampled in May (CV) allowed obtaining a leachate with high DOC able to perform nitrate reduction but with a partial DNRA mechanism. In these vegetal materials initial nitrogen is important and is associated to chlorophyll. This material and some soils with degraded reeds could contribute also to a small load of nitrate or release ammonium.

5. ACKNOWLEDGEMENTS

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