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Original

Availability:
This version is available at: 11583/2624367 since: 2015-11-30T09:20:13Z

Publisher:
Elsevier

Published
DOI:10.1016/j.jconhyd.2015.06.006

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Integrating NZVI and Carbon Substrates in a Non-Pumping Reactive Wells Array for the Remediation of a Nitrate Contaminated Aquifer

Published in

Journal of Contaminant Hydrology

Volume 179, August 2015, Pages 182–195

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1. Bottles used in batch experiments

![Bottles used for batch experiments](image)

Figure 1: Bottles used for the batch experiments.

2. Numbers of Denitrification Bacteria

The abundance of denitrifying populations in the contaminated water in different treatments (A to F) was assessed by counting the cell number (CN) using the traditional plate count method on an agar background in Petri dish (García-Armesto et al., 1993). The denitrifying CNs were analyzed before starting the experiment (average value in the natural groundwater), and at the end of the experiment (13th day) for the six tests separately. The initial average abundance of denitrifying cells for six treatments was \((6.03\pm0.15)\times10^4\) CFU/ml, whereas the values of CN at the end of the batch experiments ranged from \(7.2\times10^4\) CFU/ml for test F (containing only NZVI) to \(157.8\times10^4\) CFU/ml for test D (containing mixed carbon substrate and NZVI) (Figure 2). Therefore, the number of denitrifier bacteria did not grow significantly (19.4%) when the NZVI was the only reactive agent (bottle F). When the carbon substrate was used as the only reductant agent (bottle A) an increase of 37.7% was observed. Conversely, a dramatic increase of the denitrifier bacteria community was observed in bottle D (437%), which corresponds to the mixture of carbon substrates and NZVI with 20g Maize cobs, 10g beech sawdust, and 2.0g NZVI (Figure 3). In addition, for a specific mass of carbon substrates, it was observed that increasing the NZVI concentration resulted in a more relevant growth of the denitrifier bacteria community (treatments from B to C to D).
Figure 2: Number of denitrification cells for different treatments.

![Bar chart showing cell numbers for different treatments.]

- Treatment A: 8.3 x 10^4 CFU/ml
- Treatment B: 13.9 x 10^4 CFU/ml
- Treatment C: 87.0 x 10^4 CFU/ml
- Treatment D: 157.8 x 10^4 CFU/ml
- Treatment E: 148.7 x 10^4 CFU/ml
- Treatment F: 7.2 x 10^4 CFU/ml

Figure 3: Denitrifier bacteria colonies in Petri dish for solution: (a) at start of experiment, and at the end of (b) treatment A, (c) treatment F, and (d) treatment D.
3. Nitrate removal in the downstream piezometers

Figure 4: Average cumulative percentage of nitrate removal rate (ACPNR) from groundwater by NPRWs system observed in the downstream piezometers (P3 to P7).
4. Kinetic model estimation for nitrate reduction and ammonium production and stripping

Figure 5: Pseudo first-order kinetic model estimation (solid lines) for nitrate reduction and ammonium production and stripping for different treatments. Observed values are shown as colored circles and triangles.
Figure 5 (continued).