Introduced biodenitrification of nitrate-polluted groundwater: engineering strategies and assessment of chemical, microbial and isotope effects

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INDUCED BIODENITRIFICATION OF NITRATE-POLLUTED GROUNDWATER: ENGINEERING STRATEGIES AND ASSESSMENT OF CHEMICAL, MICROBIAL AND ISOTOPE EFFECTS

PHD THESIS

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A PhD dissertation presented by

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to obtain the degree of Doctor in Earth Sciences

Research work conducted in the "Mineralogia Aplicada I Medi Ambient" research group (MAiMA), with the collaboration of the Department of Microbiology, Faculty of Biology, and D D'ENGINY BIOREM S.L., under the supervision of

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ABSTRACT

Nitrate pollution is a widespread problem that affects water bodies in many regions of the world, undermining water quality and therefore its safe use. Despite the application of improved management practices, nitrate pollution seems to increase, particularly in groundwater. The Nitrate Vulnerable Zone (NVZ) designation in Europe, for instance, has increased from 35.5% of the EU-15 territory at the end of 1999 to 44% at the end of 2003, and the Commission's report for the period 2004-2007 revealed that 15% of groundwater monitoring stations in the EU-27 territory showed nitrate levels above the limit of 50 mg of nitrates per liter. Some trends towards nitrate attenuation are observed, but at least 33% of water bodies will clearly fail in achieving the 2015 goals set by the Water Framework Directive.

Several efforts have been addressed to either reduce nitrogen inputs or to decrease its already accumulated levels, particularly by designing nitrate-removal technologies aimed at recovering drinking-water standards. This PhD thesis, hence, focuses on the optimization of an already existing technology for nitrate-removal: enhanced *in situ* biodenitrification (EISB), which is now regaining attention due to its economic and environmental benefits and its potential for scale-up and design of case-specific solutions. EISB is an engineered application of microbial heterotrophic denitrification aimed at in situ nitrate removal from groundwater. Aimed at stimulating facultative denitrifiers, EISB is based on the injection of a C source into the aquifer. Microbial denitrification is then enhanced in a designated area of the aquifer, creating a biologically active zone (often referred as biowall) which removes nitrate from the naturally-flowing groundwater.

Among the different factors that affect the technical feasibility of EISB, the type and quantity of the injected C source is a key issue, particularly due to its influence upon the microbial processes that determine the treatment performance. The understanding of the subsurface geology and hydrogeology is also an issue of concern, particularly if highly heterogeneous media, such as fractured aquifers, are meant to be remediated.

Aimed at achieving our research goal, several EISB experiments were developed at different scales -batch, flow-through column and pilot scale- and involving different geological media -granular and fractured-. Combined chemical, microbial and isotope monitoring tools where applied to gain a better insight on the denitrification process and thus improve technology design and optimization.

The first set of batch-scale experiments focused on testing the viability of in situ heterotrophic denitrification and determining the most suitable biostimulants for a case-specific scenario in the Osona region, a Catalan NVZ showing historic nitrate pollution up to 200 mg/L. Native microbiota was stimulated and nitrate reduction was effectively achieved by addition of a carbon source (ethanol or glucose) as well as a phosphorous source (disodium hydrogen phosphate). Transient nitrite accumulation was observed, especially when using glucose as the C source. The N and O isotope fractionation was determined to be -13.0‰ and -17.1‰ for εN and -8.9‰ and -15.1‰ for εO in ethanol and glucose-amended experiments respectively, resulting in εN/εO values of 1.46 (ethanol-amended experiment), and 1.13 (glucose-amended).

Organic carbon (OC) consumption in batch-scale experiments, expressed as $\Delta C/\Delta NO_3^-$, varied slightly depending on the type of C source used: 1.6 mmolOC/mmolNO_3^- for

ethanol and 2.2 for the glucose, similarly to stoichiometric values associated with nitrate respiration (0.83 and 1.25 mmolOC/mmolNO_3^- respectively). When deriving stoichiometric reactions that accounted not only for the amount of electron donor used for nitrate respiration but also for cell synthesis, the following values were determined: 1.9 and 2.0 mmolOC/mmolNO_3^- for ethanol and glucose-induced biodenitrification respectively. These values were used for the numerical modeling of batch-scale experiments, aimed at quantifying microbial kinetics by applying the modified Monod expression. The (geochemical) numerical model also indicated a different effect of mineral precipitation on ethanol or glucose-induced denitrification, an effect that is linked to a different alkalinity production. Such effect could be taken into account when designing and/or optimizing EISB systems, particularly as a way to control geochemical clogging.

A pilot-scale application was then performed at the site, aimed at assessing the viability of EISB in a fractured aquifer. Ethanol was now used as the main C source, and based on lab-scale results, P was also added. Again, transient nitrite accumulation was detected, and evidences for incomplete denitrification and coexistence of other respiration processes (such as iron or sulfate reduction) and autotrophic denitrification were observed. Sulfate isotope characterization proved that autotrophic denitrification linked to sulfide oxidation could be occurring along with heterotrophic denitrification, while sulfate-reduction couldn't be verified.

Overall, results suggested that stimulated heterotrophic denitrification could be applied as a remedial alternative in a fractured media and despite the complexity of the formation. However, a deep understanding of the system is required and efforts must be addressed to control microbial population and stability as a key issue to avoid the decrease of groundwater quality due to incomplete denitrification or secondary respiratory processes. Different engineering approaches such as feeding or pumping strategies could help improving the system performance.

Aimed at testing the impact of such engineering approaches upon resulting water quality, a second study-case was studied, now in an alluvial media. . A flow-through experiment was built to simulate an EISB system and assess the influence of different C addition strategies upon the denitrification process. Heterotrophic denitrification was stimulated by the periodic addition of a C source (ethanol), and 4 different addition strategies were evaluated, being the first-one a weekly injection, and the others a daily injection with decreasing amounts of C.

Enhanced denitrification was stimulated following the first C addition, easily achieving drinking water standards for both nitrate and nitrite. Water quality in terms of remaining C, denitrification intermediates and other anaerobic respiration products varied during the experimental time. Ethanol, for instance, showed a cyclic behavior during the weekly feeding strategy while it was completely depleted when injected daily. A quasi steady-state nitrate outflow, similar to ethanol's, was obtained in daily injection scenarios, with nitrate levels ranging from non-detected values and up to 10 mg/L, and nitrite's remaining undetected. No dissimilatory nitrate reduction to ammonium was ever detected and some secondary microbial respiration processes, mainly manganese reduction, were suspected to occur temporarily.

Overall, results showed that biodenitrification could be successfully achieved by a daily addition of a C source slightly higher than the stoichiometric value, diminishing the

accumulation of non-desired products and the biofilm growth and still obtaining the required denitrification results. Reducing the C/N ratio enables us to reduce treatment costs while achieving a better water quality in terms of remaining C and residual microflora, and potentially reducing the biofouling effect due to the increase of endogenous respiration. Endogenous activity –that provides internal C for denitrification- may become important when low C/N values are used, keep denitrification temporarily ongoing and reducing the biofilm growth, but may affect the biodenitrification performance at longer operation times. Such aspects should be further evaluated using modeling and/or experimental tools. Furthermore, results suggested that not only the feeding strategy but also the biofilm life-time have a direct effect on microbial population structure and hence on the biodenitrification performance, reducing the accumulation of nitrite over time.

The obtained εN/εO fractionation values for the flow-through experiment (1.01) fell within the low-end of previously reported data (varying from 0.9 to 2.3), an effect that may be linked to faster microbial kinetics in enhanced vs. natural biodenitrification. Similar low values were observed in our previous batch-scale experiments as well as in other work conducted in our lab. Concerning ethanol's fractionation, on the other side, a two-trend behavior was observed, probably indicating a change in the dominating C-consuming population. Interestingly, the second trend suggests an inverse fractionation of the C source that got depleted while being consumed.

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INTRODUCTION

1 INTRODUCTION

1.1 THESIS GOALS AND WORK PLAN

1.1.1 PROBLEM STATEMENT

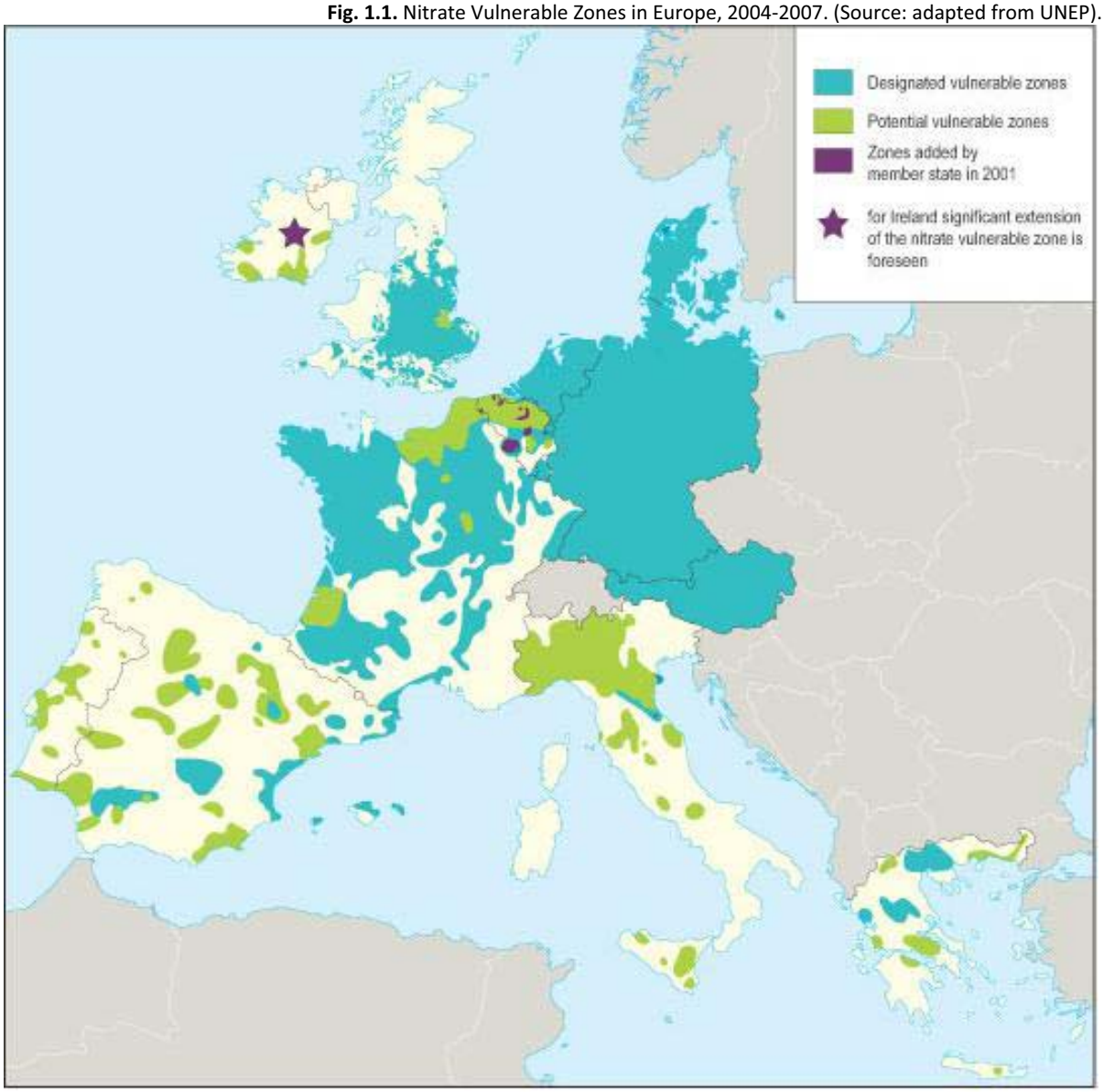
Nitrate pollution is a widespread problem that affects water bodies in many regions of the world, undermining water quality and therefore its safe use. Indeed, nitrate has showed to cause adverse health effects in animals and humans (Forman, 1989, Goodrich et al.,1991; Fan and Steinberg, 1996), such asmethemoglobinemia (the baby blue syndrome) in infants and young children (Comly, 1945) and potentially causing cancer in adults, although evidences about carcinogenicity in literature are inconclusive and conflicting (EPA, 1991 and 2006). This is why drinking water standards have been long ago established, commonly set at 50 mg/L (the World Health Organization guideline value) and adopted in Europe through the Directive 98/83/CE on the quality of water intended for human consumption. More recently, the European Water Framework Directive (WFD, 2000/60/EC), and its related Groundwater Directive (2006/118/CE) required to achieve such standards also in groundwater, a goal that should be attained by the end of 2015.

Knowing that the major causes of nitrate pollution are linked to intensive farming and agricultural activities, as well as to the leaking from sewage systems, efforts to diminish nitrate accumulation are usually focused on reducing nitrogen inputs into the subsurface, often by promoting the use of good farming practices. In this context, the Nitrates Directive (91/676/EEC) appears as one of the earliest pieces of EU legislation aimed at controlling pollution and improving water quality, requiring the member states to designate Nitrate Vulnerable Zones (NVZs) -as areas of land that drain into nitrate-polluted waters- and to develop specific action programs for compulsory implementation in these nitrate-vulnerable zones. The NVZ designation has increased ever since (see Fig. 1.1), from 35.5% of the EU-15 territory at the end of 1999 to 44% at the end of 2003. From 2003 onwards, further designations were made in Italy, Spain, Portugal and United Kingdom. Belgium established a procedure to increase its designation to include 42% of Wallonia territory and all Flanders, while Austria, Denmark, Finland, Germany, Ireland, Lithuania, Luxembourg, Malta, the Netherlands and Slovenia decided to provide the same level of protection to their whole territory, rather than designate nitrate-vulnerable zones. On the other hand, in terms of detected nitrate affection in groundwater, the Commission's report for the period 2004-2007 revealed that 15% of groundwater monitoring stations in the EU-27 territory showed nitrate levels above the limit of 50 mg of nitrates per liter. Some trends towards nitrate attenuation are observed, but at least 33% of water bodies will clearly fail in achieving the 2015 goals set by the Water Framework Directive.

Of particular concern in areas in which water is already a scarce resource, nitrate pollution in groundwater generates social tension and increases watershed and environmental disequilibrium. However, good up-stream practices have not yet lead to a noticeable recover in groundwater quality. Therefore other solutions, mainly the search for alternative water sources, have been commonly adopted, thus abandoning the focus on the underground resource. These solutions, however, do not address resource recoverability, and, often, increase the environmental costs required for water transfer and distribution. Also, drinking water is often imported at higher prices, and a 10-

fold increase is expected to occur in some areas in the next 15 years. Local governments and final users will have to internalize such increasing costs. .

Fig. 1.1. Nitrate Vulnerable Zones in Europe, 2004-2007. (Source: adapted from UNEP).



In these areas, alternative management tools must therefore be considered, not only aimed at reducing nitrogen inputs but also at actively recovering groundwater quality to enable access to a useful resource. Several remedial strategies and technological alternatives are known to achieve denitrification and allow groundwater to reach drinking water standards. Among these, enhanced *in situ* biological denitrification (EISB) appears to be the most economical and viable for small communities and private users. In this research, we believe that EISB design and management could be improved to scale-up approaches and provide aquifer-scale groundwater recovery as well as addressing mid and long-term climate-change water-related challenges. With such a goal, and framed on a future large-scale application, this thesis aims at studying different engineering approaches to optimize *in situ* biodenitrification alternatives to diminish non-

point nitrate pollution and face aquifer recovery and exploitation at different scales. Furthermore, traditional and innovative monitoring tools are applied to deepen into the microbial process.

1.1.2 RESEARCH GOALS

Among the different factors that affect the technical feasibility of EISB, the type and quantity of the injected C source is a key issue, particularly due to its influence upon the microbial processes that determine the treatment performance. Site characteristics, existing microbial populations and groundwater flow are also factors of concern. In this context, and defined as our **main research hypothesis**, we understand that EISB design parameters can be modified and case-specifically adapted to achieve viability of EISB in different geological formations. Furthermore, by combining chemical with isotopic analytical tools (the latter being of a more recent development), we should be able to evaluate technology behavior, assessing both reactive and non-reactive involved processes that can affect system performance.

Therefore, the main **research goal** is to test the feasibility of heterotrophic EISB in two different geological media, an alluvial sandy aquifer and a fractured formation, and assess its performance through the combined use of chemical, microbial and isotopic tools. The application of EISB in a fractured media has not been previously reported. The hydrogeological complexity of such a formation poses a challenge to both site characterization and remediation, mainly due to its intrinsic heterogeneity.

Aimed at achieving the stated research goal, several EISB experiments were developed at different scales (batch, 1-dimension column and pilot scales) and involving different geological media (granular and fractured). This research is meant to be a one-step forward for the nitrate-pollution research developed across the world and in particular by the **Grup de Mineralogia Aplicada i Medi Ambient** (MAIMA) at the University of Barcelona, that focuses on groundwater management and nitrate attenuation. MAIMA research projects have been a very helpful approach to prove the validity of isotopic tools to assess nitrate pollution and its attenuation in groundwater. It is now our goal to apply them in enhanced biodenitrification systems aimed at improving the treatment design a field scale. This thesis' goal is to achieve a knowledge transfer between previous research at lab and field scale and identify innovative approaches for biodenitrification technologies.

Furthermore, beyond our main generic research goal, several **specific goals** were defined:

1. design, construct and operate heterotrophic enhanced biodenitrification tests at different scales;

2. assess biodenitrification kinetics at different scales;

3. evaluate the technology feasibility at flow-through and pilot-scale experiments by applying different amendment strategies; and finally,

4. combine the use of chemical, microbial and isotopic tools to characterize the denitrification process and assess other chemical and/or biochemical reactions that may be involved. And in particular, the determination of case-specific fractionation factors (ε) for EISB is pursued.

Finally, it is pertinent mentioning that the results obtained in this research are being used

for another PhD thesis aimed at biodenitrification modeling (including chemical, microbial and isotopic processes), a thesis that will be submitted to the *Universitat Autònoma de Barcelona*. Both research works were developed at D D'ENGINY BIOREM S.L., an environmental EISB pioneer firm, with the collaboration and participation of the university (*Universitat de Barcelona*, *Universitat Autònoma de Barcelona*) and the financial support of D D'ENGINY BIOREM S.L., the *Agència Catalana del'Aigua* (Catalan Water Agency), CIDEM and Catalan and Spanish Governments.

1.1.3 WORK PLAN

In order to fulfill main and specific research goals, several tasks were developed at different scales, including lab and field experiments:

1. **site selection**: two sites were selected for assessing the technical viability of EISB. Both selected cases represent real-case scenarios in which different stakeholders were involved and different groundwater management goals were established. Thus, selection criteria included technical as well as socio-political and economic factors. Site selection was approved by the Catalan Water Agency, the main responsible Administration;

2. **site characterization**: both sites were characterized in terms of geology, hydrogeology, land-use and site constrictions in order to (a) define site conceptual model and (b) obtain site-specific parameters for EISB design;

3. **biodenitrification treatability-assessment**: a first set of batch experiments was developed aimed at assessing the treatment characteristics so that further detailed experiments could be successfully designed and developed. Required experimental time, monitoring schedule and nutrient needs were among the studied parameters during this task;

4. **batch-scale experiments for study case 1**: EISB was tested at study case 1 by developing different batch-scale experiments aimed at assessing microbial kinetics and determining isotope enrichment factors for two different carbon sources;

5. **numerical-modeling for study case 1**: kinetics parameters from batch-scale experiments were quantified by numerical modeling, applying the Monod kinetic approach commonly used to describe the relationship between bacterial growth and substrate concentration;

6. **pilot-scale application for study case 1**: based on site characterization and lab-scale experimental results, an EISB system was designed, installed and implemented at a pilot-scale in Roda de Ter (Osona, Barcelona). The system was operated and monitored for five months by periodic addition of a C and a P source. Nitrate isotope enrichment factors determined at the lab-scale (during task 4) were applied to assess the biodegradation efficiency, while sulfate isotope analysis were used to study other microbial processes (such as autotrophic denitrification or sulfate-reduction) potentially occurring at the site;

7. **design of a column lab-scale aquifer:** a 70-cm long and 8-cm diameter column was designed and constructed to reproduce a one-dimension (1-D) aquifer at the lab-scale;

8. **flow-through experiment for study case 2:** EISB was assessed at site 2 during a 10-month flow-through experiment using different C-addition strategies and ethanol as the carbon source. Chemical and microbial parameters were monitored, and nitrate isotope enrichment factors determined aimed at a future field-scale application.

1.1.4 THESIS CONTENTS AND STRUCTURE

This PhD thesis is structured around two study-cases, located both in Catalonia. The first one corresponds to a pilot-scale EISB application in a fractured aquifer in Roda de Ter (Osona, Barcelona) -the first EISB field application conducted in the country and the first reported worldwide in a fractured media- and the latter to a lab-scale column experiment aimed at designing a future EISB application in the Argentona aquifer (Maresme, Barcelona).

Most relevant results of the conducted research work have been already published or are currently under review for publication. Related thesis papers are referenced here and attached as appendixes:

- Vidal-Gavilan, G., Folch, A., Otero, N., Solanas, A. M., Soler, A. 2013. Isotope characterization of an *in situ* biodenitrification pilot-test in a fractured aquifer. Appl. Geochem. 32, 153–163.

- Rodríguez-Escales, P., van Breukelen, B., Vidal-Gavilan, G., Soler, A., Folch, A. Integrated modeling of biogeochemical reactions and associated isotope fractionations at batch-scale: a tool to monitor enhanced biodenitrification applications.

- Vidal-Gavilan, G., Carrey, R., Solanas, A. M., Soler, A. Amendment strategies for groundwater heterotrophic denitrification: chemical, microbial and isotopic assessment of a 1-D flow-through experiment. Submitted to the Science of the Total Environment journal.

Main developed tasks, lab and field-scale results, conclusions and other relevant information are then reported in this thesis as follows:

- **CHAPTER 1: INTRODUCTION**, a contextual framework of nitrate pollution in groundwater, its management needs, remedial alternatives and the fundaments of EISB as a remedial technology and of isotopic tools for denitrification assessment. Research goals are also presented in this chapter.

- **CHAPTER 2: CASE DESCRIPTION AND SITE CHARACTERIZATION**, where the two study sites are described and the main results of site characterization are presented. Site characterization, in terms of geology, hydrogeology, microbiology, land-use and site constrictions, together with remedial goals, are key needs for subsequent EISB design and operation.

- **CHAPTER 3: RESULTS, DISCUSSION AND MAIN CONCLUSIONS**. This chapter summarizes the main results, discussions and conclusions of the published[1] research work, including the lab and pilot-scale tests of an EISB application at site #1, the numerical model built to quantify and evaluate batch-scale microbial kinetics occurring at case #1, and the flow-through experiment developed to assess the technical viability of EISB at site #2. Main conclusions related to defined research goals are also included.

[1]Already published work or in press.

