Batch Test Evaluation of Four Organic Substrates Suitable for Biological Groundwater Denitrification

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Nitrates pollution represents nowadays a serious issue related to the quality of groundwater; continuous growth of industrial-scale agricultures lead to an increase of nitrates content in groundwater in the last years. Several technologies have been validated as capable to promote in situ biological nitrates remediation, such as permeable reactive barriers (PRB), biotrench, biobarriers etc. These technologies are all characterised by the use of organic substrate that act as a slow release carbon source. In free dissolved oxygen absence, such organic carbon is further oxidised, by heterotrophic bacteria naturally present in soil, in compliance to anoxic metabolism by using nitrates bound oxygen. Such dissimilatory reaction converts nitrates in elemental nitrogen. Organic substrates capable to sustain this reaction are various and easily recoverable (e.g. sawdust, cotton, woodchips etc.); thus, several carbon source has been already tested. The present paper reports the results of batch test carried out on four organic substrates used to promote biological denitrification; in details the organic matters tested were: sawdust, pine bark, cork and olive pomace. The first step of experimental study was focused to evaluate the organic carbon release capability of each substrate; particularly, organic matter has been keep in contact with tap water for almost 10 days; thus, samples of water has been periodically collected to measure Total Organic Carbon (TOC). Further, microcosm batch test has been carried out to reproduce in situ biological groundwater denitrification. In details, each batch reactor was prepared with a mixture of organic matter and agricultural soil, used to provide heterotrophic bacteria capable to promote biological denitrification, kept in contact with tap water artificially spiked at 60 mg NO_3-N L^{-1}. Batch test were realized in slow agitation condition, by using a vibratory plate, and were carried out for 12 days. All organic matters tested provided good results, in terms of removal efficiency; further, specific denitrification rate has been computed and ranged from 0.06 (olive pomace) to 0.51 (sawdust) mg NO_3-N L^{-1} $d^{-1}g^{-1}_{sub}$. Column test are actually in progress to evaluate the biological denitrification in continuous.

1. Introduction

Groundwater pollution by nitrates is nowadays one of the most frequently discussed subjects by scientific community as proved by the fact that the managing of the Nitrogen Cycle has been recognised as one of the 14 Grand Challenges for Engineering in the 21ST century (see www.engineeringchallenges.org) (**Schipper et al., 2010**). Such an interest is related basically to the dangerous effect that nitrate can cause to human health; in details, if assumed in rich concentration by youth organisms, presence of nitrates in water can cause methemoglobinemia; furthermore, in the intestine, nitrates can be reduced in nitrites which has been recognised as precursor of nitrosamines, compounds well known for their carcinogenic effects (**Soares, 2000**). Another key issue of nitrates contamination is related also to the effects for the environmental ecosystem; in details, the enrichments in nitrates can contribute to eutrophication, hypoxia, toxic algal blooms, shifts in the food chain, loss of biodiversity, loss of fish stocks and habitat degradation (**Galloway et al., 2003; Warneke et al., 2011**). In the last years the continuous growth of industrial-scale agricultures lead to an increase of use of fertilizers containing nitrogen and, as a consequence, the nitrates content in groundwater has significantly raised up (**Benyoucef et al., 2013**). Available technologies for groundwater denitrification can be divided in in-situ and ex situ treatments; particularly, in situ biological

treatments of groundwater denitrification are achieving growing consideration tanks to the good efficiency guaranteed and also to the fact that such a technologies usually are easily applicable and also at affordable costs (**Ying Xu et al., 2011**). Among the process capable to operate the biological denitrification in situ, those based on passive treatment such as permeable reactive barriers (PRB), bio barriers or bio trench has been validates by several application realized in pilot scale (inter alia **Gibert et al., 2008; Benyoucef et al., 2013**) and also in field scale (*inter alia* **Robertson et al., 2000; Schipper et al., 2005**). The denitrification process is operated by heterotrophic biomass, that are almost ubiquitous in nature (**Gamble et al., 1977; Zumft, 1992**); bacteria utilize the nitrates bound oxygen to oxidise the available organic carbon. The dissimilatory reaction that transforms nitrate in elementary nitrogen occurs in compliance with anoxic metabolism. One of the key issue for the correct application of this technology is the proper selection of the organic carbon source; indeed, as the process is drove by the carbon availability, the release of organic substrates strongly influence the process. The main features that a carbon source has to comply are the slow release and also the endurance. It is to be stressed that the slow release is a key point for the proper evolution of the reaction; indeed, if the amount of carbon available is too much higher than nitrates, high C:N ratio, it could be favoured the antagonistic reaction of dissimilatory nitrate reduction to ammonium (DNRA) (**Gibert et al., 2008)**, that convert nitrates in ammonium instead of elementary nitrogen; condition required for heterotrophic denitrification and DNRA are similar, but the fermentative process that leads to ammonium production is promoted by bacteria species (e.g. *Clostridia, Desulfovibrio, Vibrio, and Pseudomonas)* capable to use more efficiently the available carbon source, indeed DNRA process occurs with the transfer of eight electron while the heterotrophic denitrification requires transfer of five electron (**Burgin & Hamilton, 2007**). A wide range of materials are capable to release organic carbon by means of leaching process (e.g. cotton (**Della Rocca et al., 2006**), newspapers (**Volokita et al., 1996**), wood and his derived materials, as sawdust, softwood, coniferous and mulch (*inter alia* **Schipper et al., 2005; Greenan et al., 2006; Robertson et al., 2008; Gibert et al., 2008**). It has to be stressed that it is important to simulate the denitrification process at lab-scale highlighting the capacity of a selected carbon source for removing the contaminant of interest; indeed, to operate the in situ biological denitrification by using PRBs technology, the first step of the design process is to achieve information with batch and column test (**Gavaskar, 1999**). In such context, the present paper reports the results of batch test carried out on four organic substrates capable to release organic carbon for in situ biological groundwater denitrification. The criteria for selection of organic substrates consisted of the ability to release organic carbon, carbon vs nitrogen ratio (C:N) for woody material ranges from 30:1 to 300:1 (**Gibert at al., 2008; Vogan, 1993**), in good availability and low cost of retrieval. The first experimental step was focused to evaluate the carbon release capacity of the selected substrates; further, microcosm test **(Ovez, 2006; Gibert et al., 2008)** were carried out with artificial groundwater contaminated by nitrates to analyze the biodegradation process.

2. Materials and Methods

2.1 Organic carbon release
The organic substrates selected for the experimental study were sawdust, pine bark, cork and olive pomace; the particle size of the selected substrates was analysed with sieves ranging from 0.075 to 12.7 mm, the thinner material was found to be the pine bark, followed by olive pomace, cork and sawdust. In details, sample of each carbon source were dried in 105 °C oven for 24 h; further, 15 g of each carbon source were put in a 500 ml vessel filled with deionized water, up to avoid head space formation, and closed; each vessel has been kept in slow agitation by means of a vibratory plate. The release test were carried out at room temperature (22 ± 1,5 °C). The carbon release test was carried out for 10 days and daily samples of liquid phase were collected from each vessel to evaluate the total organic carbon (TOC) concentration chart by means of TOC-Vcsn Shimadzu Total Organic carbon Analyzer; liquid volume spilled to perform the analysis was replaced with deionized water.

2.2 Microcosm test
Four batch test were carried out for 12 days to observe the denitrification process sustained by different carbon source. Four solid blend, containing agricultural soil and organic carbon, were kept in contact with artificially nitrates contaminated water. In details, an apparent volume of 250 ml was obtained manually blending 200 ml, measured in a graduated glass cylinder of 500 ml, of each carbon source with 50 ml of agricultural soil, employed to provide heterotrophic bacteria to the batch reactors. In details, density of each substrates, measured with picnometer, characteristic diameter d60, mass and the correspondent organic carbon initial content, measured in accordance with **UNI10780:1998** standard, are summarised in table 1 together with agricultural soil.

Table 1: Batch test composition and features of substrates

Batch test	Substrate	Mass [g]	Organic carbon [%]	density [g L^{-1}]	d$_{60}$ [mm]
1	Sawdust	19	39.76±1.63	79	4.12
2	Pine bark	54	36.96±1.33	270	2.76
3	Cork	22	53.46±0.96	110	3.94
4	Olive pomace	93	43.03±1.67	465	3.74
	Soil	42	14.01±0.44	700	0.94

Each batch reactor was further fed with tap water artificially spiked at 60 mg NO_3-N L^{-1}, added to tap water as potassium nitrate (KNO_3), furthermore potassium hydrogen phosphate (K_2HPO_4) has been added to the solution to guarantee a phosphorus concentration equal to 2 mg PO_4-P L^{-1} to avoid limiting effect on bacteria metabolism. Further the microcosm test were carried out at room temperature (~21°C) and in slow agitation condition, similar to what described for release test. Liquid samples (15 ml) was collected at 0, 2, 6 and 24 h and further at 2, 4, 7, 10 and 12 days; volume collected for sampling was replaced with tap water. Collected samples were analysed for nitrogen forms, NH_4-N, NO_2-N, NO_3-N, by means of MERCK test cuvette and MERCK Spectroquant NOVA 60; TOC was measured by using TOC-Vcsn Shimadzu Total Organic carbon Analyzer; dissolved oxygen (DO), pH and ox-reduction potential (Redox) were measured by using WTW Multi 340-I multimeter and specific probes WTW Cell-ox 325, WTW Sentix ORP and WTW pH-electrode Sentix 41-3. Each analytic measure was twice performed and mean result was reported. Removal efficiency has been computed at the end of the experimentation and maximum nitrates removal rate, computed as ΔNO_3-N max vs Δt, has been evaluated and referred to the mass of substrate available in the batch test.