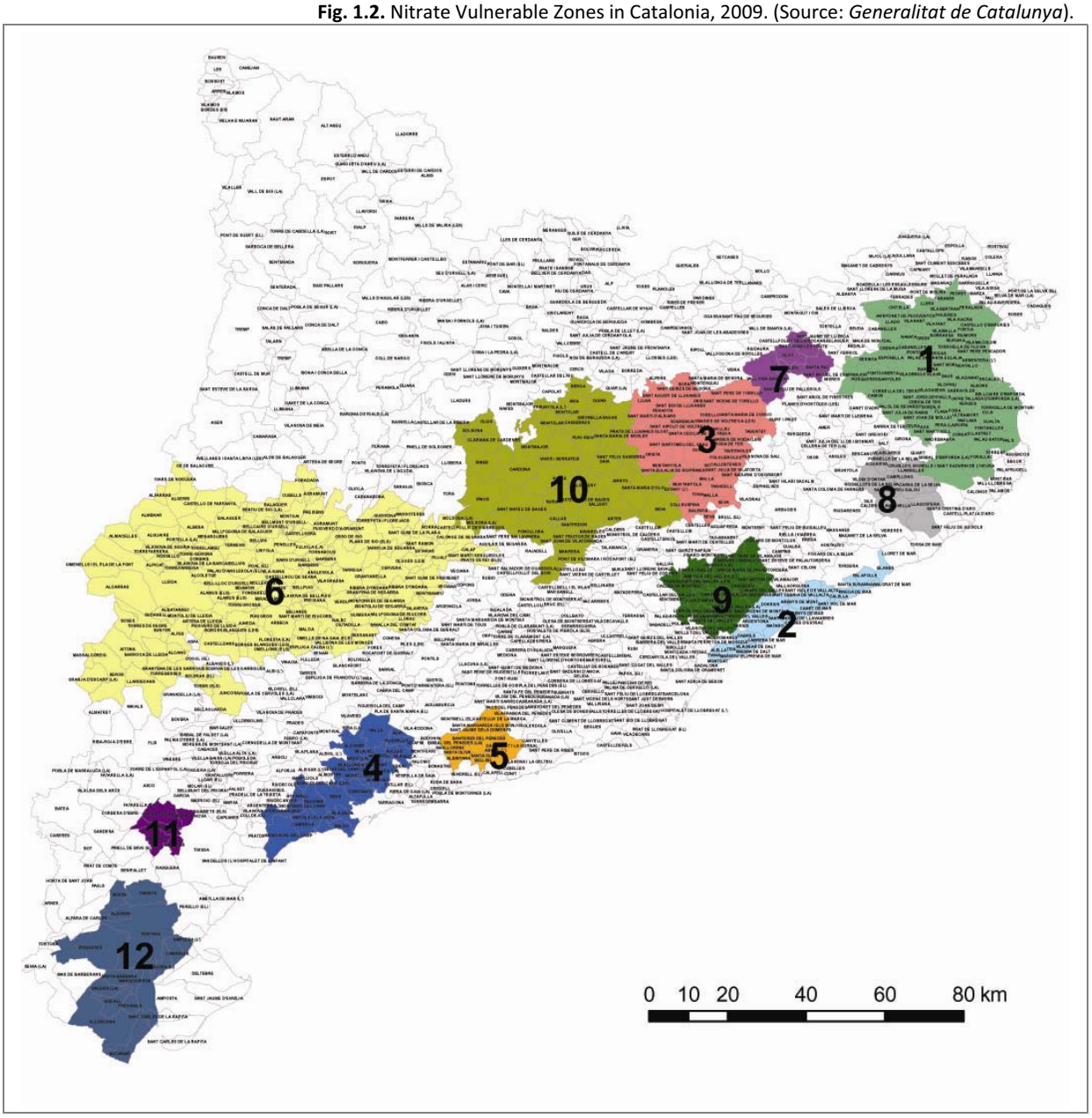
Experimental and analytical methodology is described on each attached paper. For site characterization, adopted methodology is included on chapter 2. A PhD thesis executive summary is included at the beginning of the document.

1.2 NITRATE POLLUTION IN GROUNDWATERS: A LONG-LASTING ENVIRONMENTAL PROBLEM

Nitrate pollution in groundwater has been constantly reported as one of the main environmental issues threatening water quality due to its potential health effects in drinking water (Comly, 1945, Forman, 1989, Fan and Steinberg, 1997, Goodrich et al., 1991, Warm et al.,2005), nutrient enrichment of terrestrial and aquatic ecosystems (Vitousek et al., 1997; Rabalais, 2002;Galloway et al.,2003) and contribution to global warming (Vitouseket al., 1997; Groffmanet al., 2000). In most naturally occurring environments, nitrate concentrations in ground water are usually <3 mg/L (Smith et al.,1987).However, the nitrate drinking water standard, commonly set as 50 mg/L, is often exceeded in private and domestic wells. In agricultural areas of North America, for example, 5 to 46% of the domestic water wells exceeded the drinking water quality standard in different studies conducted among its territories (EPA, 1992; Meyer, 1994; Hamilton and Helsel 1995; Gosset al., 1998). In Europe, Commission's report for the period 2004-2007 revealed that 15% of groundwater monitoring stations in the EU-27 territory showed nitrate levels above the limit of 50 mg of nitrates per liter, and at least 33% of water bodies will clearly fail in achieving the 2015 goals set by the Water Framework Directive. In Catalonia, 12 areas were designated as Nitrate Vulnerable Zones (NVZ) according to the 91/676/EC Directive (the Nitrates Directive) on the 2009 update (*Acord de Gorvern* GOV/128/2009, de 28 de *juliol*) (see Fig. 1.2), thus increasing the vulnerable territory from the original 6 areas designated in 1988 (*Decret* 238/1998). Assessing historical trends in nitrate contamination in groundwater is actually problematic due to the scarcity of long-term nitrate-concentration data. However, studies show a progressive increase of nitrate levels, despite the potential occurrence of natural attenuation through denitrification (Puckett et al, 2011 and references herein).This trend seems parallel to the increase in the use of industrially fixed N-fertilizer, animal manure and atmospheric deposition.

Several causes have been reported as the main sources of nitrate pollution in groundwater, their contribution varying across the territory. In general, they are linked to agricultural and farming activities (fertilizers and manure spreading, manure lagoons) as well as to urban (mainly septic systems and sewage networks). The impact of sewage on groundwater can be due to direct input from leaking or to indirect from the use of treated wastewater for irrigation. Overall, the particular distribution of nitrate in a water body will be determined by its long-term history of nitrate inputs, the groundwater flow system, and the occurring nitrate attenuation processes.

Fig. 1.2. Nitrate Vulnerable Zones in Catalonia, 2009. (Source: *Generalitat de Catalunya*).



1.3 REMEDIAL AND MANAGEMENT STRATEGIES

The accumulation and persistence of nitrate in groundwater is a long-lasting and widely-discussed problem in need of local and regional solutions, solutions that have been traditionally aimed at reducing nitrogen inputs into the subsurface. Due to the increasing demand for drinking water in rural communities and urban centers, water managers and agricultural organizations also started to focus their efforts on reducing existing nitrate contamination in local and regional aquifers, so thus groundwater could be safely used. In this context, the European Water Framework Directive (2000/60/EC), together with its developing Groundwater Directive (2006/118/CE), set the nitrate quality standards to be attained by 2015, establishing a value of 50 mg/L, the same as the drinking water standard.

When managing nitrate-polluted water supplies for water-distribution needs, end-pipe alternatives have commonly considered the application of 4 different strategies: (1) well closure -and hence, resource loss-; (2) water blending -that is, the combination of different nitrate-level waters-; (3) connection to an alternative water supply system, and (4) the use of *ex situ* treatment technologies whenever the previous alternatives are not feasible. Less frequent is (5) the use of *in situ* denitrification technologies, that is, enhanced biodenitrification.

Among *ex situ* treatment technologies, the most common include ion exchange and reverse osmosis, and less often electrodialisys, biological denitrification or wetlands. These nitrate-removal technologies differ mainly in design, performance and cost. Ion exchange and reverse osmosis, both with a long market-level experience, produce high-quality water, with removals ranging from 95 to 99% in the case of ion exchange, and from 85 to 95% in reverse osmosis, varying depending on the initial quality of the water, the system pressure, and the water temperature. But both require high maintenance costs. Although simple and stable to operate, ion exchange must deal with waste-management issues, and may not be effective if chloride reduction is also required. In the case of reverse osmosis, the high energy consumption, added to the above-mentioned maintenance costs, increases the overall cost of the treatment.

Recently, due to the constant effort to find a definitive and sustainable nitrate-removal alternative, other technologies have been developed: selective catalytic hydrogenation, currently undergoing pilot-scale applications, and microbial cell fuels (Pouset al., 2012), an *ex situ* bioremediation alternative still at bench-scale. Selective catalytic hydrogenation, tested at a semi-industrial scale in a water-supply well in the Catalan basin, pursues the catalytic reduction of nitrate and nitrite to nitrogen gas by using hydrogen. Its main advantage refers to the avoidance of by-products and waste, thus reducing environmental costs; however, biofouling issues have been reported to affect final water quality. The main drawback is the high investment costs and the high energy consumption needed for hydrogen production.

Enhanced *in situ* biodenitrification is a recognized nitrate-removal technology derived, as its *ex situ* alternative, from previous wastewater nutrient-removal applications. It is aimed at optimizing heterotrophic nitrate respiration through the addition of a carbon source into the aquifer, thus acting *in situ*. Microbial activity is thus enhanced in an enriched area of the aquifer, creating a biologically active zone (often referred as biowall), which removes nitrate from the naturally-flowing groundwater. Requiring simple equipment, low energy consumption and with negligible waste production, it is thought to offer an economic alternative for nitrate-removal. Several pilot and small field-scale applications have been developed (Janda et al., 1988; Mercado et al., 1988; Breaster and Martinelli; 1990, Hamon and Fustec, 1991; Nuttall et al., 2001; Nutall et al., 1999; Tartakovsky et al., 2003; Khan and Spalding, 2003; Khan and Spalding, 2004), mostly in Europe, often aimed at recovering a previously-abandoned supply well. In Spain, there is only one reported application, developed at pilot-scale by D D'ENGINY BIOREM with the collaboration of the *Universitat Autònoma de Barcelona* and the *Universitat de Barcelona*, and reviewed in this thesis. This technology is currently regaining attention based on its economic advantage and potentiality for achieving the WFD groundwater goals, since, opposite to *ex situ* alternatives, may be used not only for well

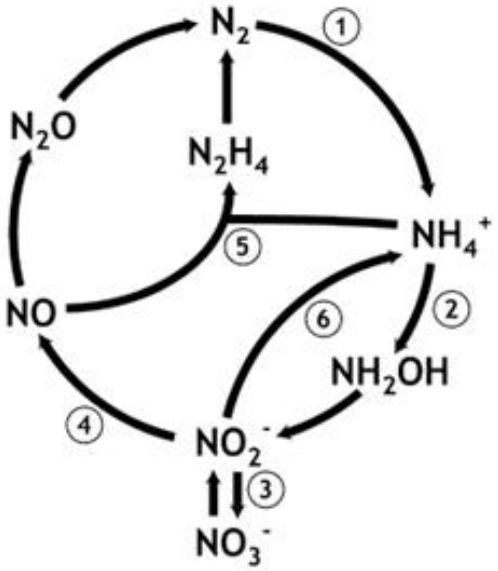
recovery but for aquifer restoration, thus pursuing a wider environmental benefit.

1.4 ENHANCED *IN SITU* BIODENITRIFICATION: FUNDAMENTS AND STATE OF THE ART

1.4.1 MICROBIAL DENITRIFICATION AND N CYCLE

Microbial denitrification refers to the microbial-mediated reduction of nitrate to nitrogen gas, a process that occurs subsequently through the reduction of nitrate via nitrite to nitric oxide, nitrous oxide and finally dinitrogen gas. In bacteria, this process is used as an alternative to oxygen respiration under low oxygen or anoxic conditions, and means returning the most-oxidized form of nitrogen (nitrate) to the most-reduced one (nitrogen gas) in the nitrogen cycle (Gayon and Dupetit, 1886). Once known to be a quite-simple three-step process including (1) dinitrogen gas fixation (that provides ammonium for assimilation (Beijerinck, 1888), (2) oxidation of ammonium to nitrate via nitrite (that is, nitrification (Winogradsky, 1890), and (3) denitrification, the microbial N cycle is currently recognized to be quite more complex, including the above-mentioned reactions as well as (4) anaerobic ammonium oxidation (anammox) (Strous et al., 1999), (5) aerobic nitrite oxidation, and (6) dissimilatory nitrate and nitrite reduction to ammonium (see Fig. 1.3), processes that may coexist with enhanced denitrification. Other recent findings related to the N cycle include the aerobic ammonium oxidation by archaea (Koenneke et al., 2005; Francis et al., 2007), nitrate reduction to dinitrogen gas by foraminifera (Risgaard-Petersen et al., 2006), nitrite-oxidizing phototrophy (Griffin et al., 2007), nitrite-dependent anaerobic methane oxidation (N-DAMO) (Raghoebarsing et al., 2006), and hyperthermophilic N_2-fixing methane-producing archaea (Mehta and Baross, 2006).

Fig. 1.3. Reactions of the microbial nitrogen cycle. (Source: Jetten, 2008).



(1): dinitrogen gas fixation.
(2): aerobic ammonium oxidation by bacteria and archea.
(3): aerobic/anaerobic nitrite oxidation.
(4) denitrification.
(5) anaerobic ammonium oxidation.
(6): dissimilatory nitrate and nitrite reduction to ammonium

Denitrification can occur via heterotrophic -through the oxidation of an organic C source- and autotrophic –through the oxidation of sulfur and the use of CO_2 as the main carbon source- metabolisms. Most denitrifying heterotrophic microorganisms are actually facultatively anaerobic, switching from oxygen to nitrate respiration at O_2 levels of less than about 0.5 mg/L (Hübner, 1986). However, chemo-autotrophic denitrification by anaerobic bacteria such as *Thiobacillus denitrificans* can also be important (Batchelor and Lawrence 1978).

It is commonly assumed that microbial heterotrophic denitrification, that is, the reduction of nitrate, is the major nitrate removal pathway in anoxic environments over

other processes such as nitrate assimilation or dissimilatory nitrate or nitrite reduction to ammonium (DNRA) that would also lead to nitrate decrease. However, several environmental conditions seem to affect the expression of the involved denitrifying proteins in different microbial species, mainly the oxygen levels, the organic carbon, the presence of denitrification intermediates, and the solution pH, while others are still unknown (Kraft et al., 2011), and thus the degree and completion of denitrification may vary in different media. As an example, DNRA could occur at a much noticeable level that denitrification when nitrate is limited in comparison to organic carbon (Cole and Brown, 1980). In the presence of oxygen, on the other side, denitrification is also reported to occur for several isolated bacterial species (Robertson and Kuenen, 1984; Robertson et al., 1989; Bell et al., 1990; Lesley et al., 1995). However, aerobic denitrification is often incomplete and leads to the accumulation of N_2O, a greenhouse effect gas, particularly when conditions switch from anaerobic to aerobic (Patureau et al., 1994; Frette et al., 1997). Finally, it is also known that nitrite oxidation can occur under anaerobic conditions, incorporating oxygen from the water molecule to form nitrate (Aleem et al., 1965; Wunderlich, 2013).

1.4.2 KINETICS OF MICROBIAL DENITRIFICATION

Denitrifying bacteria, as a group, are genetically diverse and metabolically versatile. Several attempts have been made to determine the kinetic parameters of nitrate utilization. However, and as in many other microbial-growth situations, these are determined not only by the substrate concentration but also by the environmental parameters. Tiedje et al., 1982, for instance, noted that the denitrifying enzyme activity of bacterial populations from several habitats seemed to be influenced more by oxygen and carbon availability than by nitrate concentration, while many other authors reported varying influences by the type of C source (Constantin and Fick, 1997; Weier et al., 1993; Ge et al., 2012; Senbayram et al., 2012; Welti et al., 2012) or the solution pH values, that may sometimes be inhibitory (Glass and Silverstain, 1998). Furthermore, in biofilm-type applications such EISB, solute transport and mass-flux across the biofilm may hinder microbial kinetics, thus affecting observed results at the macroscopic scale.

Following the experimental quantification of kinetic parameters, many theoretical and empirical approaches have been developed to describe and numerically-model the denitrification process (and other biogeochemical processes that may be involved). The most-common one, aimed at defining bacterial-growth kinetics, is the Monod approach (Monod 1949), used to describe the relationship between bacterial growth and substrate concentration:

$$\mu = \mu max \cdot [S/(S+K_s)] \quad (1.1)$$

where μ and μ_{max} ($time^{-1}$) are the specific growth rate and the maximum growth rate of a biomass population, respectively, S (moles/volume) is the substrate concentration limiting the growth, and K_s (moles/volume) is the saturation coefficient for substrate, which refers to the substrate concentration at which the growth rate is half its maximum rate.

Although not suited for all situations and showing significant limitations (André et al., 2011 and references herein), it is commonly used for batch-type microbial kinetics, particularly in denitrification systems (MacQuarrie and Sudicky, 2001; Rittmann and McCarty, 2001; Chen and MacQuarrie, 2004;

Lee et al., 2006). Usually, a modified approach is actually applied, accounting for the several substrates that may limit the microbial growth. It is then referred as the multiple-Monod expression.

Other reported models include the zero-order kinetics (Glass and Silverstein, 1998; Starr and Gillham, 1993), in which the growth rate is independent of the substrate concentration, and the first-order kinetics, in which nitrate concentration depends on the nitrate concentration itself (Ocampo et al., 2006) or on the substrate concentration (Sheibley et al., 2003).More recently, sophisticated approaches such us that described by André et al., 2011, that considers both geochemical and thermodynamic processes, are being developed.

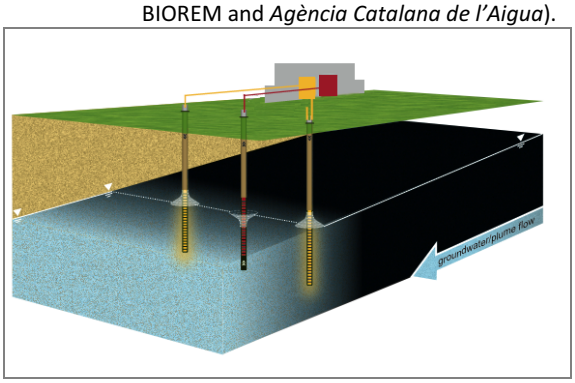
1.4.3 ENHANCED *IN SITU* BIODENITRIFICATION

Enhanced *in situ* biodenitrification is an engineered application of microbial heterotrophic denitrification aimed at *in situ* nitrate removal from groundwater. Derived from the previously-known wastewater denitrification, EISB is considered a viable, and often cheaper alternative for nitrate removal and groundwater recovery.

Aimed at stimulating facultative denitrifiers, EISB is based on the injection of a C source into the aquifer. In order for this injection to be effective, a detailed understanding of the subsurface geology and hydrogeology must be acquired prior to its design. Microbial denitrification is then enhanced in an enriched area of the aquifer, creating a biologically active zone (often referred as biowall) which removes nitrate from the naturally-flowing groundwater (see Fig. 1.4).

Different approaches have already been tested at the field-scale (Breaster and Martinelli; 1990, Hamon and Fustec, 1991; Jandaet al.,1988; Mercado et al.,1988; Nutallet al.,1999; Nuttallet al.,2001; Khan and Spalding, 2003; Tartakovsky et al., 2003; Khan and Spalding, 2004) mainly in Europe and often aimed at recovering a previously-abandoned water supply well, (Jechlinger et al, 1991; Chevron et al., 1998; Khan and Spalding, 2004). The technology is currently regaining attention based on its economic advantage and potentiality for achieving the WFD groundwater goals, and new and innovating approaches could be designed to optimize existing solutions. However, results and recent findings on N-related microbiology suggest that studies must be developed before further technology development.

Fig. 1.4. Schematics of a biowall. (Source: D D'ENGINY BIOREM and *Agència Catalana de l'Aigua*).



Both microbial and hydrogeological factors are key issues for EISB performance. Among the microbiological, the type and quantity of C are of particular concern. Concerning the first, different C sources have been used for EISB, including ethanol, acetic acid and glucose, and different stoichiometric reactions have been defined:

Ethanol: $5C_2H_6O + 12NO_3^- \rightarrow 6N_2 + 10HCO_3^- + 2OH^- + 9H_20$ (Lewandowski, 1985)

Acetic acid: $5CH_3COOH + 8NO_3^- \rightarrow N_2 + 8HCO_3^- + 2CO_2 + 6H_20$ (Lemoine et al., 1988)

Glucose: $5/6C_6H_{12}O_6 + 4NO_3^- + 5CaCO_3 + 4H^+ \rightarrow 4N_2 + 10HCO_3^- + 5Ca^{2+} + 2H_20$ (Trudell et al., 1986)

The type of carbon source seems to affect biomass growth and biofilm development, but different authors report different findings (Constantin and Fick, 1997; Matejuet al., 1992, Gómez et al., 2000). Furthermore, other amendments such as P, metals and/or oxygen have been sometimes added in order to increase denitrification rates or as a final-step cleaning process.

1.5 ISOTOPIC TOOLS APPLIED TO DENITRIFICATION PROCESSES

1.5.1 NITRATE ISOTOPE FINGERPRINT AND FRACTIONATION

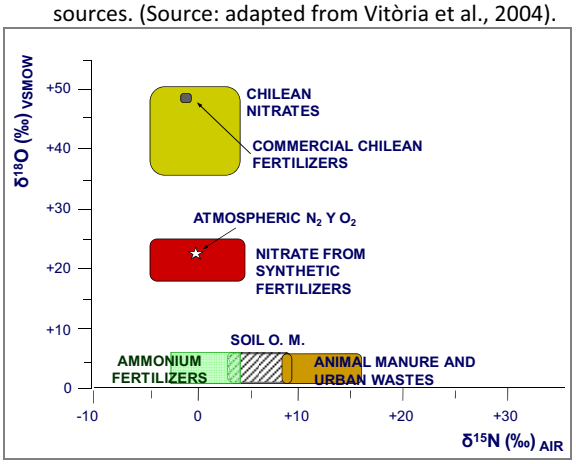
The natural presence of different isotopes – stable isotopes- in the nitrate molecule and its *concentration* change due to reactive processes, allows us to use isotopic tools to evaluate not only nitrate sources but also the processes that determine nitrate fate in groundwater (as well as in other environmental pools). Both N and O from the nitrate molecule can be isotopically determined. Nitrogen has two stable isotopes: ^{14}N and ^{15}N, the first one being the most abundant in natural conditions. The abundance of ^{14}N in atmospheric nitrogen, for instance, is 99.64%. Oxygen, on the other hand, has three stable isotopes: ^{16}O, the most dominant (with a natural abundance of 99.76%), ^{17}O, the least (0.037%) and ^{18}O (0.1995%). The isotopic characterization of the nitrate molecule in nitrate-related isotopic studies is then focused on the quantification of ^{15}N vs ^{14}N and ^{18}O vs ^{16}O, and is reported in delta (δ) per mil (‰) units relative to the international reference materials and determined as follows:

$$\delta^{15}N = [(R_{sa} - R_{std}) / R_{std}] \times 1000 \quad (1.2)$$

where $R = {}^{15}N/{}^{14}N$ in the sample (sa) and the standard (std). Reference materials for N and O are atmospheric nitrogen and V-SMOW (standard mean ocean water), respectively, and analytical precision is close to 0.1 and 0,5‰. Detailed information about current laboratory protocols for ^{15}N and ^{18}O analyses can be found in Silva et al., 2000, Sigman et al., 2001, Casciotti et al., 2002, and Coplen et al., 2004.

When nitrogen-containing compounds are formed, N and O are incorporated, establishing a particular isotopic composition that depends on the sources and processes that determine their formation. Subsequently, the isotopic composition of the nitrogen compounds in the nitrogen cycle will be determined by the isotope composition of the sources as well as by the processes that affect the N-pools (Aravena and Mayer, 2010).For the nitrate in groundwater, concerning our pool of interest, a wide range for $\delta^{15}N$ and $\delta^{18}O$ values have been reported, linking the isotope composition of the dissolved nitrate to the external (fertilizers and animal or domestic waste) or internal (soil nitrogen) origins (see Figure 1.5).

Fig. 1.5. Nitrate isotopic composition from different sources. (Source: adapted from Vitòria et al., 2004).



Following its formation, the isotopic composition of an element in the nitrate molecule (as well as in other compounds) may change due to reactive processes. These processes preferentially select the lighter or the heavier isotope, and based on this, the isotopic enrichment factor, that is, the

quantification of the evolution of nitrate isotopic composition, can be used to distinguish between reactive and not-reactive processes, both potentially leading to nitrate level reduction in groundwater. Furthermore, the enrichment factor is used to calculate the rate of biodegradation in both natural attenuation and engineered systems. The dual-isotope method, that is, the complementary measurement of $\delta^{15}N$ and $\delta^{18}O$in the nitrate molecule, has been employed for the evaluation of denitrification in groundwater, commonly in natural attenuation conditions (Böttcher et al., 1990; Smith et al., 1991; Aravena and Robertson, 1998; Fukada, et al., 2004). During denitrification, the isotopically light nitrate molecules tend to be reduced faster that the isotopically heavier. This occurs for both N and O, and it is due to the smaller energy required to break the molecular bond in the case of the lighter isotopes, that originates a difference in reaction rate in comparison to the heavy isotope. As a result, residual nitrate becomes enriched in the heavy isotopes (both ^{15}N and ^{18}O) (Mariotti 1986; Mariotti et al., 1988; Aravena and Robertson 1998; Devito et al., 2000). The enrichment trend follows a Rayleigh distillation process, that is, a straight line when $\delta^{15}N$ or $\delta^{18}O$ are plotted against the natural logarithm of the remaining nitrate:

$$\delta X_{res} = \delta X_o + \varepsilon \ln (C_{res}/Co) \quad (1.3)$$

where o and res represent the initial and residual nitrate, respectively, δX is the $\delta^{15}N$ or the $\delta^{18}O$ value of NO_3^-, C is the nitrate concentration and ε is the enrichment factor for ^{15}N or ^{18}O and related to the fractionation factor α by $\varepsilon = (\alpha - 1) \times 1{,}000$. Values of -5‰ (Bryan et al., 2013) to -39‰ (Toyoda et al., 2005) for $\varepsilon^{15}N\text{-}NO_3^-$and -27.6‰ (Torrentó et al., 2011), to +32‰ (inversed isotopic fractionation, Toyoda et al., 2005) for $\varepsilon^{18}O\text{-}NO_3^-$ have been reported.

This enrichment behavior for both atoms provides a distinctive signature for denitrification in front of other attenuation processes. Actually, reported results for denitrification indicate a linear relationship between $\varepsilon^{15}N\text{-}NO_3^-$ and the $\varepsilon^{18}O\text{-}NO_3^-$, with a εN/εO slope ranging between 0.9 to 2.3 (Otero et al, 2009 and references herein), implying that the isotopic composition of both nitrogen and oxygen increases during denitrification in a ratio of about 2:1 (Böttcher et al., 1990; Aravena and Robertson, 1998; Mengis et al., 1999; Devito et al., 2000; Fukada et al., 2004). And although the individual isotopic enrichment factors for $\delta^{15}N$ and $\delta^{18}O$ in the nitrate molecule during denitrification may vary in site-specific field conditions (Létolle, 1980; Olleros, 1983; Hübner, 1986), the fractionation factor εN/εO appears to remain constant (Fukada et al., 2003).

Several are the factors that may, in practice, affect denitrification isotopic fractionation in particular environments and scenarios:

- non-isotopic processes such as dilution or diffusion that modify nitrate concentrations while not changing its isotopic composition;

- co-occurring processes, such as nitrite reoxidation, pyrite oxidation (that is, autotrophic denitrification) and/or new nitrogen inputs that generate or eliminate nitrate in groundwater. If nitrite is reoxidized to nitrate (in both aerobic –incorporating oxygen dissolved in water- and anaerobic –incorporating oxygen from water), the isotopic composition of the latter may be hindered by all these processes and thus differ noticeably from that one from the source (Wunderlich et al., 2013).
  These processes may occur both in natural conditions, thus reflecting real processes,

as well as during sample storage. In this latter situation, care should be taken in sample storing time and conditions to avoid these processes to occur and therefore unrealistically affect fractionation. It is worth mentioning, furthermore, that nitrite interferes with most methods of nitrate isotopic analysis. Thus, it is then often eliminated from those samples used to determine nitrate isotopic composition;

- factors that affect denitrification rates such as temperature, C availability and oxygen levels. In this sense, Mariotti et al.,1982, found that the higher the denitrification rate (due to, for instance, a high temperature or a high electron donor concentration), the lower the isotopic effect;

- at a microbial-scale, non isotopically-sensitive steps preceding the isotopically sensitive denitrification reaction that may affect the kinetics of a degradation process, thus reducing the observed isotope fractionation at a macroscopic scale. Examples of such processes are substrate mass-transfer to the cell due to, for instance, diffusion through biofilm, bioavailability, transport of substrate within the cell or binding of substrate to the enzyme;

- the type of C source, a factor that some authors (Wunderlich et al.,2012) attribute to the potential change of the kinetics of nitrate transport across the cell (compared to the kinetics of intracellular nitrate reduction). In this sense, the kinetic isotope effect occurring inside the cytoplasm is modified by the bottle-neck of nitrate transport, and thus the measured isotope fractionation is an apparent kinetic isotope effect. In general, cultures with more complex compounds (such as toluene or benzene) produce less negative enrichment factors than simple compounds (acetate).

The influence of these factors upon observed fractionation values will of course depend on the degree of every one of them; that is, their relative reaction rate. Thus, in enhanced biodenitrification, some factors are expected to have a minor effect as they will occur at a noticeably lower rate than that of microbial denitrification.

1.5.2 OTHER MOLECULES INVOLVED IN THE DENITRIFICATION REACTIONS

The isotopic characterization of other denitrification-involved compounds offers a chance to assess microbial-mediated processes and, particularly, those processes affecting the biodenitrification performance. Among these, we include the carbon source, alkalinity (a product of the microbial heterotrophic denitrification) and sulfate (involved in both autotrophic denitrification by pyrite oxidation and sulfate heterotrophic reduction due to remaining available C).

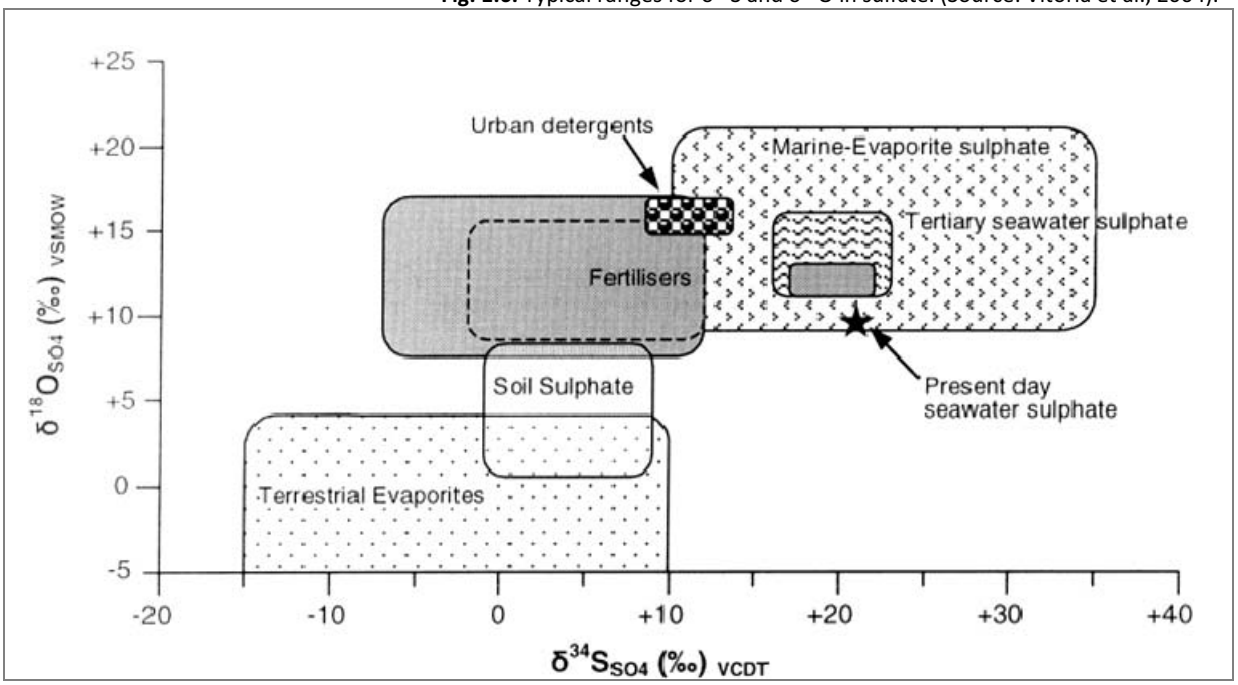
In heterotrophic denitrification, the organic C source is consumed and bicarbonate is produced, resulting in an expected enrichment of the remaining C source (as well as in NO_3^-) and a decrease in δ^{13}C-DIC coupled with an increase in bicarbonate levels. This effect, however, could be buffered by the aquifer lithology as well as by the water-air equilibrium, which may alter bicarbonate levels in solution. In such situations, the just-mentioned isotope effects on δ^{13}C-DIC are difficult to observe.

Sulfate, on the other side, is not directly related to the heterotrophic denitrification but to the autotrophic and, also, to sulfate

reduction, an anaerobic respiration process that occurs if an organic C source is still available when more favorable electron acceptors (such as nitrates) have been depleted. Its isotope composition depends, as nitrate's, on its original source (biotic or abiotic, see Fig. 1.6) as well as on its posterior reactive processes. Interestingly, microbially-mediated processes leading to a potential sulfate increase in solution (autotrophic denitrificarion) or to a decrease (sulfate respiration), generate an opposite isotope effect.

Sulfur has four stable isotopes: ^{32}S, ^{33}S, ^{34}S and ^{36}S, being the ^{32}S and ^{34}S the two most abundant, and $^{34}S/^{32}S$ is used for isotope analysis. During sulfate-producing autotrophic denitrification, the reduction of nitrate coupled to the oxidation of pyrite in anaerobic conditions, sulfate becomes depleted in ^{34}S as sulfur oxidation proceeds, while $\delta^{18}O$ tends to decrease, as oxygen is incorporated from water (Balci et al., 2007). During the sulfate respiration, on the contrary, the lighter sulfur isotope (^{32}S) is preferentially metabolized upon ^{34}S (Harrison and Thode, 1958; Mizutani and Rafter, 1969) and consequently, the remaining sulfate becomes progressively enriched in ^{34}S as sulfate concentrations decrease. Similarly, the lighter oxygen isotope is also preferred, and thus sulfate also becomes enriched in ^{18}O.

Fig. 1.6. Typical ranges for $\delta^{34}S$ and $\delta^{18}O$ in sulfate. (Source: Vitòria et al., 2004).



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2

SITE DESCRIPTION

AVÍS IMPORTANT

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IMPORTANT NOTICE

The text of this chapter has been withdrawn on the instructions of the author, as there is participation of undertakings, confidentiality agreement or the ability to generate patent

3

RESULTS, DISCUSSION AND CONCLUSIONS

3 RESULTS, DISCUSSION AND CONCLUSIONS

The objective of this chapter is to present the main results as well as our discussion and to offer conclusions from the following already published research work:

STUDY CASE #1:

- Vidal-Gavilan, G., Folch, A., Otero, N., Solanas, A. M., Soler, A., 2013. Isotope characterization of an *in situ* biodenitrification pilot-test in a fractured aquifer. Appl. Geochem. 32, 153–163.

- Rodríguez-Escales, P., van Breukelen, B., Vidal-Gavilan, G., Soler, A., Folch, A. 2014. Integrated modeling of biogeochemical reactions and associated isotope fractionations at batch-scale: a tool to monitor enhanced biodenitrification applications. The kinetic model is considered for this dissertation.

STUDY CASE #2:

- Vidal-Gavilan, G., Carrey, R., Solanas, A. M., Soler, A. In press. Amendment strategies for groundwater heterotrophic denitrification: chemical, microbial and isotopic assessment of a 1-D flow-through experiment. Submitted to the Science of the Total Environment journal.

Main results and discussions are detailed by study case, while conclusions are integrated together.

3.1 STUDY CASE #1

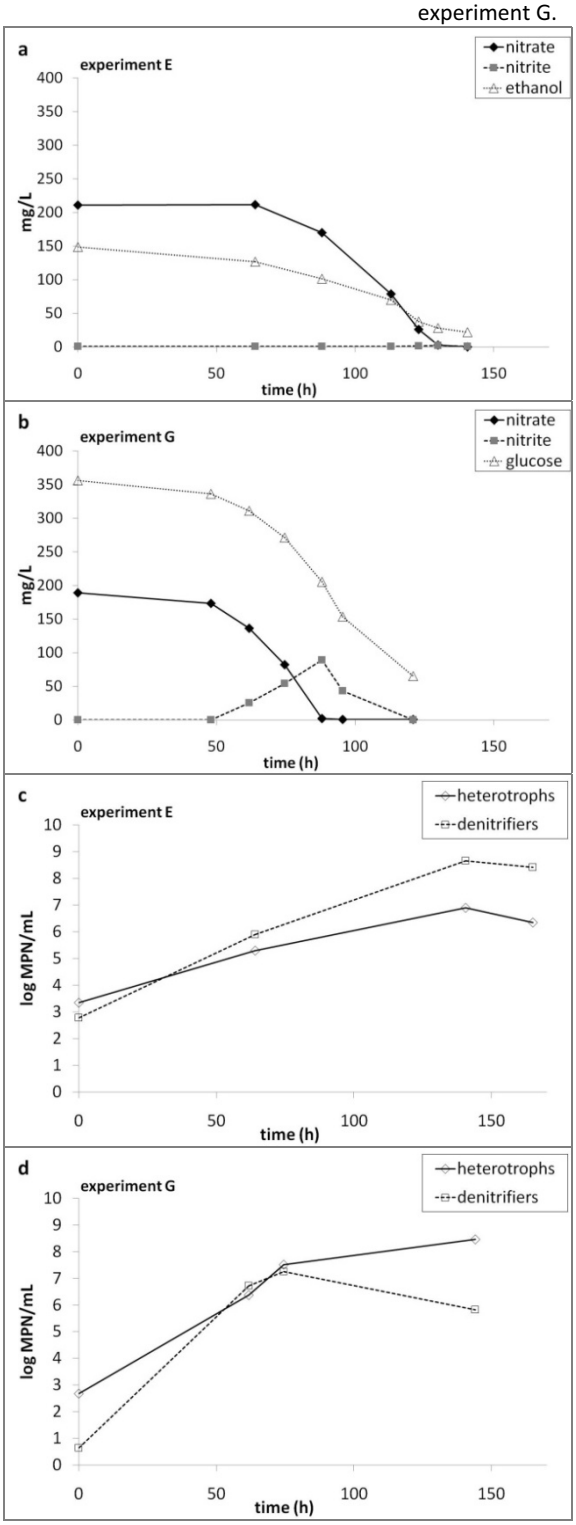
Batch-scale biodenitrification tests were developed to assess the potential of enhanced *in situ* biodenitrification (EISB) at site #1 (Roda de Ter). Groundwater from La Muntanyeta well (nitrate concentration around 200 mg/L) was obtained and used for the tests. After an initial pre-treatability test aimed at selecting most-suitable biostimulants, ethanol and glucose were chosen as the main C sources to be used in batch-scale experiments. The sole use of a C source, however, didn't proved to be effective, and thus P source was also added to the systems in form of disodium hydrogen phosphate. Enhanced biodenitrification, conducted at aquifer temperature (15 °C), was then monitored by means of chemical, microbial and isotopic tools.

After completing the batch-scale experiments, EISB was tested at the field site, at a pilot-test experiment that lasted for 5 months. Ethanol and P were periodically injected into the aquifer. One piezometer was used as the injection point (IP), and the remaining 5 as monitoring wells (MW1 to MW5, see chapter 2 for location). Groundwater for biostimulant addition was extracted from the old municipal well (La Muntanyeta well) existing at the site, mixed with biostimulants, and injected through the IP.

3.1.1 RESULTS

Heterotrophic denitrification was effectively induced at batch experiments by the addition of a C and a P source, and regardless the type of C source used (ethanol (E) or glucose (G)). Both C sources were added in excess compared to stoichiometric C/N needs, so thus carbon content wouldn't be a limiting factor. Following the biostimulant addition, an initial lag phase was observed in all experiments, a phase that lasted for about 3 days and that was attributed to acclimation, the time taken to increase the microbial population. Afterwards, nitrate reduction was observed, proceeding to complete depletion of nitrate in less than 2 days (see Fig 3.1. A and B).

Fig. 3.1. Kinetics of biodenitrification at lab-scale experiments. A: Nitrate, nitrite and ethanol levels in experiment E. B: Nitrate, nitrite and glucose levels in experiment G. C: microbial population counts in experiment E. D: microbial population counts in experiment G.



Maximum nitrate consumption rates, determined by linear regression of nitrate concentration vs. time, were similar in both cases, with values of 0.05 and 0.07 mmol NO_3^- /L·h for ethanol and glucose experiments respectively. Nitrite was temporarily accumulated, achieving a maximum of 88.8 mg/L when using glucose, but being completely consumed afterwards (once nitrate had been depleted). Finally, organic carbon (OC) consumption, expressed as $\Delta C/\Delta NO_3^-$, varied slightly depending on the type of C source: 1.6 mmolOC/mmolNO_3^- for ethanol, and 2.2 for the glucose, similarly to stoichiometric values (0.83 and 1.25 mmolOC/mmolNO_3^- according to equations (1) and (2)).

Ethanol: $5C_2H_6O + 12NO_3^- \rightarrow 6N_2 + 10HCO_3^- + 2OH^- + 9H_20$ (3.1)

Glucose: $5/6C_6H_{12}O_6 + 4NO_3^- + 5CaCO_3 + 4H^+ \rightarrow 4N_2 + 10HCO_3^- + 5Ca^{2+} + 2H_20$ (3.2)

Kinetic parameters (K_{max}, $K_{s,ED}$, $K_{s,EA}$, and b) and OC consumption from these batch-scale experiments were also quantified by numerical modeling, considering the modified Monod kinetics, one of the most common expressions in biodenitrification models (MacQuarrie and Sudicky, 2001; Rittmann and McCarty, 2001; Chen and MacQuarrie, 2004; Lee et al., 2006; Calderer et al., 2010), and by using the parameter estimation software PEST (Doherty, 2005) for calibration. Nitrate removal was modeled as a single step reaction when nitrite had not been noticeably detected (ethanol experiment) and as a two-step reaction when it had been accumulated (glucose experiment). Results are presented in Table 3.1.

Table 3.1. Kinetic parameters optimized in the numerical model.

parameter	units	calibrated value (ethanol experiment)	calibrated value (glucose experiment)
k_{max}, NO_3^-	mol OC / mol C-biomass.day	53.95	12.25
k_{max}, NO_2^-	mol OC / mol C-biomass.day		8.78
K_{sat}, organic carbon	mol OC/day	$7.28x10^{-2}$	$6.04x10^{-3}$
K_{sat}, NO_3^-	mol nitrate/L	$1.86x10^{-4}$	$1.55x10^{-3}$
b	day^{-1}	$1.50x10^{-1}$	$8.79x10^{-3}$
K_{sat}, NO_2^-	mol nitrite/L		$5.05x10^{-3}$
K_{inh}	mol nitrate/L		$8,51x10^{-5}$

New stoichiometric reactions were derived and used in the numerical model in order to take into account not only the amount of electron donor used for nitrate respiration but also that one for cell synthesis (equations 3 and 4). The McCarty (1975) thermodynamic and bioenergetic principles and the calculation protocol of Rittmann and McCarty (2001) were applied. Resulting electron fractions (f_s, that is, the fraction of electrons from the electron donor that is used for cell synthesis) were 0.682 for ethanol and 0.727 and 0.812, respectively, for each of the two glucose redox steps. The remaining electrons (f_e, considering $f_e+f_s = 1$) are then used for energy production. Overall, new determined stoichiometric values required for nitrate reduction to nitrogen gas, now accounting for both nitrate respiration and biomass synthesis, were 1.9 and 2.0 $mmolOC/mmolNO_3^-$ for ethanol and glucose respectively.

Ethanol: $0.083\ C_2H_60 + 0.088\ NO_3^- + 0.043\ H^+ \rightarrow 0.024\ C_5H_7O_2N + 0.032\ N_2 + 0.045\ HCO_3^- + 0.164\ H_2O$ (3.3)

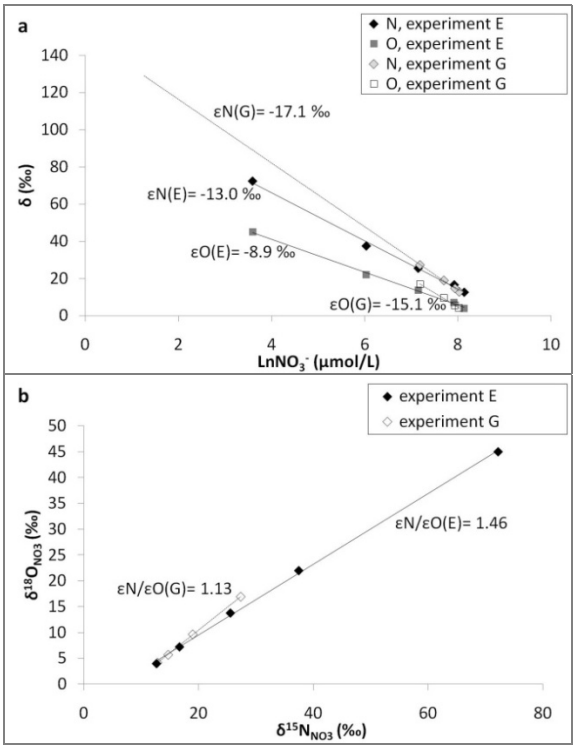
Glucose: $0.042\ C_6H_{12}0_6 + 0.162\ NO_3^- \rightarrow 0.026\ C_5H_7O_2N + 0.136\ NO_2 + 0.098\ H^+ + 0.120\ HCO_3^- + 0.052\ H_2O$ (3.4)

$0.042\ C_6H_{12}0_6 + 0.063\ NO_2^- + 0.029\ NO_3 \rightarrow 0.029\ C_5H_7O_2N + 0.031\ N_2 + 0.018\ H^+ + 0.105\ HCO_3^- + 0.089\ H_2O$ (3.5)

Effectiveness of biostimulant addition was also observed at the microbial level, with populations increasing exponentially in both experiments, up to maximum values of 10^8MPN/mL. Microbial populations shifted from an initial non denitrifying community to a dominating denitrifying community afterwards, as expressed by the % of denitrifiers over total heterotrophs (see Fig. 3.1 C and D). In general terms, higher counts for denitrifiers were obtained in the ethanol-amended experiment despite a lower initial C/N ratio. At the end of the experiments, once nitrate was completely removed, microbial counts tended to decrease for both total heterotrophic and denitrifiers and in both type of experiments. This behavior was reflected in the numerical modeling, showing an increase of endogenous nitrate respiration (vs. exogenous) when nitrate levels decreased substantially. Following complete nitrate depletion, the biomass entered a decay phase, and could be oxidized by other electron acceptors available (such as sulfate).

Isotopic fractionation was determined at the batch-scale with no interference from other attenuation process (mainly diffusion and dispersion). Obtained nitrogen and oxygen isotopic fractionation values (ε) were -13.0‰ and -17.1‰ for $\varepsilon N\text{-}NO_3^-$ in the ethanol and glucose experiments respectively, and -8,9‰ and -15.1‰ for $\varepsilon O\text{-}NO_3^-$, resulting in $\varepsilon N/\varepsilon O$ values of 1.46 (ethanol-amended experiment), and 1.13 (glucose-amended).

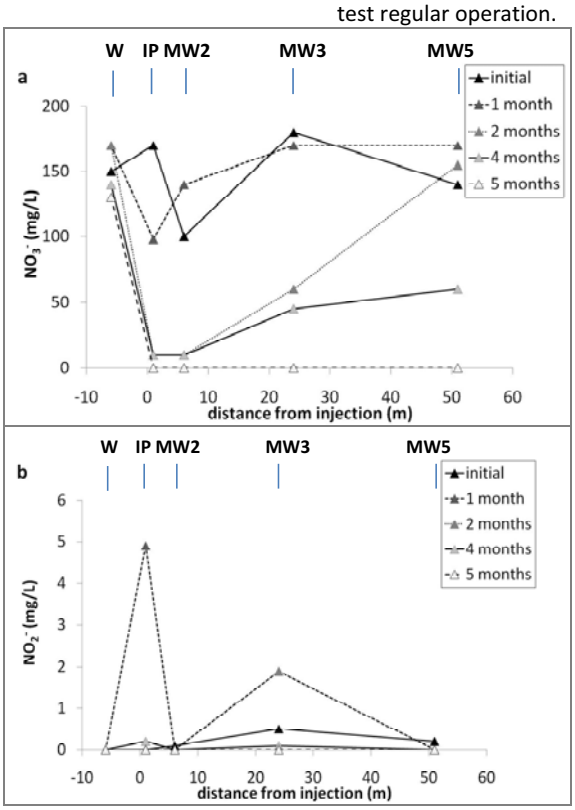
Fig. 3.2. . Isotopic results from lab-scale experiments. A: $\delta^{15}N$ and $\delta^{18}O$ vs. nitrate levels. B: $\delta^{15}N$ vs $\delta^{18}O$.