3. Results and discussion

3.1 Release test
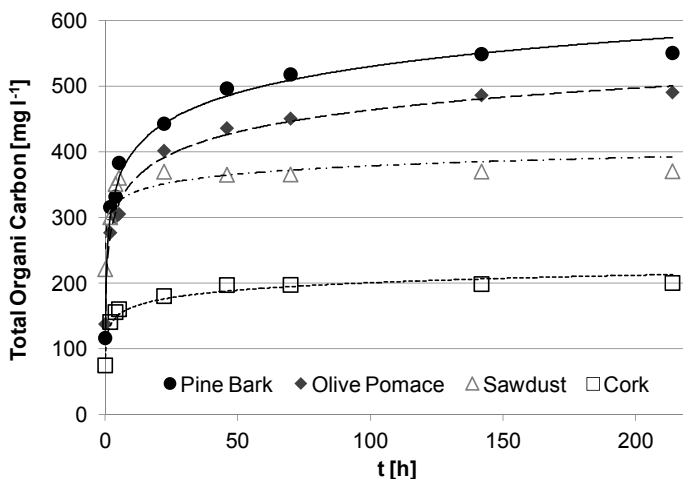Organic carbon release results are shown in figure 1 with the interpolating trends.

Figure 1: Carbon release results

In details, it's worth noting that the higher increase in the release occurs in the initial hours of liquid-solid contact and further, after about 24 hours, the measured organic carbon concentration slowly increase fitting a logarithmic regression. In details, with the same mass of substrate available (15 g), the higher concentration value was reached by pine bark, that supplied about 500 mg TOC L^{-1} after about 50 hours of contact; at the same time, cork supplied only half of the TOC concentration reached by pine bark batch test. Such differences are probably due to the influence of the organic carbon content, shown in table 1, and also to the different particle size of the selected substrates; indeed, pine cork results to be the thinnest

of the substrates followed by olive pomace, on the contrary, the organic carbon content of oil pomace was higher than the pine bark, suggesting that influence of the particle size is more stringent than the organic carbon content; such aspect it is also remarked by the relatively low release achieved by cork, substrate with the higher organic carbon content but also with an high particle size.

3.2 Microcosm test

All the microcosm batch test carried out shown significant denitrification; in details, the achieved efficiency in nitrates removal, at the end of the considered period, were measured equal to 59,7 %, 60,3 %, 77,7 % and 80,8% for Pine bark, Olive Pomace, Sawdust and Cork respectively; furthermore, specific denitrification rate, for mass of substrate, has been computed for each batch test equal to 0,05 mg NO_3-N L^{-1} d^{-1} g_{sub}^{-1}, 0,09 mg NO_3-N L^{-1} d^{-1} g_{sub}^{-1}, 0,28 mg NO_3-N L^{-1} d^{-1} g_{sub}^{-1} and 0,37 mg NO_3-N L^{-1} d^{-1} g_{sub}^{-1} for Olive pomace, pine bark, cork and sawdust respectively. The concentration values measured for the whole experimental set, with the interpolating logarithmic trend, throughout the 12 days are shown in the chart of figure 2.
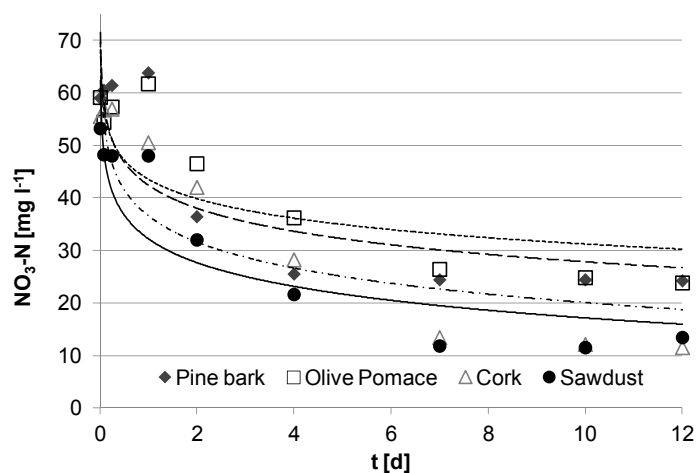
Figure 2: Microcosm batch test results