Batch-scale data was then used to design and monitor the pilot-test application. Nitrate levels were observed to decrease following the biostimulation, a decrease that was first noticed at monitoring wells close to the injection point (MW 1 and 2) and later on at the remaining monitoring wells (3, 4 and 5) (see Fig. 3.3.A). Nitrate fell easily below the drinking water standard (50 mg/L). Five months after initial operation, nitrate was not detected in most monitoring wells. Temporarily accumulation of nitrite was observed, achieving maximum values of 5 mg/L, although it also tended to decrease over time. Nitrite levels remained below the drinking water standard (0.5 mg/L) at most points, except for at MW3 (Fig. 3.3.B).

Redox potential easily decreased to negative values, indicating the achievement of reducing conditions. Dissolved manganese was detected to increase, but no such variation or decrease was observed in neither iron nor sulfate contents. A rotten-egg (H_2S) smell, however, was temporally noticed at monitoring wells 1, 2, and 3, indicating potential stimulation of sulfate-reducing activity.

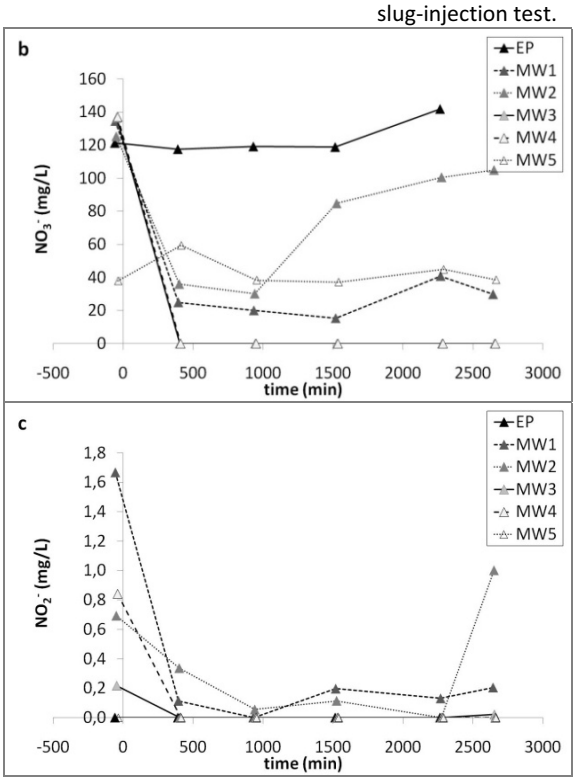
Fig. 3.3. Nitrate (A) and nitrite (B) levels during pilot-test regular operation.



At the end of the 5thmonth of operation, an injection event was closely characterized to get an insight of biostimulant addition as well as the system response to this addition. Injected C was consumed (or wash out) from the system in less than 16 hours, and nitrate removal proceeded fast (see Fig. 3.4) thanks to an already stimulated microflora. Again, temporary accumulation of denitrification intermediates (nitrite and nitrous oxide –which was now analyzed-) was detected, as well as increasing levels of dissolved-iron and H_2S smell. All parameters showed a peak behavior, and tended to disappear. Concerning the isotope fractionation, nitrate became enriched in both nitrogen and oxygen as expected, and $\delta^{15}N$ and $\delta^{18}O$ for the nitrate molecule tended to increase to maximum values of +25‰ and

+12‰ respectively. No clear trend was observed for $\delta^{34}S\text{-}SO_4^{2-}$ data, except for MW5, that showed a decrease of $\delta^{34}S\text{-}SO_4^{2-}$ linked to a small increase in sulfate levels.

Fig. 3.4. Nitrate (A) and nitrite (B) levels during the slug-injection test.



3.1.2 DISCUSSION

Heterotrophic denitrification was stimulated at batch and pilot-scales, achieving nitrate and nitrite complete depletion in all situations. At the pilot-scale, diminishing values of nitrate and nitrite were interpreted as an increase in the area of influence of the treatment, linked to a stabilization of microbial activity. Nitrate consumption was accompanied by an enrichment of both N and O isotopes, resulting in batch-determined εN/εO values of 1.46 (ethanol-amended experiment), and 1.13 (glucose-amended), lower than most previously reported values (Otero et al., 2009 and references herein). As already discussed (see introduction chapter), several factors may affect isotope fractionation. In this case, higher εN/εO values in the ethanol-amended experiment compared to glucose's could be explained by different microbial behavior and lower nitrite accumulation.

Microbial populations at the batch-scale were observed to increase exponentially once biostimulants were added, and to slightly decrease once the electron acceptor was depleted. The biomass increase is expected higher for glucose amended applications (compared to ethanol's) based on electron fractions (fs) computed results, although these results do not exactly match the experimental observations, a difference that could be attributed to small variations in experiment conditions and/or to difficulties in microbial counts. As known, biomass build-up could result in clogging situations, affecting the system hydraulics and performance. On the other side, however, clogging is believed to be diminished by using pulse application of the carbon source instead of a continuous supply regime (Soares et al., 1990).

Based on lab-scale results, ethanol was preferred over glucose as a C source for field application due to the lower organic carbon consumption and the lower observed nitrite accumulation. Injected C was used for nitrate respiration as well as for cell synthesis. In the pilot-test application it was also required for oxygen depletion, since oxygen was initially present at the site and it is a preferred electron acceptor over nitrate. Furthermore, if organic C was still available once the heterotrophic denitrification was completed, it could have stimulated other metabolisms, such us manganese, iron or sulfate reduction. In all cases, outflow water quality would be diminished.

Indeed, indication of anaerobic activity other than nitrate reduction, particularly sulfate reduction, was observed in all experiments, both at lab and field tests. However, values of $\delta^{34}S\text{-}SO_4^{2-}$ were not conclusive enough to prove

that sulfate-reduction was actually occurring. On the contrary, some autotrophic sulfide oxidation was detected at the field site by a decrease of $\delta^{34}S\text{-}SO_4^{2-}$ linked to a small increase in sulfate levels. Accumulation of denitrification intermediates was noticed as well, at least temporarily, affecting also the water quality. Due to analytical limitations, however, complete or incomplete denitrification could not be proved. Dissimilatory nitrate reduction to ammonia (DNRA) was, on the contrary, proved not to be quantitatively important. All these issues are of great concern if denitrified groundwater is meant to be used for drinking water standards. Therefore these concerns were particularly addressed in the subsequence study case.

3.2 STUDY CASE #2

In this second study case, a flow-through experiment was built and operated to simulate and test the viability of an EISB treatment at site #2 (Argentona aquifer). Based on the results obtained from the study case #1, the flow-through experiment for site #2 was particularly aimed at assessing the influence of different C addition strategies upon the denitrification process and the resulting groundwater quality. The experiment lasted for 10 months and 4 different feeding strategies were tested: a weekly injection strategy (#1), and 3 different daily injection strategies varying on the C/N value (strategies # 2, 3 and 4). No biostimulant other than C (ethanol) was used. Results are mainly referred to the column outflow.

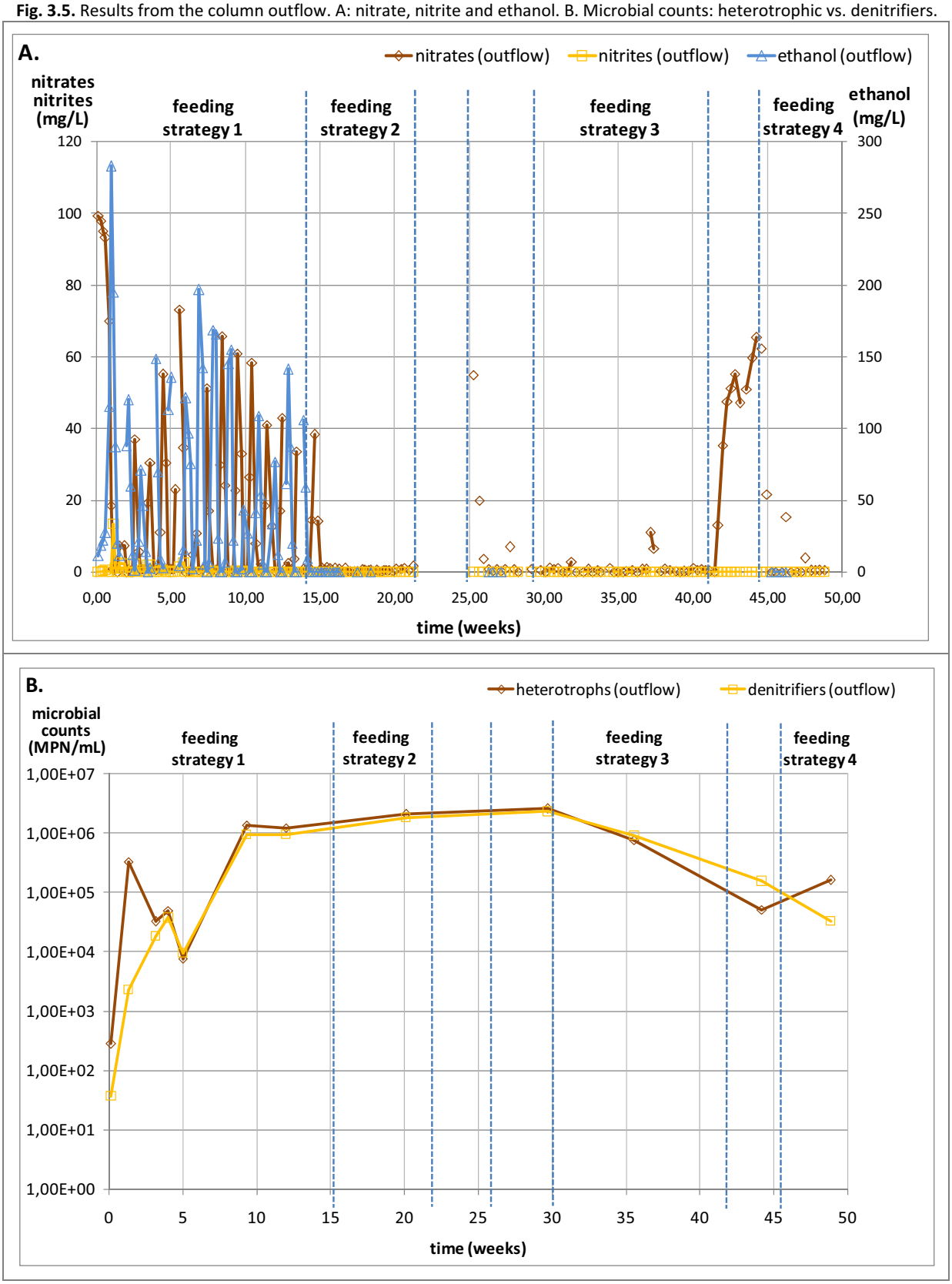
3.2.1 RESULTS

Denitrification was observed to occur following the first ethanol injection. A cyclic nitrate behavior was observed for the weekly feeding strategy (see Fig. 3.5), decreasing to non-detected values after the C addition but recovering to above drinking water standards one to two days after the injection. Once the feeding was changed to daily injections, a more steady-state outcome was observed, regardless the amount of C added to the system. Nitrate levels at the outflow then kept at values below 10 mg/L and often close to non-detected values (Fig. 3.5). A similar pattern to that of nitrate was observed for the C source, showing a cyclic behavior during the weekly feeding strategy (although inverse to that of nitrate's), and being completely consumed afterwards (during the daily injection strategies).

Nitrite production initially followed a similar cyclic pattern to that of nitrate; however, it soon decreased to non-detectable values, even during the weekly injection, and no nitrite was further detected throughout the rest of the experiment. Other denitrification intermediates could not be analyzed. Ammonia was never detected, so DNRA was not considered to take place. Finally, and concerning electron acceptors as indicators of other reductive processes potentially occurring, only dissolved manganese was detected at the outflow at higher concentrations that at the inflow, while iron and sulfate showed no clear variation.

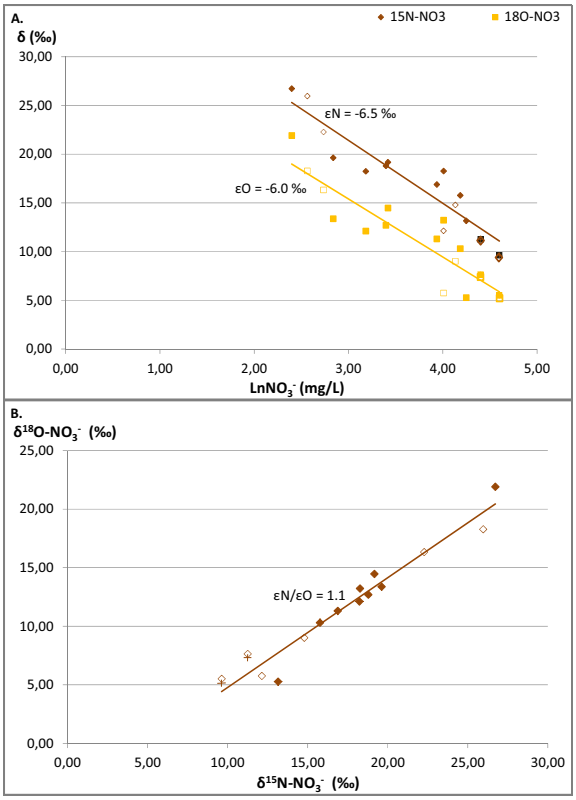
As expected, microbial populations did increase due to the addition of a C source, achieving maximum values around the injection point. Microbial counts at this area decreased progressively when so did the C/N ratios, although such a change was hardly noticed further downstream. Regarding microbial community structure, heterotrophic populations shifted towards a dominant denitrifying community once biodentrification was enhanced, similarly to the study case #1.

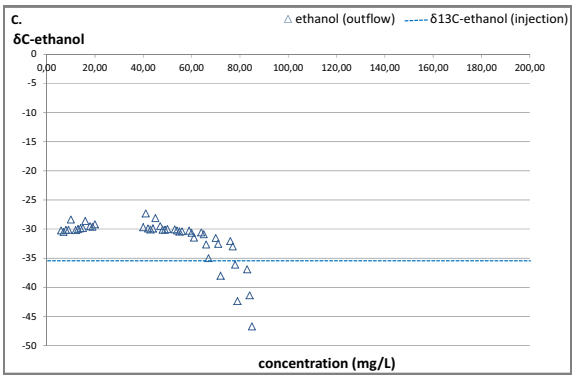
Fig. 3.5. Results from the column outflow. A: nitrate, nitrite and ethanol. B. Microbial counts: heterotrophic vs. denitrifiers.



During this enhanced biodenitrification, nitrate isotopic composition increased in both N and O heavy isotopes, from an initial +9.6‰ and +5.5‰ for δ^{15}N-NO_3^- and δ^{18}O- NO_3^- respectively - corresponding to nitrate derived from the ammonium oxidation of manure or wastewater effluents (Vitoria et al., 2004; Kendall et al., 2007; Xue et al., 2010)-, to a detected maximum of +26.7‰ for δ^{15}N-NO_3^-, and +21.9‰ for δ^{18}O-NO_3^- (Fig. 3.6). This enrichment resulted in fractionation values (ε) of -6.5‰ for the N and -6.0‰ for the O, and a fractionation ratio (εN/εO) of 1.01. Concerning the C source isotope composition, δ^{13}C-ethanol values ranged from an enriched -27.3‰ to a depleted -46.7‰ (Fig. 3.6). Depleted values were obtained from day 69 of the experiment (feeding strategy 1), decreasing over time.

Fig. 3.6. Isotope results from the column experiment. A. δ^{15}N-NO_3^- and δ^{18}O-NO_3^- vs. nitrate concentration. B. δ^{15}N-NO_3^- vs. δ^{18}O-NO_3^-. Inflow samples (cross-filled), outflow samples from the weekly injection (complete filling) and outflow samples from daily injections (no filling). C. Ethanol vs. d13C-ethanol. Samples from the column outflow.





3.2.2 DISCUSSION

Denitrification was induced by the addition of a C source, since *natural* C content was too low to allow effective natural attenuation to happen. Ethanol addition seemed to sustain heterotrophic denitrification (as well as oxygen consumption and cell synthesis) in all tested feeding strategies, even at low C/N values, although a more stable output was obtained when daily-injection strategies were applied (compared to weekly injections). Nitrate was consumed within 20 to 30 cm downstream of the injection point, corresponding to a retention time of 1.8 to 2.7 days. A quasi steady-state outflow was obtained in daily injection scenarios, with nitrate levels ranging from non-detected values and up to 10 mg/L, and nitrite's remaining undetected. With such resulting water quality, water blending alternatives could be applied for water-supply systems, mixing denitrified groundwater with nitrate-containing water, still achieving drinking water standards while increasing the amount of water potentially entering the supply system.

Nitrite accumulation with respect to nitrate consumption was an issue of concern, and different results had been previously reported: in some cases, nitrate was depleted before achieving complete nitrite removal (Betlach and Tiedje, 1981; Almeida et al., 1995), while in other cases nitrite depletion occurred prior to nitrate removal (Gómez et al., 2010). The fact that in our flow-through experiment nitrite

depletion was not always linked to complete nitrate consumption suggests that nitrite accumulation in a flow-through engineered system can be diminished over time, due to the evolution of the microbial population, an effect that is not observed in batch-scale experiments. These findings would be in accordance with those obtained by Martin et al., 2009.

Remaining C source after the biodenitrification is also a parameter of concern since it may favor undesired microbial growth and thus affect drinking water quality. The cyclical-peak ethanol distribution obtained during the weekly addition indicated an unbalanced ethanol distribution, with carbon excess in some points and (potentially) carbon deficit in others. During daily injections, on the contrary, a more homogeneous distribution and complete ethanol consumption were attained while not affecting resulting water quality. Reducing the C/N ratio, furthermore, enabled us to reduce the amount of injected C, thus reducing the treatment costs. Also, by reducing the amount of C source, we were able to reduce microbial populations at the active zone, improving water quality while still obtaining the required denitrification results. Hence, a better water quality –in terms of remaining C and residual microflora- and a smaller biofouling effect are expected when reducing C addition. However, endogenous activity –providing internal C for denitrification- may become important when low C/N values are used and keep denitrification temporarily ongoing. Endogenous respiration, actually, may act as a positive effect for clogging control but, on the contrary, may affect the biodenitrification performance at longer operation times. Such aspects will be further evaluated using modeling tools.

Biofilm formation was considered to be the main cause of hydrogeological alterations detected in the system, decreasing porosity and increasing the dispersion coefficient, although no clogging effect was observed to affect water movement. Other geochemical processes, such as mineral precipitation due to alkalinity production and/or pH modification -both linked to heterotrophic denitrification-, could have also contributed to clogging. Induced calcite precipitation in biodenitrification experiments, actually, has been previously reported (Barbieri et al., 2011; Mastrocicco et al., 2011), although it could also tend to dissolve when other C sources inducing different alkalinity production are used (Rodríguez-Escales et al., 2014).

Finally, concerning the application of isotope tools, the obtained $\varepsilon N/\varepsilon O$ fractionation values fell within the low-end of previously reported data (varying from 0.9 to 2.3, Otero et al., 2009 and references herein), an effect that may be linked to faster microbial kinetics in enhanced vs. natural biodenitrification. Similar low values were observed in enhanced denitrification systems developed in our lab (Carrey et al., 2014 and Vidal-Gavilan et al., 2013) as well as on Mariotti et al., 1988, that reported that the higher the denitrification rate (due to a high temperature or a high electron donor concentration), the lower the isotopic effect. Concerning ethanol's fractionation, on the other side, a two-trend behavior was observed, probably indicating a change in the dominating C-consuming population. Interestingly, the second trend suggests an inverse fractionation of the C source that got depleted while being consumed. A similar inverse behavior was observed by Goevert et al., (2008) when studying the acetate enrichment factors from different acetate-utilizing sulfate-reducing bacteria, but no previously published data is available for ethanol fractionation.

3.3 CONCLUSIONS

As presented, two different sites were selected to test the technical viability of EISB and assess the influence of engineering strategies upon the resulting water quality. A choice was made to apply isotopic tools, often used to assess nitrate natural attenuation and to establish water management practices, to enhanced engineering systems. Combined with chemical and microbial monitoring, isotope analysis were applied aimed at quantifying microbial denitrification in front of other attenuation processes and to gain an insight on the microbial process and the other microbial, geochemical of physical processes that could be influencing the performance of EISB systems.

The feasibility of EISB was evaluated at different scales: batch, flow-through and pilot. In all cases, heterotrophic denitrification was successfully stimulated by addition of biostimulants, mainly C, but also P (required in study case #1). In this sense, the sole use of C addition is preferable in terms of both system complexity and cost optimization. But despite that it is commonly accepted that most groundwaters contain enough elements (other than C) to support microbial activity for denitrification (Champ et al., 1979), initial treatability tests should be performed for every new site to assess if other nutrients (P or else) would be required.

The degree of denitrification was almost complete in all tested scenarios, although the resulting water quality varied. The accumulation of denitrification intermediates and/or the stimulation of other anaerobic processes due to an excess of C addition should be diminished. Based on batch and pilot-scale results from study case #1, we thought that intermediate accumulation and water quality could be controlled by feeding strategies and evolution of microbial populations, aspects that were assessed and proved potentially correct at study case #2. Furthermore, we suggest that hydraulic (pumping)control combined by optimization of feeding strategies such as reported in this thesis should help minimizing undesired processes and optimize EISB performance at field-scale applications. Similar approaches have been tested by Khan and Spalding, 2003. Otherwise, a post-treatment phase would be required as a polishing step if denitrified groundwater is meant for drinking water purposes.

Biofilm growth, which plays an important role for the denitrification efficiency and the potential clogging of the system, should be controlled by the injection strategy. In this sense, both the type of C source and the frequency of the injection are of interest: pulse-type injection and low C/N ratios would help reducing the biofilm by stimulating the endogenous activity. On the other hand, by alternating different types of C sources with different impact on solution alkalinity and pH, such as ethanol and glucose, we could somehow control geochemical processes, mainly calcite precipitation, that also contribute to potential clogging. Such effects should be further studied by both experimental and modeling tests.

Overall, EISB seems a suitable technology for nitrate removal in different scenarios, even applicable in complex geological media such as the fractured aquifers. In this latter case, this thesis reports the very first field-case application worldwide.

The combined application of chemical, microbial and isotope tools proved valuable to get detailed insight of the denitrification process and other microbial metabolisms affecting EISB system performance.

Specifically, these tools should help detect site-specific heterogeneities (such as preferential flow-paths or C source accumulation), potential occurrence of other respiration processes, or the extension of enhanced denitrification, particularly if large-scale applications are pursued. Overall, by applying isotope tools a better decision-making process could be undertaken for both system design and optimization.

Nitrate fractionation followed previously published values, but falling in the low end of published data for both N and O. All obtained results seem to indicate that this is due to the fast microbial kinetics, as reported in Mariotti et al., 1988, compared to denitrification kinetics under natural attenuation conditions and/or to other attenuation processes such as dispersion. The faster the microbial kinetics are, the lower the isotope effect is. Furthermore, it is known that denitrification is overestimated when field-derived isotopic fractionation factors are used (Mariotti et al., 1988; Böttcher et al., 1990), and thus batch-scale isotope fractionation should be used to assess denitrification at the field scale. Concerning these two issues, this PhD applicant believes that 1D-column experiments could also be used to determine nitrate isotopic fractionation in enhanced biodenitrification systems, due to the noticeable difference between microbial kinetics and other attenuation processes. Other processes potentially affecting nitrate isotopic composition, such as nitrite reoxidation (Wunderlich et al, 2012), may also be minimized in 1D EISB experiments due to either smaller oxygen diffusion or higher denitrification rates than those observed at field-scale natural attenuation. Actually, a similar situation could be occurring at field-scale EISB applications, noticeably differing from natural attenuation studies except for nitrate behavior at EISB fringes.

Despite the valid results and increasing isotope data available worldwide, some issues need further study. During this work, ethanol fractionation was reported for the first time, and, surprisingly, two different behaviors were observed within the same experiment. Although suspected to be linked to a change in microbial community, this issue requires deeper attention, and both experimental and modeling and efforts could be developed to provide definitive answers. And as for the study of δ^{13}C-DIC also conducted in this experimental work, no clear trends were observed, probably due the instability of the samples and the influence of the storage time. These results are being further assessed by modeling tools (in another research work linked, as mentioned at the introduction, to this present thesis). An interesting integrative approach is applied for this DIC modeling, relating isotope fractionation to microbial activity and geochemical interactions, that is, considering the whole C cycle. Again, some complementary experimental work could help generating more data.

It is finally worth mentioning that some uncertainties remain unsolved, such as the occurrence of complete or uncompleted denitrification, or the role of co-existing microbial processes. In this sense, and based on obtained results, monitoring and analytical plans could be optimized to help answering some of these remaining concerns. It is also recommended to immediately include analytical results on the decision-making process in order to modify system performance.

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ANNEXE S

ANNEX A

GRAPHIC REPORT: CORE SAMPLES FROM SITE CHARACTERIZATION

SITE #1: CORE SAMPLES FROM DRILLINGS

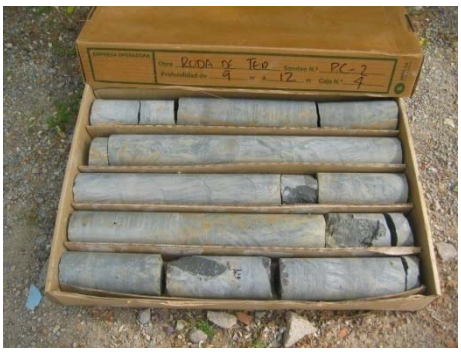
IP

MW1

0 m
3 m
6 m
9 m
12 m
15 m

SITE #1: CORE SAMPLES FROM DRILLINGS

MW2



MW3

0 m
3 m
6 m
9 m
12 m
15 m

SITE #2: CORE SAMPLES FROM DRILLINGS

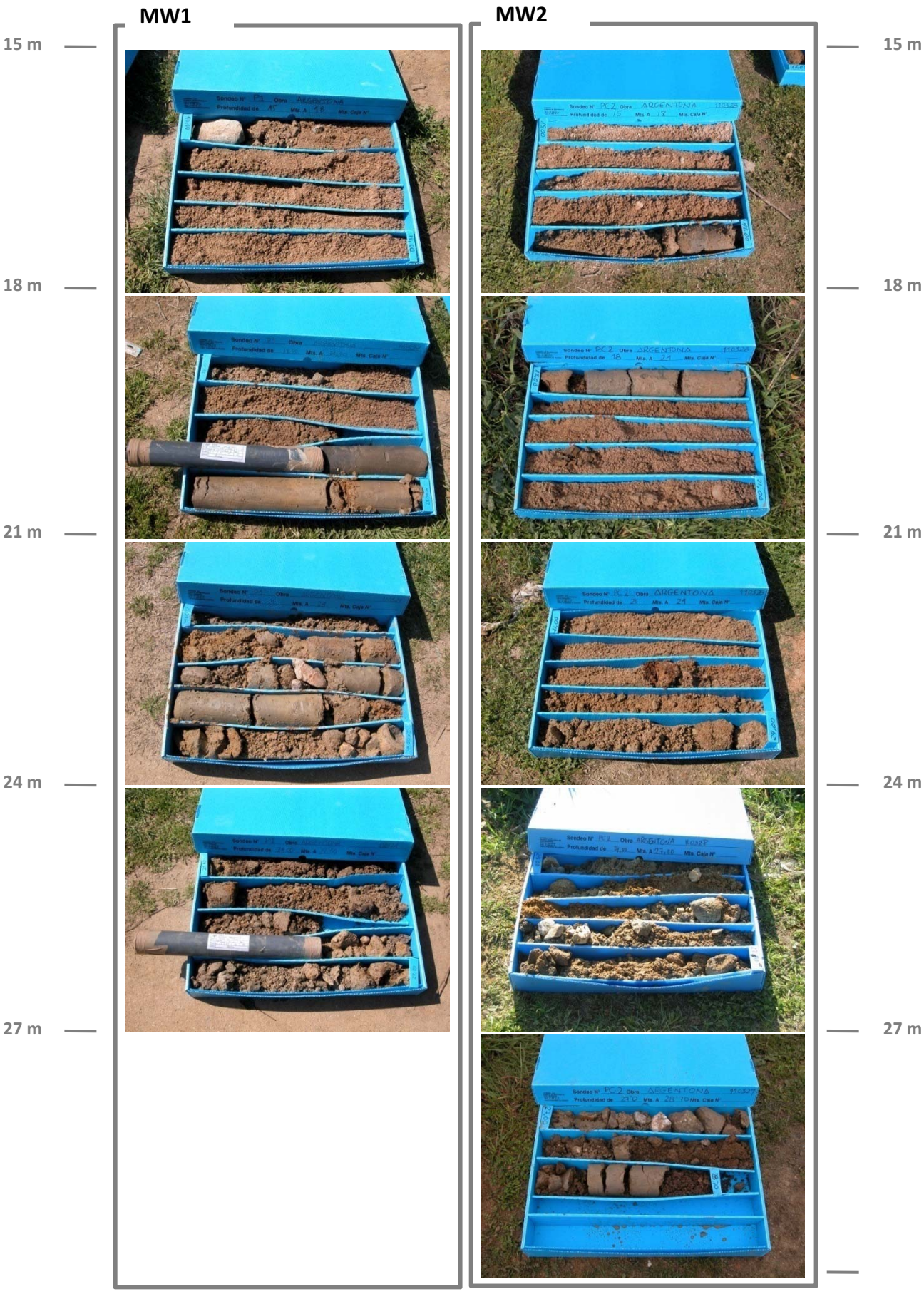
MW1

MW2

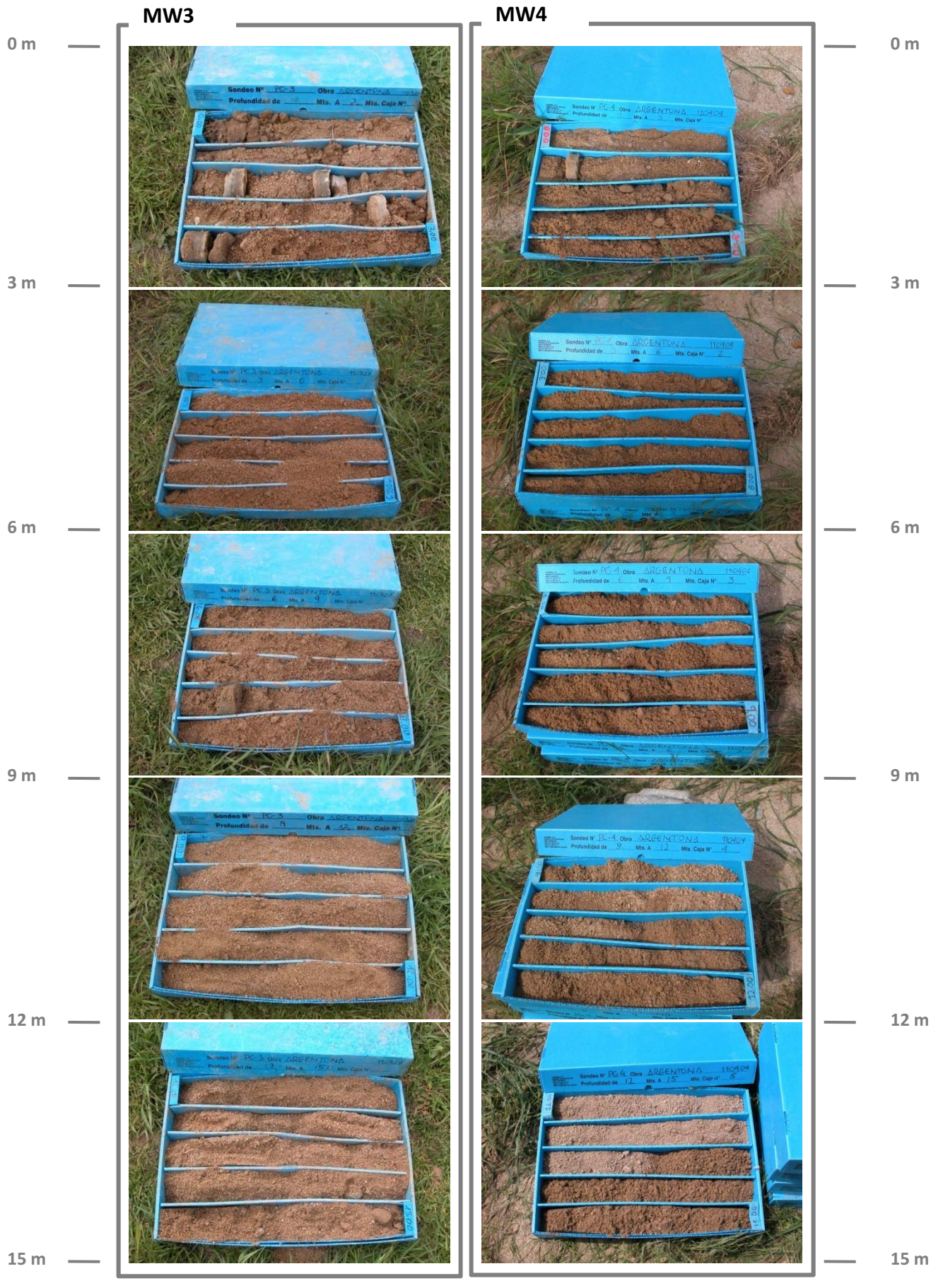
0 m — 3 m — 6 m — 9 m — 12 m — 15 m

0 m — 3 m — 6 m — 9 m — 12 m — 15 m

SITE #2: CORE SAMPLES FROM DRILLINGS



SITE #2: CORE SAMPLES FROM DRILLINGS



SITE #2: CORE SAMPLES FROM DRILLINGS

MW3

15 m

18 m

21 m

24 m

27 m

MW4

15 m

18 m

21 m

24 m

27 m

ANNEX **B**

ISOTOPE CHARACTERIZATION ON AN IN SITU BIODENITRIFICATION PILOT-TEST IN A FRACTURED AQUIFER

Vidal-Gavilan, G., Folch, A., Otero, N., Solanas, A.M.,Soler, A.

2013

Applied Geochemistry 32, 153-163

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ANNEX C

INTEGRATED MODELING OF BIOGEOCHEMICAL REACTIONS AND ASSOCIATED ISOTOPE FRACTIONATIONS AT BATCH-SCALE: A TOOL TO MONITOR ENHANCED BIODENITRIFICATION APPLICATIONS

Rodríguez-Escales, P., van Breukelen, B.M, Vidal-Gavilan, G., Soler, A., Folch, A.

2014

Chemical Geology 365, 20-29

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ANNEX D

FEEDING STRATEGIES FOR GROUNDWATER ENHANCED BIODENITRIFICATION: CHEMICAL, MICROBIAL AND ISOTOPIC ASSESSMENT OF A 1D FLOW-THROUGH EXPERIMENT

Vidal-Gavilan, G., Carrey, R., Solanas, A.M., Soler, A.

Submitted to the Science of the Total Environment journal

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ANNEX E

SUPPLEMENTARE DATA

Table E1. Analytical results from biodenitrification batch-scale experiments.

sample	time (h)	pH	heterotrophs (log MPN/mL)	denitrifiers (log MPN/mL)	C source (mg/L)	DOC (mg/L)	HCO_3^- (mg/L)	δ^{13}C-TIC	NO_3^- (mg/L)	δ^{15}N-NO_3^-	δ^{18}O-NO_3^-	NO_2^- (mg/L)	PO_4^{2-} (mg/L)	SO_4^{2-} (mg/L)	NH_4^+ (mg/L)	Fe (mg/L)	Mn (µg/L)
Ethanol-amended experiment (experiment E)																	
E0	0.0	7.2	3.33	2.77	148.5	64.3	386.0	-13.0	210.6	12.7	4.0	0.2	168.3	129.8	0.20	0.01	5.1
E1	64.0	7.2	5.29	5.89	126.8	69.6	407.0	-15.9	211.5	n.d.	n.d.	0.6	167.4	123.8	0.20	0.01	9.1
E2	88.0	7.4	n.d.	n.d.	101.0	61.8	429.0	-16.0	169.8	16.7	7.2	0.2	170.0	130.1	0.17	0.01	10.4
E3	113.0	7.6	n.d.	n.d.	69.4	44.3	502.9	-16.9	78.5	25.5	13.8	0.3	169.4	130.4	0.18	0.02	10.5
E4	123.0	7.9	n.d.	n.d.	37.1	36.3	537.4	-17.6	25.8	37.5	22.0	0.7	169.5	128.2	0.19	0.01	10.3
E5	130.0	8.1	n.d.	n.d.	27.6	32.0	567.1	-18.5	2.3	72.2	45.0	2.0	113.4	122.9	0.19	0.00	8.8
E6	140.5	n.d.	6.88	8.64	21.7	29.6	n.d.	-18.0	b.d.l.	n.d.	n.d.	0.1	113.7	127.4	0.17	0.01	8.5
E7	165.0	n.d.	6.34	8.41	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Glucose-amended experiment (experiment G)																	
G0	0.0	7.20	2.67	0.64	356.0	146.50	392.6	-12.9	189.1	12.8	4.2	b.d.l.	n.d.	91.7	0.16	0.01	2.6
G1	48.0	7.27	n.d.	n.d.	336.0	138.90	392.0	-12.6	173.0	14.7	5.7	b.d.l.	n.d.	103.2	0.15	0.00	4.8
G2	61.8	7.21	6.38	6.72	311.0	128.60	417.4	-14.1	136.4	19.0	9.6	25.1	n.d.	96.4	0.03	0.00	6.0
G3	74.5	7.15	7.51	7.26	271.0	109.40	428.9	-11.9	81.8	27.3	16.9	53.6	n.d.	94.8	0.02	0.00	4.2
G4	88.0	7.10	n.d.	n.d.	205.0	79.58	477.4	-11.7	2.0	n.d.	n.d.	88.8	n.d.	120.4	0.15	0.01	5.4
G5	95.5	7.19	n.d.	n.d.	153.0	66.02	538.9	-11.8	0.4	n.d.	n.d.	43.0	n.d.	92.9	0.16	0.00	5.4
G6	121.0	7.17	n.d.	n.d.	65.0	42.27	593.0	-12.6	0.2	n.d.	n.d.	b.d.l.	n.d.	100.1	0.14	0.00	5.9
G7	144.0	n.d.	8.47	5.82	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

n.d.: not determined. b.d.l.: below detection limit.

Table E2. Analytical results from the EISB pilot-test at site #1.

sample	time (day)	pH	conductivity (mS/cm)	Eh (mV)	O_2 (mg/L)	TOC (mg/L)	HCO_3^- (mg/L)	NO_3^- (mg/L)	$\delta^{15}N\text{-}NO_3^-$ (‰)	$\delta^{18}O\text{-}NO_3^-$ (‰)	NO_2^- (mg/L)	PO_4^{2-} (mg/L)	SO_4^{2-} (mg/L)	$\delta^{34}S\text{-}SO_4^{2-}$ (‰)	Fe^{2+} (mg/L)	Mn (µg/L)
EP-0	-30	6.8	1.3	295	3.3	3	380	150	12.7	5.0	b.d.l.	b.d.l.	98	2.0	b.d.l.	b.d.l.
IP-0	-30	7.0	1.9	280	2.5	59	300	170	12.3	4.5	0.3	b.d.l.	95	1.9	b.d.l.	b.d.l.
MW1-0	-30	7.0	1.9	260	2.4	n.d.	n.d.	n.d.	n.d.	n.d.	b.d.l.	b.d.l.	n.d.	2.2	n.d.	n.d.
MW2-0	-30	6.8	1.8	273	2.4	43	260	100	12.1	5.3	0.1	b.d.l.	92	1.7	0.20	<l.d.
MW3-0	-30	6.8	1.9	278	2.8	67	310	180	12.6	5.0	0.5	b.d.l.	100	1.9	b.d.l.	b.d.l.
MW4-0	-30	n.d.	n.d.	n.d.	n.d.	51	310	150	14.7	6.7	0.5	b.d.l.	130	-1.9	b.d.l.	0.05
MW5-0	-30	6.7	1.8	278	2.8	70	300	140	11.2	5.2	0.2	b.d.l.	140	-4.5	b.d.l.	b.d.l.
EP-1	33	6.8	1.2	283	3.3	5	300	170	n.d.	n.d.	b.d.l.	n.d.	n.d.	n.d.	n.d.	n.d.
IP-1	33	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
MW1-1	33	7.1	1.9	262	2.1	n.d.	380	98	21.1	11.1	4.9	n.d.	n.d.	n.d.	n.d.	n.d.
MW2-1	33	6.8	1.9	273	2.0	n.d.	310	140	n.d.	n.d.	b.d.l.	n.d.	n.d.	n.d.	n.d.	n.d.
MW3-1	33	6.7	1.9	278	2.7	n.d.	310	170	13.8	6.6	1.9	n.d.	n.d.	n.d.	n.d.	n.d.
MW4-1	33	n.d.	n.d.	n.d.	n.d.	n.d.	300	165	n.d.	n.d.	b.d.l.	n.d.	n.d.	n.d.	n.d.	n.d.
MW5-1	33	6.7	1.8	280	2.8	n.d.	310	170	n.d.	n.d.	b.d.l.	n.d.	n.d.	n.d.	n.d.	n.d.
EP-2	65	n.d.	n.d.	n.d.	n.d.	5	300	170	n.d.	n.d.	b.d.l.	b.d.l.	100	2.5	n.d.	n.d.
IP-2	65	6.6	2.7	171	4.1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
MW1-2	65	n.d.	n.d.	n.d.	n.d.	76	460	10	n.d.	n.d.	b.d.l.	b.d.l.	97	2.2	n.d.	n.d.
MW2-2	65	n.d.	n.d.	n.d.	n.d.	260	310	10	n.d.	n.d.	b.d.l.	b.d.l.	95	n.d.	n.d.	n.d.
MW3-2	65	6.9	3.2	83	1.4	6	320	60	n.d.	n.d.	1.9	b.d.l.	110	-3.9	n.d.	n.d.
MW4-2	65	n.d.	n.d.	n.d.	n.d.	6	310	150	n.d.	n.d.	b.d.l.	b.d.l.	120	n.d.	n.d.	n.d.
MW5-2	65	7.2	2.0	49	2.3	3	300	155	n.d.	n.d.	b.d.l.	b.d.l.	110	n.d.	n.d.	n.d.
EP-3	95	6.8	1.9	283	3.3	n.d.	n.d.	150	n.d.	n.d.	b.d.l.	n.d.	n.d.	n.d.	n.d.	n.d.
IP-3	95	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
MW1-3	95	6.9	3.2	-99	0.3	n.d.	n.d.	120	n.d.	n.d.	0.2	n.d.	n.d.	n.d.	n.d.	n.d.
MW2-3	95	6.7	3.0	-105	0.1	n.d.	n.d.	115	n.d.	n.d.	0.6	n.d.	n.d.	n.d.	n.d.	n.d.
MW3-3	95	6.8	2.7	-5	0.3	n.d.	n.d.	95	n.d.	n.d.	0.4	n.d.	n.d.	n.d.	n.d.	n.d.
MW4-3	95	6.9	2.8	10	0.6	n.d.	n.d.	65	n.d.	n.d.	0.2	n.d.	n.d.	n.d.	n.d.	n.d.
MW5-3	95	7.0	2.6	22	0.6	n.d.	n.d.	80	n.d.	n.d.	0.1	n.d.	n.d.	n.d.	n.d.	n.d.
EP-4	120	6.8	1.8	290	3.5	4	330	140	n.d.	n.d.	b.d.l.	b.d.l.	88	n.d.	n.d.	n.d.
IP-4	120	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
MW1-4	120	6.8	3.2	-170	0.3	6	460	10	n.d.	n.d.	0.2	b.d.l.	100	n.d.	n.d.	n.d.
MW2-4	120	6.7	3.2	-260	0.1	20	310	10	n.d.	n.d.	b.d.l.	b.d.l.	104	n.d.	n.d.	n.d.

Table E2. Analytical results from the EISB pilot-test at site #1.

sample	time (day)	pH	conductivity (mS/cm)	Eh (mV)	O_2 (mg/L)	TOC (mg/L)	HCO_3^- (mg/L)	NO_3^- (mg/L)	$\delta^{15}N\text{-}NO_3^-$ (‰)	$\delta^{18}O\text{-}NO_3^-$ (‰)	NO_2^- (mg/L)	PO_4^{2-} (mg/L)	SO_4^{2-} (mg/L)	$\delta^{34}S\text{-}SO_4^{2-}$ (‰)	Fe^{2+} (mg/L)	Mn (µg/L)
MW3-4	120	7.0	3.1	-88	0.2	6	320	45.0	n.d.	n.d.	0.1	b.d.l.	98	n.d.	n.d.	n.d.
MW4-4	120	6.9	3.2	-94	0.6	6	310	60	n.d.	n.d.	0.2	b.d.l.	101	n.d.	n.d.	n.d.
MW5-4	120	7.0	2.9	-33	0.2	4	300	60	n.d.	n.d.	b.d.l.	b.d.l.	114	n.d.	n.d.	n.d.
EP-5	145	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	130	n.d.	n.d.	b.d.l.	n.d.	n.d.	n.d.	n.d.	n.d.
IP-5	145	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
MW1-5	145	6.8	2.1	-150	0.3	n.d.	n.d.	b.d.l.	n.d.	n.d.	b.d.l.	n.d.	n.d.	n.d.	n.d.	n.d.
MW2-5	145	6.7	2.0	-70	0.1	n.d.	n.d.	b.d.l.	n.d.	n.d.	b.d.l.	n.d.	n.d.	n.d.	n.d.	n.d.
MW3-5	145	6.9	1.9	-45	0.3	n.d.	n.d.	b.d.l.	n.d.	n.d.	b.d.l.	n.d.	n.d.	n.d.	n.d.	n.d.
MW4-5	145	6.9	1.9	-45	0.5	n.d.	n.d.	5	n.d.	n.d.	0.1	n.d.	n.d.	n.d.	n.d.	n.d.
MW5-5	145	6.9	1.8	-30	0.3	n.d.	n.d.	b.d.l.	n.d.	n.d.	b.d.l.	n.d.	n.d.	n.d.	n.d.	n.d.

n.d.: not determined. b.d.l.: below detection limit.

Table E3. Environmental parameters during the EISB slug-injection-test at site #1.

sample	time (min)	pH	conductivity (mS/cm)	Eh (mV)	O_2 (mg/L)
EP-0		n.d.	n.d.	n.d.	n.d.
EP-1		n.d.	n.d.	n.d.	n.d.
EP-2		n.d.	n.d.	n.d.	n.d.
EP-3		n.d.	n.d.	n.d.	n.d.
EP-4		n.d.	n.d.	n.d.	n.d.
MW1-0	-200	6.7	3.0	205.0	2.8
MW1-1	60	7.1	2.4	130.0	2.6
MW1-2	870	6.6	2.3	-66.0	0.2
MW1-3	1,455	6.8	2.3	-11.5	0.1
MW1-4	2,235	6.6	2.3	-14.0	0.1
MW1-5	2,610	6.9	2.3	-13.0	0.1
MW2-0	-195	6.7	2.9	81.5	0.3
MW2-1	65	6.7	2.4	117.0	1.6
MW2-2	875	6.8	2.3	-14.0	0.2
MW2-3	1,460	6.7	2.4	17.8	0.2
MW2-4	2,240	6.7	2.4	48.0	0.2
MW2-5	2,615	6.7	2.4	30.0	0.1
MW3-0		n.d.	n.d.	n.d.	0.9
MW3-1	70	6.7	2.4	145.0	4.0
MW3-2	880	6.8	2.4	-20.8	0.2
MW3-3	1,465	6.8	2.3	-45.0	0.1
MW3-4	2,245	6.8	2.3	-99.0	0.2
MW3-5	2,620	6.8	2.3	-67.0	0.3
MW4-0		n.d.	n.d.	n.d.	n.d.
MW4-1	75	6.7	2.4	137.0	0.7
MW4-2	885	6.8	2.3	-17.0	1.6
MW4-3	1,470	6.8	2.3	-11.2	1.3
MW4-4	2,250	6.8	2.3	-21.0	0.1
MW4-5	2,625	6.8	2.3	-148.0	0.1
MW5-0		n.d.	n.d.	n.d.	n.d.
MW5-1	80	6.8	2.3	120.0	0.3
MW5-2	890	6.7	2.3	45.0	0.6
MW5-3	1,475	6.8	2.3	37.0	0.6
MW5-4	2,255	6.7	2.3	5.9	0.4
MW5-5	2,630	6.7	2.3	-16.0	0.2

n.d.: not determined. b.d.l.: below detection limit.