Chart reported in figure 2 shows that for Pine bark and Olive pomace batch reactors there was an initial nitrification phase; indeed nitrates concentration, spiked for each batch test at 60 mg NO_3-N L^{-1}, rose up to 61,7 and 63,8 mg NO_3-N L^{-1} for olive pomace and pine bark respectively. Such circumstance is probably related to the initial DO content of the tap water, measured equal to 8,12 and 8,06 mg DO L^{-1} for olive pomace and pine bark respectively, that allowed an oxidative process of the organic nitrogen present in the substrates by bacteria nitrifying community present in soil; indeed a certain amount of organic nitrogen is present in the substrate, **D'Angelo (2010)** measured in olive pomace an amount of nitrogen equal to 0,96%, **Trois et al. (2010)** reported an amount of nitrogen equal to 0,53% in pine bark; minor amount are reported for cork and sawdust. Another important aspect to discuss is related to the apparent discrepancy between the carbon release test and the microcosm results; in details, carbon release test shown that pine bark and olive pomace were the substrates capable to release the higher amount of organic carbon available, on the contrary the highest denitrification efficiency was achieved by the lowest releasers of carbon, cork and sawdust. Such aspect is probably due to the biodegradability of organic carbon released; effectively, for all the batch test carried out the complete denitrification was never achieved, significant concentration of nitrates were measured in all the batch reactors at the end of the experimentation (24,2, 23,8, 13,4 and 11,5 mg NO_3-N L^{-1} for pine bark, olive pomace, sawdust and cork respectively) suggesting a probably lake of organic carbon available in spite of the high amount releasable. Such aspect should be better investigated by evaluating the carbon biodegradability checking the biochemical oxygen demand (BOD_5) vs Chemical Oxygen Demand (COD) ratio. In any case, the confirm that the biological denitrification process took place in each batch reactor is provided by the trends of nitrites reported in figure 3.
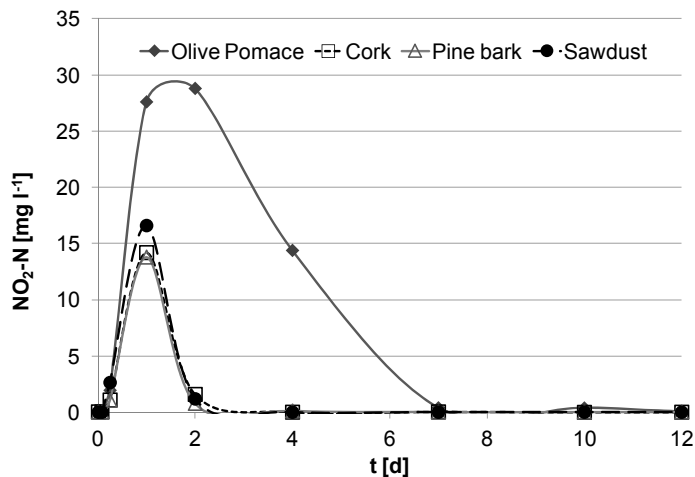
Figure 3: Nitrites concentration measured in microcosm batch test

The concentration reported in figure 3 highlights the conventional trend of nitrites in a biological sequential process as the denitrification process. Indeed, denitrification dissimilatory reaction proceed step by step in accordance with the path described by **Soares** (2000) in Eq (1).

$$NO_3^- \rightarrow NO_2^- \rightarrow N_2 \qquad\qquad (1)$$

The initial formation, the progressive temporary storage and the further dissolution of nitrites confirm the occurrence of the biological process.

The others measured parameters did not shown significant trend throughout the whole experimentation; DO measure confirmed the anoxic condition together with the ORP measured (< 50 mv in all the batch test since 24 h from the beginning); pH variation were not significant and ammonium measurement shown that the DNRA process did not significantly occurred throughout the experimentation, less than 5% of available nitrogen were converted in ammonium rather than in elementary nitrogen.

4. Conclusion

The present study reports the results of batch test carried out on four different organic carbon source capable to promote biological denitrification. In details, although it exists a consolidate state of art relative at the groundwater denitrification, frequently it is necessary to recur to technologies very expensive and that generate a concentrate liquid waste to be further treated (**Soares, 2000**). From such point of view the in situ biological denitrification can surely play the role of the most environmental friendly technology; Furthermore when the substrate used to promote the denitrification process is conventionally considered a waste, like the substrates tested in the present paper.

Batch and microcosm test shown significant features of the selected materials. It has been highlighted that the particle size of the material can play a role more stringent of the carbon content itself. The biological process that occurred in the batch test revealed that it is important also the knowledge of the biodegradability of the released carbon. Such experimental study intend to be the first step of a wider study focused on the in situ biological denitrification, promoted by the reported substrates and observed in steady condition by means of column test.

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