Table E4. Analytical results from the EISB slug injection-test at site #1.

sample	time (min)	DOC (mg/L)	d13C-TIC (‰)	NO_3^- (mg/L)	$\delta^{15}N\text{-}NO_3^-$ (‰)	$\delta^{18}O\text{-}NO_3^-$ (‰)	NO_2^- (mg/L)	N_2O (µgN/L)	PO_4^{2-} (mg/L)	SO_4^{2-} (mg/L)	$\delta^{34}S\text{-}SO_4^{2-}$ (‰)	Fe^{2+} (mg/L)	Mn (µg/L)
EP-0	-60	3.1	-10.5	121.4	12.7	n.d.	b.d.l.	n.d.	b.d.l.	82.8	3.2	0.03	1.0
EP-1	390	4.7	-11.4	117.5	13.1	3.7	b.d.l.	n.d.	b.d.l.	76.0	2.5	0.02	5.2
EP-2	930	3.7	-11.4	119.2	12.8	2.7	b.d.l.	n.d.	b.d.l.	77.2	2.4	0.03	2.6
EP-3	1.515	4.0	-7.8	118.9	12.3	2.9	b.d.l.	n.d.	b.d.l.	75.3	2.1	0.02	0.2
EP-4	2.265	2.3	-9.4	141.9	13.0	3.9	b.d.l.	n.d.	b.d.l.	95.9	1.9	0.02	1.0
MW1-0	-55	6.1	-11.4	134.5	12.6	4.3	1.7	n.d.	b.d.l.	101.2	1.7	0.02	17.5
MW1-1	395	41.6	-12.5	25.0	n.d.	n.d.	0.1	n.d.	b.d.l.	86.0	n.d.	0.02	66.7
MW1-2	935	5.7	-13.2	20.1	n.d.	n.d.	<l.d.	12.8	b.d.l.	72.7	n.d.	0.02	229.7
MW1-3	1.520	6.4	-10.4	15.4	n.d.	n.d.	0.2	n.d.	b.d.l.	87.7	n.d.	0.02	165.4
MW1-4	2.270	6.4	-7.8	40.9	25.0	11.0	0.1	n.d.	b.d.l.	82.2	2.1	0.02	245.3
MW1-5	2.645	5.7	-13.6	29.8	22.4	11.5	0.2	30.3	b.d.l.	95.5	2.2	0.02	179.2
MW2-0	-50	19.3	-11.8	125.2	16.2	8.0	0.7	n.d.	b.d.l.	103.6	1.2	0.02	50.5
MW2-1	400	7.5	-9.7	36.0	21.0	11.1	0.3	n.d.	b.d.l.	105.5	-0.8	0.01	471.6
MW2-2	940	5.8	-9.6	30.2	24.4	12.0	0.1	9.9	b.d.l.	98.0	1.3	0.01	583.2
MW2-3	1.525	5.3	-9.2	84.7	13.5	5.8	0.1	17.8	b.d.l.	101.8	1.3	0.02	370.2
MW2-4	2.275	3.9	-11.5	100.5	15.1	7.5	b.d.l.	n.d.	b.d.l.	97.9	1.0	0.02	44.1
MW2-5	2.650	4.3	-10.4	104.9	15.1	6.9	1.0	24.5	b.d.l.	97.1	1.2	0.02	57.7
MW3-0	-45	14.6	-10.2	136.5	12.4	n.d.	0.2	n.d.	b.d.l.	91.4	1.4	0.01	2.0
MW3-1	405	47.7	-11.3	b.d.l.	n.d.	n.d.	b.d.l.	n.d.	b.d.l.	52.0	n.d.	0.13	45.8
MW3-2	945	9.9	-14.4	b.d.l.	n.d.	n.d.	b.d.l.	0.1	b.d.l.	68.4	n.d.	0.45	242.1
MW3-3	1.530	16.8	-10.0	b.d.l.	n.d.	n.d.	b.d.l.	0.1	b.d.l.	94.1	n.d.	0.02	5.7
MW3-4	2.280	6.7	-10.4	b.d.l.	n.d.	n.d.	b.d.l.	n.d.	b.d.l.	89.0	n.d.	0.01	135.9
MW3-5	2.655	4.7	-13.4	b.d.l.	n.d.	n.d.	0.0	0.3	b.d.l.	88.6	2.0	0.01	0.9
MW4-0	-40	8.5	-11.8	137.0	12.4	4.4	0.8	n.d.	b.d.l.	101.2	0.7	0.02	2.6
MW4-1	410	25.1	-12.5	b.d.l.	n.d.	n.d.	b.d.l.	n.d.	b.d.l.	87.8	n.d.	0.02	1,356.0
MW4-2	950	6.8	-14.7	b.d.l.	n.d.	n.d.	b.d.l.	n.d.	b.d.l.	98.4	n.d.	0.01	2,413.8
MW4-3	1.535	5.2	-14.3	b.d.l.	n.d.	n.d.	b.d.l.	1.8	b.d.l.	100.6	n.d.	0.01	2,076.2
MW4-4	2.285	5.4	-14.2	b.d.l.	n.d.	n.d.	b.d.l.	n.d.	b.d.l.	101.2	n.d.	0.02	2,003.6
MW4-5	2.660	6.2	-13.5	b.d.l.	n.d.	n.d.	b.d.l.	0.2	b.d.l.	101.9	n.d.	0.01	1,660.0
MW5-0	-35	3.5	-11.8	37.9	20.9	10.7	b.d.l.	n.d.	b.d.l.	114.9	-0.2	0.01	842.6
MW5-1	415	3.1	-10.4	59.4	22.3	10.1	b.d.l.	n.d.	b.d.l.	126.5	-1.5	0.01	250.6
MW5-2	955	4.6	-12.4	38.4	24.5	13.3	b.d.l.	1,243.3	b.d.l.	138.4	-0.9	0.01	45.9

Table E4. Analytical results from the EISB slug injection-test at site #1.

sample	time (min)	DOC (mg/L)	d13C-TIC (‰)	NO_3^- (mg/L)	$\delta^{15}N\text{-}NO_3^-$ (‰)	$\delta^{18}O\text{-}NO_3^-$ (‰)	NO_2^- (mg/L)	N_2O (µgN/L)	PO_4^{2-} (mg/L)	SO_4^{2-} (mg/L)	$\delta^{34}S\text{-}SO_4^{2-}$ (‰)	Fe^{2+} (mg/L)	Mn (µg/L)
MW5-3	1,540	4.1	-8.7	37.2	27.6	10.8	b.d.l.	n.d.	b.d.l.	143.3	-4.4	0.02	596.8
MW5-4	2,290	3.2	-9.2	44.8	27.8	12.3	b.d.l.	n.d.	b.d.l.	144.4	-4.7	0.02	323.9
MW5-5	2,665	5.4	-11.1	38.5	29.0	13.4	b.d.l.	687.0	b.d.l.	140.5	-4.2	0.01	75.1

n.d.: not determined. b.d.l.: below detection limit.

Table E5. Flow-through experiment: column's inflow analytical data.

time (weeks)	feeding strategy	sample	pH	NPOC (mg/l)	TIC/DIC (mg/l)	d13C-TIC/DIC	NO_3^- (mg/l)	NO_3^- (mmol/l)	NO_2^- (mg/l)	NO_2^- (mmol/l)	SO_4^{2-} (mg/l)	$\delta^{15}N\text{-}NO_3^-$	$\delta^{15}N\text{-}NO_3^-$	NH4+ (mg/l)
0,00	1	GVG-13-01-E	7.60	2.11	405.00	-11.61	99.36	1.60	b.d.l.	b.d.l.	119.43	9.63	5.51	b.d.l.
5,30	1	GVG-13-32-E	7.30	n.d.	n.d.	n.d.	109.07	1.76	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
8,28	1	GVG-13-48-E	7.40	n.d.	385.00	n.d.	102.79	1.66	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
9,03	1	GVG-13-52-E	n.d.	n.d.	330.00	-16.96	n.d.	n.d.	b.d.l.	b.d.l.	n.d.	n.d.	n.d.	b.d.l.
10,43	1	GVG-13-60-E	n.d.	n.d.	407.00	n.d.	109.37	1.76	b.d.l.	b.d.l.	n.d.	n.d.	n.d.	b.d.l.
11,43	1	GVG-13-66-E	n.d.	n.d.	319.40	-14.18	86.50	1.40	b.d.l.	b.d.l.	n.d.	n.d.	n.d.	b.d.l.
14,61	2	GVG-13-83-E	n.d.	n.d.	n.d.	n.d.	80.39	1.30	b.d.l.	b.d.l.	n.d.	n.d.	n.d.	b.d.l.
16,00	2	GVG-13-91-E	6.82	0.13	n.d.	n.d.	76.16	n.d.	b.d.l.	b.d.l.	104.04	n.d.	n.d.	b.d.l.
26,59	2	GVG-13-123-E	7.47	2.43	307.65	-15.43	81.43	1.31	b.d.l.	b.d.l.	105.06	11.25	7.63	b.d.l.
34,30	3	GVG-13-145-E	7.48	n.d.	n.d.	n.d.	82.50	1.33	b.d.l.	b.d.l.	84.01	n.d.	n.d.	b.d.l.
40,86	3	GVG-13-167-E	7.40	n.d.	n.d.	n.d.	83.58	1.35	b.d.l.	b.d.l.	113.05	n.d.	n.d.	b.d.l.
44,60	4	GVG-13-180-E	7.35	n.d.	n.d.	n.d.	78.95	1.27	b.d.l.	b.d.l.	106.73	n.d.	n.d.	b.d.l.
47,26	4	GVG-13-188-E	7.35	n.d.	297.20	-14.66	87.17	1.41	b.d.l.	b.d.l.	109.72	n.d.	n.d.	b.d.l.

n.d.: not determined. b.d.l.: below detection limit.

Table E6. Flow-through experiment: ethanol injection.

time (weeks)	feeding strategy	injected ethanol (mg)	injected ethanol (mmol)	injected C-ethanol (mmol)	C/N
0,00	1				
0,13	1	136,00	2,96	5,91	2,98
0,28	1				
0,43	1				
0,60	1				
0,86	1				
0,99	1				
1,15	1	136,00	2,96	5,91	3,05
1,29	1				
1,43	1				
1,55	1				
1,86	1				
2,00	1				
2,14	1	136,00	2,96	5,91	3,10
2,29	1				
2,43	1				
2,57	1				
2,85	1				
3,00	1				
3,14	1	136,00	2,96	5,91	3,00
3,28	1				
3,42	1				
3,60	1				
3,86	1				
4,01	1				
4,14	1	136,00	2,96	5,91	2,98
4,28	1				
4,44	1				
4,68	1				
4,85	1				
5,00	1				
5,15	1	136,00	2,96	5,91	3,55
5,30	1				
5,30	1				
5,44	1				
5,59	1				
5,86	1				
6,00	1				
6,15	1	136,00	2,96	5,91	3,11
6,30	1				
6,44	1				
6,70	1				
6,87	1				
7,11	1	136,00	2,96	5,91	2,38
7,29	1				
7,44	1				
7,55	1				
7,84	1				
8,00	1				
8,15	1	136,00	2,96	5,91	2,64
8,28	1				
8,44	1				
8,61	1				

Table E6. Flow-through experiment: ethanol injection.

time (weeks)	feeding strategy	injected ethanol (mg)	injected ethanol (mmol)	injected C-ethanol (mmol)	C/N
8,87	1				
9,03	1				
9,15	1	136,00	2,96	5,91	2,44
9,29	1				
9,44	1				
9,74	1				
9,84	1				
10,13	1	120,00	2,61	5,22	1,99
10,31	1				
10,43	1				
10,71	1				
10,86	1				
11,03	1				
11,16	1	120,00	2,61	5,22	2,44
11,29	1				
11,43	1				
11,75	1				
11,96	1				
12,16	1	120,00	2,61	5,22	2,39
12,29	1				
12,44	1				
12,59	1				
12,74	1				
12,87	1				
12,99	1				
13,13	1	120,00	2,61	5,22	2,51
13,29	1				
13,43	1				
13,75	1				
13,87	1				
14,02	1				
14,16	2	16,00	0,35	0,70	2,32
14,29	2	16,00	0,35	0,70	2,19
14,43	2	16,00	0,35	0,70	1,83
14,61	2	24,00	0,52	1,04	2,55
14,83	2	24,00	0,52	1,04	3,08
14,87	2				
15,03	2	16,00	0,35	0,70	3,16
15,16	2	16,00	0,35	0,70	2,90
15,30	2	16,00	0,35	0,70	3,35
15,44	2	16,00	0,35	0,70	1,95
15,68	2	16,00	0,35	0,70	2,33
15,83	2	24,00	0,52	1,04	4,56
15,87	2				
16,00	2	16,00	0,35	0,70	2,82
16,15	2	16,00	0,35	0,70	3,35
16,30	2	16,00	0,35	0,70	3,07
16,43	2	16,00	0,35	0,70	2,02
16,68	2	16,00	0,35	0,70	2,33
16,87	2	16,00	0,35	0,70	2,82
17,01	2	16,00	0,35	0,70	2,75
17,16	2	16,00	0,35	0,70	2,75
17,31	2	16,00	0,35	0,70	1,88

Table E6. Flow-through experiment: ethanol injection.

time (weeks)	feeding strategy	injected ethanol (mg)	injected ethanol (mmol)	injected C-ethanol (mmol)	C/N
17,54	2	16,00	0,35	0,70	2,75
17,69	2	16,00	0,35	0,70	2,82
17,83	2	16,00	0,35	0,70	2,55
18,01	2	16,00	0,35	0,70	2,98
18,15	2	16,00	0,35	0,70	3,70
18,26	2	16,00	0,35	0,70	3,35
18,39	2	12,00	0,26	0,52	2,01
18,59	2	12,00	0,26	0,52	1,34
18,82	2	12,00	0,26	0,52	2,18
18,96	2	12,00	0,26	0,52	1,68
19,17	2	12,00	0,26	0,52	2,60
19,28	2	12,00	0,26	0,52	1,87
19,46	2	12,00	0,26	0,52	2,24
19,60	2	16,00	0,35	0,70	3,16
19,73	2	12,00	0,26	0,52	1,10
19,87	2	12,00	0,26	0,52	2,12
20,01	2	12,00	0,26	0,52	1,18
20,12	2	12,00	0,26	0,52	2,06
20,29	2	12,00	0,26	0,52	1,10
20,43	2	20,00	0,43	0,87	2,06
20,68	2	20,00	0,43	0,87	3,05
20,87	2	12,00	0,26	0,52	2,51
21,00	2	12,00	0,26	0,52	2,37
21,14	2	12,00	0,26	0,52	2,51
21,27	2	12,00	0,26	0,52	n.a.
NO FLOW (SYTEM BREAKDOWN)					
25,01	2	12,00	0,26	0,52	1,92
25,14	2	12,00	0,26	0,52	1,87
25,30	2	12,00	0,26	0,52	2,12
25,44	2	12,00	0,26	0,52	0,97
25,68	2	16,00	0,35	0,70	n.a.
25,84	2	16,00	0,35	0,70	n.a.
25,97	2	12,00	0,26	0,52	2,51
26,13	2	12,00	0,26	0,52	2,12
26,30	2	12,00	0,26	0,52	2,24
26,44	2	12,00	0,26	0,52	2,24
26,59	2	12,00	0,26	0,52	2,51
26,72	2	12,00	0,26	0,52	2,48
26,87	2	12,00	0,26	0,52	3,61
26,98	2	12,00	0,26	0,52	2,27
27,16	2	12,00	0,26	0,52	2,84
27,29	2	12,00	0,26	0,52	2,84
27,41	2	12,00	0,26	0,52	2,09
27,60	2	12,00	0,26	0,52	3,61
27,73	2	12,00	0,26	0,52	2,84
27,86	2	12,00	0,26	0,52	2,48
28,02	2	12,00	0,26	0,52	2,84
28,15	2	12,00	0,26	0,52	2,41
28,29	2	12,00	0,26	0,52	n.a.
28,44	2	12,00	0,26	0,52	n.a.
28,59	2	0,00	0,00	0,00	n.a.
28,87	2	12,00	0,26	0,52	2,48

Table E6. Flow-through experiment: ethanol injection.

time (weeks)	feeding strategy	injected ethanol (mg)	injected ethanol (mmol)	injected C-ethanol (mmol)	C/N
29,02	2	12,00	0,26	0,52	2,84
29,16	2	12,00	0,26	0,52	2,56
29,31	2	12,00	0,26	0,52	2,74
29,44	3	8,00	0,17	0,35	0,68
29,82	3	8,00	0,17	0,35	1,51
29,98	3	8,00	0,17	0,35	1,47
30,14	3	8,00	0,17	0,35	2,04
30,27	3	8,00	0,17	0,35	1,51
30,43	3	8,00	0,17	0,35	0,88
30,72	3	8,00	0,17	0,35	1,77
30,86	3	8,00	0,17	0,35	2,21
30,98	3	8,00	0,17	0,35	1,61
31,14	3	8,00	0,17	0,35	1,66
31,31	3	8,00	0,17	0,35	1,96
31,42	3	8,00	0,17	0,35	1,83
31,55	3	8,00	0,17	0,35	0,85
31,87	3	8,00	0,17	0,35	1,32
32,03	3	8,00	0,17	0,35	1,47
32,17	3	8,00	0,17	0,35	2,04
32,30	3	12,00	0,26	0,52	1,07
32,69	3	12,00	0,26	0,52	1,02
32,98	3	12,00	0,26	0,52	1,94
33,15	3	8,00	0,17	0,35	1,23
33,31	3	8,00	0,17	0,35	1,47
33,45	3	8,00	0,17	0,35	1,71
33,61	3	8,00	0,17	0,35	1,23
33,84	3	12,00	0,26	0,52	2,41
34,01	3	8,00	0,17	0,35	2,12
34,13	3	8,00	0,17	0,35	1,56
34,30	3	8,00	0,17	0,35	1,66
34,46	3	8,00	0,17	0,35	1,63
34,59	3	8,00	0,17	0,35	1,24
34,75	3	12,00	0,26	0,52	1,51
35,00	3	12,00	0,26	0,52	1,91
35,16	3	8,00	0,17	0,35	1,34
35,31	3	8,00	0,17	0,35	1,54
35,43	3	8,00	0,17	0,35	1,45
35,57	3	12,00	0,26	0,52	0,78
35,97	3	12,00	0,26	0,52	1,78
36,14	3	6,40	0,14	0,28	0,95
36,31	3	6,40	0,14	0,28	1,10
36,44	3	6,40	0,14	0,28	0,87
36,61	3	6,40	0,14	0,28	0,54
36,90	3	6,40	0,14	0,28	1,39
37,00	3	4,80	0,10	0,21	0,64
37,18	3	6,40	0,14	0,28	0,69
37,41	3	6,40	0,14	0,28	1,05
37,56	3	6,40	0,14	0,28	0,57
37,85	3	8,00	0,17	0,35	1,31
38,00	3	6,40	0,14	0,28	0,95
38,16	3	6,40	0,14	0,28	1,23
38,30	3	6,40	0,14	0,28	1,10
38,46	3	6,40	0,14	0,28	1,27

Table E6. Flow-through experiment: ethanol injection.

time (weeks)	feeding strategy	injected ethanol (mg)	injected ethanol (mmol)	injected C-ethanol (mmol)	C/N
38,59	3	6,40	0,14	0,28	0,58
38,86	3	6,40	0,14	0,28	1,13
39,01	3	6,40	0,14	0,28	1,10
39,16	3	6,40	0,14	0,28	1,23
39,30	3	6,40	0,14	0,28	1,07
39,46	3	6,40	0,14	0,28	1,23
39,59	3	6,40	0,14	0,28	1,49
39,70	3	12,00	0,26	0,52	0,84
40,06	3	6,40	0,14	0,28	1,31
40,17	3	6,40	0,14	0,28	1,00
40,31	3	6,40	0,14	0,28	1,19
40,45	3	6,40	0,14	0,28	1,16
40,59	3	6,40	0,14	0,28	0,57
40,86	3	6,40	0,14	0,28	0,51
41,17	no injection	0,00	0,00	0,00	0,00
41,46	no injection	0,00	0,00	0,00	0,00
41,72	no injection	0,00	0,00	0,00	0,00
42,03	no injection	0,00	0,00	0,00	0,00
42,33	no injection	0,00	0,00	0,00	0,00
42,60	no injection	0,00	0,00	0,00	0,00
42,84	no injection	0,00	0,00	0,00	0,00
43,17	no injection	0,00	0,00	0,00	0,00
43,44	no injection	0,00	0,00	0,00	0,00
43,61	no injection	0,00	0,00	0,00	0,00
43,98	no injection	0,00	0,00	0,00	0,00
44,30	no injection	0,00	0,00	0,00	0,00
44,60	4	6,40	0,14	0,28	0,63
44,83	4	6,40	0,14	0,28	0,95
44,99	4	6,40	0,14	0,28	1,04
45,15	4	6,40	0,14	0,28	0,99
45,31	4	6,40	0,14	0,28	1,29
45,43	4	6,40	0,14	0,28	0,95
45,60	4	6,40	0,14	0,28	0,40
45,98	4	12,00	0,26	0,52	2,10
46,12	4	6,40	0,14	0,28	1,02
46,28	4	6,40	0,14	0,28	1,12
46,42	4	6,40	0,14	0,28	0,91
46,59	4	6,40	0,14	0,28	0,50
47,01	4	6,40	0,14	0,28	1,46
47,12	4	6,40	0,14	0,28	1,12
47,26	4	6,40	0,14	0,28	0,99
47,42	4	6,40	0,14	0,28	1,04
47,56	4	6,40	0,14	0,28	1,04
47,69	4	12,00	0,26	0,52	1,03
47,95	4	6,40	0,14	0,28	1,04
48,10	4	6,40	0,14	0,28	1,10
48,24	4	6,40	0,14	0,28	0,97
48,39	4	6,40	0,14	0,28	1,04
48,54	4	6,40	0,14	0,28	1,10
48,69	4	6,40	0,14	0,28	1,16
48,84	4	6,40	0,14	0,28	n.a.

Table E7. Flow-through experiment: outflow analytical data.

time (weeks)	flow (ml/day)	feeding strategy	residence time (days)	sample	pH	ethanol (mg/l)	$\delta^{13}C$- ethanol	NPOC (mg/l)	TIC/DIC (mg/l)	$\delta^{13}C$- DIC	NO_3^- (mg/l)	NO_2^- (mg/l)	SO_4^{2-} (mg/l)	$\delta^{15}N$-NO_3^-	$\delta^{15}N$-NO_3^-	NH4+ (mg/l)	Fe (mg/l)	Mn (µg/l)
0,00																		
0,13	165,00	1	7,04	GVG-13-01	7,65	11,44	n.d.	n.d.	406,9	-11,61	99,00	b.d.l.	119,43	n.d.	n.d.	b.d.l.	n.d.	n.d.
0,28	165,00	1	7,04	GVG-13-02	n.d.	18,61	n.d.	n.d.	411,2	-7,64	97,96	b.d.l.	118,51	n.d.	n.d.	b.d.l.	n.d.	n.d.
0,43	180,00	1	6,45	GVG-13-03	7,41	21,91	n.d.	n.d.	417,2	-15,41	94,98	0,55	120,48	n.d.	n.d.	b.d.l.	n.d.	n.d.
0,60	166,96	1	6,95	GVG-13-04	7,45	27,43	n.d.	n.d.	406,5	-15,57	93,22	1,47	121,63	n.d.	n.d.	b.d.l.	n.d.	n.d.
0,86	172,02	1	6,75	GVG-13-05	7,54	115,22	-30,24	n.d.	420,1	-16,78	70,05	b.d.l.	119,05	13,15	5,26	b.d.l.	n.d.	n.d.
0,99	166,15	1	6,99	GVG-13-06	n.d.	282,73	-30,49	129,00	419,4	-17,47	18,53	b.d.l.	121,42	n.d.	n.d.	b.d.l.	0,01	3,34
1,15	172,08	1	6,75	GVG-13-07	7,41	194,95	-30,14	108,40	452,9	-17,65	0,69	33,30	120,10	n.d.	n.d.	b.d.l.	0,01	6,20
1,29	190,00	1	6,11	GVG-13-08	7,44	87,22	-30,11	49,42	455,5	-18,02	0,82	2,58	121,74	n.d.	n.d.	b.d.l.	0,01	5,27
1,43	170,00	1	6,83	GVG-13-09	7,22	19,91	-28,37	18,33	472,8	-17,82	b.d.l.	0,11	121,54	n.d.	n.d.	b.d.l.	0,02	7,60
1,55	153,44	1	7,57	GVG-13-10	7,23	11,39	n.d.	10,69	453,1	n.d.	7,37	5,94	122,46	n.d.	n.d.	b.d.l.	0,01	6,99
1,86	168,31	1	6,90	GVG-13-11	7,29	n.d.	-30,17	18,95	440,9	n.d.	7,25	5,33	120,56	n.d.	n.d.	b.d.l.	0,01	6,76
2,00	170,00	1	6,83	GVG-13-12	7,50	87,83	-30,02	66,79	442,2	n.d.	b.d.l.	b.d.l.	121,17	n.d.	n.d.	b.d.l.	0,01	21,50
2,14	181,26	1	6,41	GVG-13-13	7,53	119,88	-29,87	n.d.	446,3	n.d.	0,85	b.d.l.	123,50	n.d.	n.d.	b.d.l.	n.d.	n.d.
2,29	197,26	1	5,89	GVG-13-14	7,48	59,82	-29,81	n.d.	442,1	n.d.	b.d.l.	b.d.l.	122,68	n.d.	n.d.	b.d.l.	n.d.	n.d.
2,43	180,00	1	6,45	GVG-13-15	7,51	11,86	-28,61	n.d.	454,5	n.d.	b.d.l.	b.d.l.	122,69	n.d.	n.d.	b.d.l.	n.d.	n.d.
2,57	169,41	1	6,85	GVG-13-16	7,49	1,18	n.d.	n.d.	427	n.d.	37,08	5,67	119,43	24,91	22,48	b.d.l.	n.d.	n.d.
2,85	160,28	1	7,24	GVG-13-17	7,50	21,37	-29,51	n.d.	454,5	n.d.	5,56	1,05	122,33	n.d.	n.d.	b.d.l.	n.d.	n.d.
3,00	147,04	1	7,90	GVG-13-18	7,72	70,81	-29,60	n.d.	430,6	n.d.	0,69	b.d.l.	123,81	n.d.	n.d.	b.d.l.	n.d.	n.d.
3,14	150,52	1	7,71	GVG-13-19	8,22	46,07	-29,18	n.d.	445,3	n.d.	0,77	b.d.l.	125,08	n.d.	n.d.	b.d.l.	n.d.	n.d.
3,28	186,95	1	6,21	GVG-13-20	7,37	14,00	n.d.	n.d.	450,2	n.d.	0,87	b.d.l.	124,18	n.d.	n.d.	b.d.l.	n.d.	n.d.
3,42	170,00	1	6,83	GVG-13-21	7,38	n.d.	n.d.	n.d.	431,8	n.d.	19,44	4,73	123,29	27,50	19,03	b.d.l.	n.d.	n.d.
3,60	175,41	1	6,62	GVG-13-22	8,04	2,40	n.d.	n.d.	427,3	n.d.	30,41	4,68	123,78	24,84	17,75	b.d.l.	n.d.	n.d.

Table E7. Flow-through experiment: outflow analytical data.

time (weeks)	flow (ml/day)	feeding strategy	residence time (days)	sample	pH	ethanol (mg/l)	$\delta^{13}C$- ethanol	NPOC (mg/l)	TIC/DIC (mg/l)	$\delta^{13}C$- DIC	NO_3^- (mg/l)	NO_2^- (mg/l)	SO_4^{2-} (mg/l)	$\delta^{15}N$-NO_3^-	$\delta^{15}N$-NO_3^-	NH4+ (mg/l)	Fe (mg/l)	Mn (µg/l)
3,86	184,75	1	6,28	GVG-13-23	7,59	n.d.	n.d.	n.d.	454,5	n.d.	0,78	b.d.l.	125,19	n.d.	n.d.	b.d.l.	n.d.	n.d.
4,01	167,10	1	6,95	GVG-13-24	7,38	148,56	n.d.	78,63	426,9	-17,96	0,66	b.d.l.	123,57	n.d.	n.d.	b.d.l.	0,01	1348,60
4,14	164,12	1	7,08	GVG-13-25	7,50	69,49	n.d.	39,31	444,5	-15,90	0,77	b.d.l.	124,01	n.d.	n.d.	b.d.l.	0,16	1964,66
4,28	213,71	1	5,43	GVG-13-26	7,69	8,89	n.d.	12,54	419,3	-18,61	11,00	0,42	124,60	26,73	21,91	b.d.l.	0,02	1706,10
4,44	166,32	1	6,98	GVG-13-27	7,65	2,82	n.d.	7,92	399,8	-17,49	55,07	1,50	123,65	18,27	13,21	b.d.l.	0,01	2208,82
4,68	172,92	1	6,71	GVG-13-28	7,69	n.d.	n.d.	12,86	405,9	-17,64	30,53	0,30	123,02	19,17	14,46	b.d.l.	0,01	2239,62
4,85	179,45	1	6,47	GVG-13-29	7,51	112,93	n.d.	56,20	399,5	-17,14	0,97	b.d.l.	125,02	n.d.	n.d.	b.d.l.	0,01	1883,34
5,00	172,62	1	6,73	GVG-13-30	7,53	135,44	n.d.	56,03	396,8	-18,38	0,85	0,17	125,48	n.d.	n.d.	b.d.l.	0,01	2400,04
5,15	171,09	1	6,79	GVG-13-31	7,52	n.d.	n.d.	25,74	n.d.	-18,46	0,75	0,18	124,25	n.d.	n.d.	b.d.l.	0,01	2977,07
5,30	184,86	1	6,28	GVG-13-32	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	22,89	0,33	123,81	n.d.	n.d.	b.d.l.	n.d.	n.d.
5,30	n.a.	1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
5,44	n.a.	1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
5,59	158,45	1	7,33	GVG-13-33	7,51	4,12	n.d.	n.d.	n.d.	n.d.	73,12	3,46	118,86	n.d.	n.d.	b.d.l.	n.d.	n.d.
5,86	176,00	1	6,60	GVG-13-34	7,40	15,70	n.d.	n.d.	n.d.	n.d.	34,60	6,32	118,61	n.d.	n.d.	b.d.l.	n.d.	n.d.
6,00	177,39	1	6,55	GVG-13-35	7,99	121,39	n.d.	n.d.	n.d.	n.d.	b.d.l.	0,13	118,35	n.d.	n.d.	b.d.l.	n.d.	n.d.
6,15	161,58	1	7,19	GVG-13-36	7,59	96,70	n.d.	n.d.	n.d.	n.d.	0,31	b.d.l.	121,14	n.d.	n.d.	b.d.l.	n.d.	n.d.
6,30	205,71	1	5,64	GVG-13-37	7,54	75,62	-29,68	n.d.	426	n.d.	0,28	b.d.l.	123,59	n.d.	n.d.	b.d.l.	n.d.	n.d.
6,44	180,00	1	6,45	GVG-13-38	7,47	2,15	-27,32	n.d.	413,9	n.d.	4,79	b.d.l.	120,05	24,36	22,45	b.d.l.	n.d.	n.d.
6,70	163,64	1	7,10	GVG-13-39	7,49	21,96	-29,89	n.d.	418,8	n.d.	10,81	b.d.l.	125,71	n.d.	n.d.	b.d.l.	n.d.	n.d.
6,87	182,07	1	6,38	GVG-13-40	7,49	196,64	-30,08	n.d.	414,7	n.d.	0,52	b.d.l.	119,98	n.d.	n.d.	b.d.l.	n.d.	n.d.
7,11	161,33	1	7,20	GVG-13-41	7,49	142,24	-29,90	n.d.	417	n.d.	0,79	b.d.l.	123,86	n.d.	n.d.	b.d.l.	n.d.	n.d.
7,29	222,81	1	5,21	GVG-13-42	7,49	5,29	-28,11	n.d.	438,2	n.d.	b.d.l.	b.d.l.	121,36	n.d.	n.d.	b.d.l.	n.d.	n.d.
7,44	190,10	1	6,11	GVG-13-43	7,46	b.d.l.	n.d.	n.d.	413,9	n.d.	51,28	0,27	123,22	16,88	11,31	b.d.l.	n.d.	n.d.

Table E7. Flow-through experiment: outflow analytical data.

time (weeks)	flow (ml/day)	feeding strategy	residence time (days)	sample	pH	ethanol (mg/l)	$\delta^{13}C$- ethanol	NPOC (mg/l)	TIC/DIC (mg/l)	$\delta^{13}C$- DIC	NO_3^- (mg/l)	NO_2^- (mg/l)	SO_4^{2-} (mg/l)	$\delta^{15}N$-NO_3^-	$\delta^{15}N$-NO_3^-	NH4+ (mg/l)	Fe (mg/l)	Mn (µg/l)
7,55	216,00	1	5,38	GVG-13-44	7,58	6,41	-29,49	n.d.	426,2	n.d.	17,08	0,27	121,52	19,62	13,37	b.d.l.	n.d.	n.d.
7,84	191,33	1	6,07	GVG-13-45	7,41	168,57	-30,13	n.d.	426,1	n.d.	0,66	0,28	122,08	n.d.	n.d.	b.d.l.	n.d.	n.d.
8,00	171,43	1	6,77	GVG-13-46	7,43	165,58	-30,13	n.d.	408,9	n.d.	0,43	b.d.l.	99,62	n.d.	n.d.	b.d.l.	n.d.	n.d.
8,15	186,58	1	6,22	GVG-13-47	7,45	23,42	-30,01	n.d.	415,5	n.d.	0,77	b.d.l.	126,22	n.d.	n.d.	b.d.l.	n.d.	n.d.
8,28	227,37	1	5,11	GVG-13-48	7,45	b.d.l.	n.d.	n.d.	401,7	n.d.	29,87	b.d.l.	121,82	18,79	12,69	b.d.l.	n.d.	n.d.
8,44	171,22	1	6,78	GVG-13-49	7,44	b.d.l.	n.d.	n.d.	400,9	n.d.	65,76	b.d.l.	125,43	15,77	10,30	b.d.l.	n.d.	n.d.
8,61	183,12	1	6,34	GVG-13-50	7,46	6,80	-30,05	n.d.	417	n.d.	24,12	b.d.l.	123,26	18,23	12,11	b.d.l.	n.d.	n.d.
8,87	192,91	1	6,02	GVG-13-51	7,47	145,23	-30,23	n.d.	417,6	n.d.	0,36	b.d.l.	119,89	n.d.	n.d.	b.d.l.	n.d.	n.d.
9,03	178,82	1	6,49	GVG-13-52	7,71	155,22	-30,43	n.d.	416,5	n.d.	b.d.l.	b.d.l.	122,75	n.d.	n.d.	b.d.l.	n.d.	n.d.
9,15	188,31	1	6,17	GVG-13-53	7,53	21,87	-30,38	n.d.	417,6	n.d.	0,42	b.d.l.	121,97	n.d.	n.d.	b.d.l.	n.d.	n.d.
9,29	219,57	1	5,29	GVG-13-54	7,51	b.d.l.	n.d.	n.d.	417,6	n.d.	22,72	0,27	121,75	n.d.	n.d.	b.d.l.	n.d.	n.d.
9,44	181,22	1	6,41	GVG-13-55	7,47	b.d.l.	n.d.	n.d.	392	n.d.	60,98	0,28	122,48	n.d.	n.d.	n.d.	n.d.	n.d.
9,74	185,88	1	6,25	GVG-13-56	7,46	2,28	-30,28	n.d.	405,1	n.d.	33,04	0,23	126,07	n.d.	n.d.	n.d.	n.d.	n.d.
9,84	174,32	1	6,66	GVG-13-57	7,45	43,12	-30,65	n.d.	396	n.d.	0,72	0,36	121,99	n.d.	n.d.	n.d.	n.d.	n.d.
10,13	185,49	1	6,26	GVG-13-58	7,41	27,27	-31,47	n.d.	417,5	n.d.	0,40	b.d.l.	121,90	n.d.	n.d.	b.d.l.	n.d.	n.d.
10,31	220,28	1	5,27	GVG-13-59	7,49	b.d.l.	n.d.	n.d.	429,9	n.d.	26,49	b.d.l.	120,81	n.d.	n.d.	n.d.	n.d.	n.d.
10,43	188,85	1	6,15	GVG-13-60	7,45	b.d.l.	n.d.	n.d.	417,6	n.d.	58,30	b.d.l.	120,26	n.d.	n.d.	n.d.	n.d.	n.d.
10,71	182,86	1	6,35	GVG-13-61	7,47	41,09	-30,57	n.d.	442,3	n.d.	7,94	b.d.l.	121,69	n.d.	n.d.	n.d.	n.d.	n.d.
10,86	184,62	1	6,29	GVG-13-62	7,45	108,87	-30,89	n.d.	429,9	n.d.	0,45	b.d.l.	120,31	n.d.	n.d.	b.d.l.	n.d.	n.d.
11,03	180,00	1	6,45	GVG-13-63	7,44	53,70	-32,63	n.d.	429,9	n.d.	2,29	b.d.l.	125,31	n.d.	n.d.	n.d.	n.d.	n.d.
11,16	186,35	1	6,23	GVG-13-64	7,45	5,41	-35,00	n.d.	446,9	n.d.	0,59	b.d.l.	120,82	n.d.	n.d.	n.d.	n.d.	n.d.
11,29	206,45	1	5,62	GVG-13-65	7,45	b.d.l.	n.d.	n.d.	442,2	n.d.	18,53	b.d.l.	108,67	n.d.	n.d.	n.d.	n.d.	n.d.
11,43	193,04	1	6,01	GVG-13-66	7,58	b.d.l.	n.d.	n.d.	429,9	n.d.	40,90	b.d.l.	101,42	n.d.	n.d.	b.d.l.	n.d.	n.d.

Table E7. Flow-through experiment: outflow analytical data.

time (weeks)	flow (ml/day)	feeding strategy	residence time (days)	sample	pH	ethanol (mg/l)	δ^{13}C-ethanol	NPOC (mg/l)	TIC/DIC (mg/l)	δ^{13}C- DIC	NO_3^- (mg/l)	NO_2^- (mg/l)	SO_4^{2-} (mg/l)	δ^{15}N-NO_3^-	δ^{15}N-NO_3^-	NH4+ (mg/l)	Fe (mg/l)	Mn (µg/l)
11,75	176,60	1	6,57	GVG-13-67	7,49	33,42	-31,53	19,96	432,2	n.d.	12,65	b.d.l.	114,02	n.d.	n.d.	n.d.	n.d.	n.d.
11,96	179,17	1	6,48	GVG-13-68	7,46	76,73	-32,55	56,08	417,6	n.d.	0,31	b.d.l.	108,83	n.d.	n.d.	n.d.	n.d.	n.d.
12,16	147,31	1	7,88	GVG-13-69	7,46	11,88	-38,00	22,26	417,6	n.d.	0,33	b.d.l.	108,83	n.d.	n.d.	n.d.	n.d.	n.d.
12,29	203,48	1	5,71	GVG-13-70	7,47	b.d.l.	n.d.	7,3	429,9	n.d.	17,00	b.d.l.	106,46	n.d.	n.d.	b.d.l.	n.d.	n.d.
12,44	175,00	1	6,64	GVG-13-71	7,46	b.d.l.	n.d.	6,3	406,3	n.d.	42,90	b.d.l.	109,28	n.d.	n.d.	n.d.	n.d.	n.d.
12,59	172,43	1	6,73	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
12,74	169,41	1	6,85	GVG-13-72	7,47	61,57	-32,05	40,81	417,6	n.d.	1,32	b.d.l.	112,26	n.d.	n.d.	n.d.	n.d.	n.d.
12,87	180,00	1	6,45	GVG-13-73	7,44	141,19	-32,97	75,26	405,1	n.d.	2,59	b.d.l.	112,43	n.d.	n.d.	n.d.	n.d.	n.d.
12,99	167,30	1	6,94	GVG-13-74	7,53	87,58	-36,12	n.d.	391,4	n.d.	0,63	b.d.l.	89,86	n.d.	n.d.	b.d.l.	n.d.	n.d.
13,13	182,54	1	6,36	GVG-13-75	7,51	19,72	-42,35	39,96	406,8	n.d.	0,54	b.d.l.	108,03	n.d.	n.d.	n.d.	0,09	5870,66
13,29	181,13	1	6,41	GVG-13-76	7,48	b.d.l.	n.d.	8,53	417,6	n.d.	3,75	b.d.l.	92,57	n.d.	n.d.	n.d.	0,09	5475,06
13,43	160,00	1	7,26	GVG-13-77	7,51	b.d.l.	n.d.	6,3	405,4	n.d.	33,57	b.d.l.	112,58	n.d.	n.d.	n.d.	0,09	5475,60
13,75	153,10	1	7,58	GVG-13-78	7,51	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
13,87	169,41	1	6,85	GVG-13-79	7,44	106,08	-36,90	81,21	387,9	n.d.	0,69	b.d.l.	113,09	n.d.	n.d.	n.d.	n.d.	n.d.
14,02	150,81	1	7,70	GVG-13-80	7,40	59,10	-41,37	n.d.	413,2	n.d.	0,39	b.d.l.	109,89	n.d.	n.d.	n.d.	0,14	6345,56
14,16	163,52	2	7,10	GVG-13-81	7,89	9,06	-46,72	n.d.	380,3	n.d.	0,46	b.d.l.	113,13	n.d.	n.d.	n.d.	0,09	5834,02
14,29	178,04	2	6,52	GVG-13-81bis	n.d.	b.d.l.	n.d.	n.d.	n.d.	n.d.	1,13	b.d.l.	113,31	n.d.	n.d.	n.d.	0,09	6757,79
14,43	185,81	2	6,25	GVG-13-82	7,61	b.d.l.	n.d.	69,4	384	n.d.	14,42	b.d.l.	116,07	22,35	14,75	b.d.l.	0,09	5448,96
14,61	167,80	2	6,92	GVG-13-83	7,53	b.d.l.	n.d.	4,94	380,8	n.d.	38,30	b.d.l.	113,45	19,38	12,95	n.d.	0,10	5423,54
14,83	n.a.	2	n.a.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
14,87	174,13	2	6,67	GVG-13-84	7,49	b.d.l.	n.d.	6,19	392	n.d.	14,04	b.d.l.	119,28	21,47	16,04	n.d.	0,10	5145,41
15,03	148,65	2	7,81	GVG-13-85	7,46	b.d.l.	n.d.	23,73	423,2	n.d.	0,40	b.d.l.	110,82	n.d.	n.d.	n.d.	0,10	5939,73
15,16	184,75	2	6,28	GVG-13-86	7,42	b.d.l.	n.d.	38,59	412,2	n.d.	1,04	b.d.l.	110,31	n.d.	n.d.	b.d.l.	n.d.	n.d.

Table E7. Flow-through experiment: outflow analytical data.

time (weeks)	flow (ml/day)	feeding strategy	residence time (days)	sample	pH	ethanol (mg/l)	$\delta^{13}C$- ethanol	NPOC (mg/l)	TIC/DIC (mg/l)	$\delta^{13}C$- DIC	NO_3^- (mg/l)	NO_2^- (mg/l)	SO_4^{2-} (mg/l)	$\delta^{15}N$-NO_3^-	$\delta^{15}N$-NO_3^-	NH4+ (mg/l)	Fe (mg/l)	Mn (µg/l)
15,30	178,79	2	6,49	GVG-13-87	7,41	b.d.l.	n.d.	n.d.	413	n.d.	0,45	b.d.l.	113,01	n.d.	n.d.	n.d.	n.d.	n.d.
15,44	169,41	2	6,85	GVG-13-88	n.d.	b.d.l.	n.d.	n.d.	399,9	n.d.	1,42	b.d.l.	112,40	10,93	14,92	n.d.	n.d.	n.d.
15,68	161,30	2	7,20	GVG-13-89	n.d.	b.d.l.	n.d.	n.d.	n.d.	n.d.	1,15	b.d.l.	110,38	n.d.	n.d.	n.d.	n.d.	n.d.
15,83	n.a.	2	n.a.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
15,87	174,78	2	6,64	GVG-13-90	7,37	b.d.l.	n.d.	n.d.	n.d.	n.d.	0,33	b.d.l.	106,55	n.d.	n.d.	b.d.l.	n.d.	n.d.
16,00	151,87	2	7,65	GVG-13-91	7,36	b.d.l.	n.d.	n.d.	n.d.	n.d.	1,00	b.d.l.	103,85	n.d.	n.d.	n.d.	n.d.	n.d.
16,15	175,95	2	6,60	GVG-13-92	7,35	b.d.l.	n.d.	n.d.	n.d.	n.d.	b.d.l.	b.d.l.	105,99	n.d.	n.d.	n.d.	n.d.	n.d.
16,30	152,58	2	7,61	GVG-13-93	n.d.	b.d.l.	n.d.	n.d.	n.d.	n.d.	0,72	b.d.l.	107,79	n.d.	n.d.	n.d.	n.d.	n.d.
16,43	193,10	2	6,01	GVG-13-94	7,33	n.d.	n.d.	n.d.	n.d.	n.d.	0,33	b.d.l.	107,47	n.d.	n.d.	n.d.	n.d.	n.d.
16,68	152,03	2	7,64	GVG-13-95	7,32	n.d.	n.d.	n.d.	n.d.	n.d.	1,02	b.d.l.	108,05	n.d.	n.d.	b.d.l.	n.d.	n.d.
16,87	173,86	2	6,68	GVG-13-96	7,32	n.d.	n.d.	n.d.	n.d.	n.d.	0,27	b.d.l.	107,28	n.d.	n.d.	n.d.	n.d.	n.d.
17,01	196,13	2	5,92	GVG-13-97	7,47	n.d.	n.d.	n.d.	n.d.	n.d.	0,31	b.d.l.	106,93	n.d.	n.d.	n.d.	n.d.	n.d.
17,16	183,53	2	6,33	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
17,31	184,13	2	6,31	GVG-13-98	7,37	n.d.	n.d.	n.d.	n.d.	n.d.	0,29	b.d.l.	105,61	n.d.	n.d.	n.d.	n.d.	n.d.
17,54	181,59	2	6,39	GVG-13-99	7,33	b.d.l.	n.d.	n.d.	n.d.	n.d.	0,28	b.d.l.	105,47	n.d.	n.d.	b.d.l.	n.d.	n.d.
17,69	182,34	2	6,37	GVG-13-100	7,33	n.d.	n.d.	n.d.	n.d.	n.d.	b.d.l.	b.d.l.	105,07	n.d.	n.d.	n.d.	n.d.	n.d.
17,83	189,34	2	6,13	GVG-13-101	7,33	n.d.	n.d.	n.d.	n.d.	n.d.	0,89	b.d.l.	106,29	n.d.	n.d.	n.d.	n.d.	n.d.
18,01	171,82	2	6,76	GVG-13-102	7,33	n.d.	n.d.	n.d.	n.d.	n.d.	0,40	b.d.l.	105,94	n.d.	n.d.	n.d.	n.d.	n.d.
18,15	178,76	2	6,50	GVG-13-103	7,34	n.d.	n.d.	n.d.	n.d.	n.d.	0,25	b.d.l.	105,19	n.d.	n.d.	b.d.l.	n.d.	n.d.
18,26	180,78	2	6,42	GVG-13-104	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0,58	b.d.l.	104,42	n.d.	n.d.	n.d.	n.d.	n.d.
18,39	177,23	2	6,55	GVG-13-105	n.d.	b.d.l.	n.d.	n.d.	n.d.	n.d.	0,38	b.d.l.	103,90	n.d.	n.d.	n.d.	n.d.	n.d.
18,59	145,45	2	7,98	GVG-13-106	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0,30	b.d.l.	103,38	n.d.	n.d.	n.d.	n.d.	n.d.
18,82	185,41	2	6,26	GVG-13-107	7,40	n.d.	n.d.	n.d.	n.d.	n.d.	0,45	b.d.l.	104,60	n.d.	n.d.	b.d.l.	n.d.	n.d.

Table E7. Flow-through experiment: outflow analytical data.

time (weeks)	flow (ml/day)	feeding strategy	residence time (days)	sample	pH	ethanol (mg/l)	$\delta^{13}C$- ethanol	NPOC (mg/l)	TIC/DIC (mg/l)	$\delta^{13}C$- DIC	NO_3^- (mg/l)	NO_2^- (mg/l)	SO_4^{2-} (mg/l)	$\delta^{15}N$-NO_3^-	$\delta^{15}N$-NO_3^-	NH4+ (mg/l)	Fe (mg/l)	Mn (µg/l)
18,96	183,72	2	6,32	GVG-13-108	7,37	n.d.	n.d.	n.d.	n.d.	n.d.	0,29	b.d.l.	104,48	n.d.	n.d.	n.d.	n.d.	n.d.
19,17	167,77	2	6,92	GVG-13-109	7,38	n.d.	n.d.	n.d.	n.d.	n.d.	0,36	b.d.l.	102,64	n.d.	n.d.	n.d.	n.d.	n.d.
19,28	207,63	2	5,59	GVG-13-110	n.d.	n.d.	n.d.	23,77	n.d.	n.d.	0,58	b.d.l.	103,96	n.d.	n.d.	n.d.	0,09	8847,14
19,46	169,64	2	6,84	GVG-13-111	7,38	n.d.	n.d.	19,89	352,39	n.d.	0,64	b.d.l.	105,26	n.d.	n.d.	b.d.l.	0,09	8725,51
19,60	183,18	2	6,34	GVG-13-112	n.d.	n.d.	n.d.	34,47	365,36	-14,55	0,56	b.d.l.	100,31	n.d.	n.d.	n.d.	0,09	8824,53
19,73	189,03	2	6,14	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
19,87	194,33	2	5,98	GVG-13-113	n.d.	n.d.	n.d.	28,16	365,73	-13,57	0,36	b.d.l.	101,25	n.d.	n.d.	n.d.	0,09	8879,33
20,01	189,34	2	6,13	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
20,12	193,72	2	5,99	GVG-13-114	n.d.	n.d.	n.d.	49,56	335,41	-12,5	0,69	b.d.l.	97,11	n.d.	n.d.	n.d.	0,09	8817,52
20,29	169,67	2	6,84	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
20,43	175,48	2	6,62	GVG-13-115	n.d.	n.d.	n.d.	39,65	377,23	-11,36	0,82	b.d.l.	97,15	n.d.	n.d.	n.d.	0,09	8887,18
20,68	179,31	2	6,48	GVG-13-116	n.d.	n.d.	n.d.	37,22	347,65	-11,26	1,13	b.d.l.	96,87	n.d.	n.d.	n.d.	0,09	8932,65
20,87	170,32	2	6,82	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
21,00	170,67	2	6,80	GVG-13-117	n.d.	n.d.	n.d.	23,71	366,46	-13,85	0,66	b.d.l.	96,75	n.d.	n.d.	n.d.	0,10	9109,13
21,14	173,62	2	6,69	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
21,27	176,55	2	6,58	GVG-13-118	n.d.	n.d.	n.d.	15,92	381,62	-15,43	1,57	b.d.l.	97,88	n.d.	n.d.	n.d.	0,09	10009,54
NO FLOW (SYTEM BREAKDOWN)																		
25,01	n.a.	2	n.a.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
25,14	228,23	2	5,09	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
25,30	197,83	2	5,87	GVG-13-119	n.d.	n.d.	n.d.	5,55	337,42	-16,53	55,01	b.d.l.	91,41	12,14	5,75	b.d.l.	0,10	4745,41
25,44	187,40	2	6,20	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
25,68	249,00	2	4,66	GVG-13-120	7,11	n.d.	n.d.	3,45	351,39	-16,42	19,87	b.d.l.	104,02	12,93	7,74	n.d.	0,10	4681,51
25,84	n.a.	2	n.a.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Table E7. Flow-through experiment: outflow analytical data.

time (weeks)	flow (ml/day)	feeding strategy	residence time (days)	sample	pH	ethanol (mg/l)	$\delta^{13}C$- ethanol	NPOC (mg/l)	TIC/DIC (mg/l)	$\delta^{13}C$- DIC	NO_3^- (mg/l)	NO_2^- (mg/l)	SO_4^{2-} (mg/l)	$\delta^{15}N$-NO_3^-	$\delta^{15}N$-NO_3^-	NH4+ (mg/l)	Fe (mg/l)	Mn (µg/l)
25,97	n.a.	2	n.a.	GVG-13-121	n.d.	n.d.	n.d.	5,25	n.d.	n.d.	3,76	b.d.l.	88,92	14,17	9,25	b.d.l.	0,09	4165,55
26,15	129,08	2	7,79	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
26,30	180,59	2	7,45	GVG-13-122	7,33	b.d.l.	n.d.	2	367,46	-17,23	0,90	b.d.l.	87,03	n.d.	n.d.	n.d.	0,10	3860,78
26,44	183,83	2	6,32	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
26,59	176,33	2	6,59	GVG-13-123	7,53	b.d.l.	n.d.	3,05	382,16	-17,93	0,24	b.d.l.	86,00	n.d.	n.d.	n.d.	0,11	3316,51
26,72	171,30	2	6,78	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
26,87	147,69	2	7,86	GVG-13-124	7,49	n.d.	n.d.	11,92	382,35	-17,42	0,94	b.d.l.	97,80	n.d.	n.d.	n.d.	0,09	4868,31
26,98	148,73	2	7,81	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
27,16	137,70	2	8,43	GVG-13-125	7,34	b.d.l.	n.d.	32,6	379,97	-13,92	0,29	b.d.l.	99,33	n.d.	n.d.	b.d.l.	0,09	6475,47
27,29	152,15	2	7,63	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
27,44	140,00	2	6,91	PV8 GVG-13-126	7,30	n.d.	n.d.	66,97	420,42	-9,97	0,80	b.d.l.	99,35	n.d.	n.d.	n.d.	0,09	7173,13
27,60	167,34	2	7,96	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
27,73	123,75	2	9,38	GVG-13-127	7,31	n.d.	n.d.	52,59	361,34	-8,4	6,93	b.d.l.	103,26	9,98	23,68	n.d.	0,12	6737,34
27,86	144,00	2	8,06	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
28,02	143,55	2	8,09	GVG-13-128	7,31	n.d.	n.d.	35,9	321,16	-10,54	0,77	b.d.l.	104,24	n.d.	n.d.	b.d.l.	0,09	6659,15
28,15	n.a.	2	7,20	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
28,29	297,76	2	3,90	GVG-13-129	7,30	n.d.	n.d.	n.d.	n.d.	n.d.	b.d.l.	b.d.l.	79,66	n.d.	n.d.	n.d.	n.d.	n.d.
28,44	n.a.	2	n.a.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
28,59	n.a.	2	n.a.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
28,87	n.a.	2	n.a.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
29,02	150,59	2	7,71	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
29,16	149,33	2	7,78	GVG-13-130	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0,88	b.d.l.	104,45	n.d.	n.d.	n.d.	n.d.	n.d.
29,31	147,33	2	7,88	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Table E7. Flow-through experiment: outflow analytical data.

time (weeks)	flow (ml/day)	feeding strategy	residence time (days)	sample	pH	ethanol (mg/l)	δ^{13}C-ethanol	NPOC (mg/l)	TIC/DIC (mg/l)	δ^{13}C- DIC	NO_3^- (mg/l)	NO_2^- (mg/l)	SO_4^{2-} (mg/l)	δ^{15}N-NO_3^-	δ^{15}N-NO_3^-	NH4+ (mg/l)	Fe (mg/l)	Mn (µg/l)
29,44	149,68	3	7,76	GVG-13-PV10	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
29,82	148,18	3	7,84	GVG-13-131	7,77	n.d.	n.d.	n.d.	n.d.	n.d.	0,65	b.d.l.	84,22	n.d.	n.d.	n.d.	n.d.	n.d.
29,98	156,52	3	7,42	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
30,14	157,09	3	7,39	GVG-13-132	7,32	n.d.	n.d.	n.d.	n.d.	n.d.	b.d.l.	b.d.l.	84,90	n.d.	n.d.	n.d.	n.d.	n.d.
30,27	152,20	3	7,63	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
30,43	154,13	3	7,53	GVG-13-133	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1,03	b.d.l.	83,36	n.d.	n.d.	n.d.	n.d.	n.d.
30,72	148,45	3	7,82	GVG-13-134	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0,89	b.d.l.	78,61	n.d.	n.d.	n.d.	n.d.	n.d.
30,86	150,00	3	7,74	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
30,98	145,82	3	7,96	GVG-13-135	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1,10	b.d.l.	80,23	n.d.	n.d.	n.d.	n.d.	n.d.
31,14	141,01	3	8,23	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
31,31	142,66	3	8,14	GVG-13-136	7,39	n.d.	n.d.	n.d.	n.d.	n.d.	b.d.l.	b.d.l.	80,17	n.d.	n.d.	n.d.	n.d.	n.d.
31,42	162,68	3	7,14	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
31,55	158,18	3	7,34	GVG-13-137	7,32	n.d.	n.d.	n.d.	n.d.	n.d.	b.d.l.	b.d.l.	96,65	n.d.	n.d.	n.d.	n.d.	n.d.
31,87	140,16	3	8,28	GVG-13-138	7,32	n.d.	n.d.	n.d.	n.d.	n.d.	2,80	b.d.l.	99,18	n.d.	n.d.	b.d.l.	n.d.	n.d.
32,03	180,56	3	6,43	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
32,17	183,18	3	6,34	GVG-13-139	7,92	n.d.	n.d.	n.d.	n.d.	n.d.	b.d.l.	b.d.l.	79,36	n.d.	n.d.	n.d.	n.d.	n.d.
32,30	138,67	3	8,37	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
32,69	138,03	3	8,41	GVG-13-140	7,28	n.d.	n.d.	n.d.	n.d.	n.d.	b.d.l.	b.d.l.	80,10	n.d.	n.d.	n.d.	n.d.	n.d.
32,98	187,20	3	6,20	GVG-13-141	7,30	n.d.	n.d.	n.d.	n.d.	n.d.	0,68	b.d.l.	81,78	n.d.	n.d.	n.d.	n.d.	n.d.
33,15	179,45	3	6,47	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
33,31	183,74	3	6,32	GVG-13-142	7,28	n.d.	n.d.	n.d.	n.d.	n.d.	b.d.l.	b.d.l.	97,38	n.d.	n.d.	n.d.	n.d.	n.d.
33,45	182,54	3	6,36	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
33,61	138,63	3	8,38	GVG-13-143	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0,59	b.d.l.	96,57	n.d.	n.d.	n.d.	n.d.	n.d.

Table E7. Flow-through experiment: outflow analytical data.

time (weeks)	flow (ml/day)	feeding strategy	residence time (days)	sample	pH	ethanol (mg/l)	δ^{13}C-ethanol	NPOC (mg/l)	TIC/DIC (mg/l)	δ^{13}C- DIC	NO_3^- (mg/l)	NO_2^- (mg/l)	SO_4^{2-} (mg/l)	δ^{15}N-NO_3^-	δ^{15}N-NO_3^-	NH4+ (mg/l)	Fe (mg/l)	Mn (µg/l)
33,84	139,15	3	8,34	GVG-13-144	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	b.d.l.	b.d.l.	75,98	n.d.	n.d.	n.d.	n.d.	n.d.
34,01	137,34	3	8,45	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
34,13	142,29	3	8,16	GVG-13-145	7,36	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
34,30	144,42	3	8,04	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
34,46	144,45	3	8,04	GVG-13-146	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1,19	b.d.l.	89,75	n.d.	n.d.	n.d.	n.d.	n.d.
34,59	177,92	3	6,53	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
34,75	190,19	3	6,11	GVG-13-147	7,43	n.d.	n.d.	n.d.	n.d.	n.d.	b.d.l.	b.d.l.	72,52	n.d.	n.d.	b.d.l.	n.d.	n.d.
35,00	146,82	3	7,91	GVG-13-148	7,36	n.d.	n.d.	n.d.	n.d.	n.d.	b.d.l.	b.d.l.	91,34	n.d.	n.d.	n.d.	n.d.	n.d.
35,16	186,25	3	6,23	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
35,31	185,96	3	6,24	GVG-13-149	7,34	n.d.	n.d.	n.d.	n.d.	n.d.	b.d.l.	b.d.l.	87,86	n.d.	n.d.	n.d.	n.d.	n.d.
35,43	192,00	3	6,05	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
35,57	187,83	3	6,18	GVG-13-150	7,31	n.d.	n.d.	n.d.	n.d.	n.d.	0,24	b.d.l.	89,23	n.d.	n.d.	n.d.	n.d.	n.d.
35,97	176,47	3	6,58	GVG-13-151	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0,58	b.d.l.	86,09	n.d.	n.d.	n.d.	n.d.	n.d.
36,14	188,57	3	6,16	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
36,31	188,57	3	6,16	GVG-13-152	7,28	n.d.	n.d.	n.d.	n.d.	n.d.	b.d.l.	b.d.l.	90,48	n.d.	n.d.	b.d.l.	n.d.	n.d.
36,44	202,67	3	5,73	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
36,61	203,29	3	5,71	GVG-13-153	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0,67	b.d.l.	98,60	n.d.	n.d.	n.d.	n.d.	n.d.
36,90	192,99	3	6,02	GVG-13-154	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0,72	b.d.l.	80,01	n.d.	n.d.	n.d.	n.d.	n.d.
37,00	200,00	3	5,81	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
37,18	197,09	3	5,89	GVG-13-155	7,35	n.d.	n.d.	n.d.	n.d.	n.d.	10,93	b.d.l.	107,04	n.d.	n.d.	n.d.	n.d.	n.d.
37,41	195,63	3	5,94	GVG-13-156	7,26	n.d.	n.d.	n.d.	n.d.	n.d.	6,54	b.d.l.	144,49	n.d.	n.d.	n.d.	n.d.	n.d.
37,56	186,41	3	6,23	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
37,85	180,61	3	6,43	GVG-13-157	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0,21	b.d.l.	97,29	n.d.	n.d.	n.d.	n.d.	n.d.

Table E7. Flow-through experiment: outflow analytical data.

time (weeks)	flow (ml/day)	feeding strategy	residence time (days)	sample	pH	ethanol (mg/l)	$\delta^{13}C$-ethanol	NPOC (mg/l)	TIC/DIC (mg/l)	$\delta^{13}C$- DIC	NO_3^- (mg/l)	NO_2^- (mg/l)	SO_4^{2-} (mg/l)	$\delta^{15}N$-NO_3^-	$\delta^{15}N$-NO_3^-	NH4+ (mg/l)	Fe (mg/l)	Mn (µg/l)
38,00	192,00	3	6,05	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
38,16	192,00	3	6,05	GVG-13-158	7,29	n.d.	n.d.	n.d.	n.d.	n.d.	0,72	b.d.l.	96,80	n.d.	n.d.	n.d.	n.d.	n.d.
38,30	181,33	3	6,40	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
38,46	172,08	3	6,75	GVG-13-159	7,24	n.d.	n.d.	n.d.	n.d.	n.d.	0,50	b.d.l.	101,80	n.d.	n.d.	n.d.	n.d.	n.d.
38,59	178,65	3	6,50	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
38,86	188,51	3	6,16	GVG-13-160	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0,27	b.d.l.	97,56	n.d.	n.d.	b.d.l.	n.d.	n.d.
39,01	177,60	3	6,54	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
39,16	177,09	3	6,56	GVG-13-161	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	b.d.l.	b.d.l.	91,65	n.d.	n.d.	n.d.	n.d.	n.d.
39,30	175,48	3	6,62	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
39,46	176,05	3	6,60	GVG-13-162	7,29	n.d.	n.d.	n.d.	n.d.	n.d.	0,14	b.d.l.	93,21	n.d.	n.d.	n.d.	n.d.	n.d.
39,59	183,37	3	6,33	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
39,70	182,44	3	6,36	GVG-13-163	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0,19	b.d.l.	93,28	n.d.	n.d.	n.d.	n.d.	n.d.
40,06	186,78	3	6,22	GVG-13-164	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1,23	b.d.l.	88,01	n.d.	n.d.	n.d.	n.d.	n.d.
40,17	204,80	3	5,67	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
40,31	205,71	3	5,64	GVG-13-165	n.d.	n.d.	n.d.	5,44	n.d.	n.d.	0,62	b.d.l.	91,95	n.d.	n.d.	n.d.	n.d.	n.d.
40,45	182,61	3	6,36	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
40,59	181,89	3	6,38	GVG-13-166	7,24	n.d.	n.d.	n.d.	n.d.	n.d.	0,81	b.d.l.	101,14	n.d.	n.d.	n.d.	n.d.	n.d.
40,86	196,61	3	5,91	GVG-13-167	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0,48	b.d.l.	74,88	n.d.	n.d.	n.d.	n.d.	n.d.
41,16	194,53	no injection	5,97	GVG-13-168	7,35	n.d.	n.d.	7,78	360,1	-17,54	0,13	b.d.l.	68,29	n.d.	n.d.	b.d.l.	0,02	5999,37
41,45	190,19	no injection	6,11	GVG-13-169	n.d.	n.d.	n.d.	n.d.	354,1	-16,80	0,04	b.d.l.	92,40	n.d.	n.d.	n.d.	0,04	4568,16
41,72	191,82	no injection	6,05	GVG-13-170	7,31	n.d.	n.d.	14,92	333,2	-16,68	12,97	b.d.l.	101,11	25,96	18,28	n.d.	0,09	4262,42
42,03	177,20	no injection	6,55	GVG-13-171	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	35,26	b.d.l.	119,13	n.d.	n.d.	n.d.	n.d.	n.d.

Table E7. Flow-through experiment: outflow analytical data.

time (weeks)	flow (ml/day)	feeding strategy	residence time (days)	sample	pH	ethanol (mg/l)	$\delta^{13}C$-ethanol	NPOC (mg/l)	TIC/DIC (mg/l)	$\delta^{13}C$- DIC	NO_3^- (mg/l)	NO_2^- (mg/l)	SO_4^{2-} (mg/l)	$\delta^{15}N$-NO_3^-	$\delta^{15}N$-NO_3^-	NH4+ (mg/l)	Fe (mg/l)	Mn (µg/l)
42,33	173,67	no injection	6,69	GVG-13-172	7,22	n.d.	n.d.	n.d.	n.d.	n.d.	47,45	b.d.l.	113,90	n.d.	n.d.	n.d.	n.d.	n.d.
42,60	168,00	no injection	6,91	GVG-13-173	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	51,17	b.d.l.	105,34	n.d.	n.d.	n.d.	n.d.	n.d.
42,84	182,24	no injection	6,37	GVG-13-174	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	55,25	b.d.l.	121,95	n.d.	n.d.	n.d.	n.d.	n.d.
43,17	183,47	no injection	6,33	GVG-13-175	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	47,13	b.d.l.	87,66	n.d.	n.d.	n.d.	n.d.	n.d.
43,44	199,34	no injection	5,82	GVG-13-176	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
43,61	201,95	no injection	5,75	GVG-13-177	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	50,91	b.d.l.	84,22	n.d.	n.d.	n.d.	n.d.	n.d.
43,98	185,81	no injection	6,25	GVG-13-178	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	59,65	b.d.l.	105,02	n.d.	n.d.	n.d.	n.d.	n.d.
44,30	187,64	no injection	6,19	GVG-13-179	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	65,32	0,20	107,39	n.d.	n.d.	n.d.	n.d.	n.d.
44,60	194,92	4	5,96	GVG-13-180	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	62,36	b.d.l.	103,75	14,79	9,01	n.d.	n.d.	n.d.
44,83	200,51	4	5,79	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
44,99	208,30	4	5,57	GVG-13-181	7,27	n.d.	n.d.	7,73	309,8	-15,66	21,72	b.d.l.	88,26	n.d.	n.d.	0,1	0,03	4475,40
45,15	188,41	4	6,16	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
45,31	189,70	4	6,12	GVG-13-182	7,26	b.d.l.	n.d.	6,96	329,1	-16,70	b.d.l.	b.d.l.	106,21	n.d.	n.d.	n.d.	0,03	4369,97
45,43	202,31	4	5,74	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
45,60	204,44	4	5,68	GVG-13-183	7,20	b.d.l.	n.d.	5,72	346,5	-16,87	b.d.l.	b.d.l.	102,66	n.d.	n.d.	n.d.	0,03	4617,18
45,98	198,62	4	5,85	GVG-13-184	7,22	b.d.l.	n.d.	6,68	357	-17,02	b.d.l.	b.d.l.	98,13	n.d.	n.d.	0,1	0,03	4055,75
46,12	199,15	4	5,83	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
46,28	195,95	4	5,93	GVG-13-185	7,33	b.d.l.	n.d.	6,6	330,8	-16,59	15,40	b.d.l.	104,71	22,28	16,33	n.d.	0,02	3799,77
46,42	195,68	4	5,93	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
46,59	199,77	4	5,81	GVG-13-186	n.d.	n.d.	n.d.	6,94	330	-16,65	b.d.l.	b.d.l.	110,37	n.d.	n.d.	n.d.	0,03	3405,73
47,01	149,96	4	7,74	GVG-13-187	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	b.d.l.	b.d.l.	98,62	n.d.	n.d.	n.d.	n.d.	n.d.

Table E7. Flow-through experiment: outflow analytical data.

time (weeks)	flow (ml/day)	feeding strategy	residence time (days)	sample	pH	ethanol (mg/l)	$\delta^{13}C$- ethanol	NPOC (mg/l)	TIC/DIC (mg/l)	$\delta^{13}C$- DIC	NO_3^- (mg/l)	NO_2^- (mg/l)	SO_4^{2-} (mg/l)	$\delta^{15}N$- NO_3^-	$\delta^{15}N$- NO_3^-	NH4+ (mg/l)	Fe (mg/l)	Mn (µg/l)
47,12	203,77	4	5,70	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
47,26	196,17	4	5,92	GVG-13-188	7,47	n.d.	n.d.	5,84	347,2	-16,90	b.d.l.	b.d.l.	97,96	n.d.	n.d.	0,1	0,03	2962,28
47,42	196,16	4	5,92	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
47,56	194,04	4	5,98	GVG-13-189	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	3,84	0,11	97,57	n.d.	n.d.	n.d.	n.d.	n.d.
47,69	204,18	4	5,69	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
47,95	198,62	4	5,85	GVG-13-190	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0,47	0,07	94,76	n.d.	n.d.	n.d.	n.d.	n.d.
48,10	188,04	4	6,17	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
48,24	185,81	4	6,25	GVG-13-191	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0,53	0,16	90,51	n.d.	n.d.	n.d.	n.d.	n.d.
48,39	183,93	4	6,31	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
48,54	184,86	4	6,28	GVG-13-192	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0,39	0,12	9,40	n.d.	n.d.	n.d.	n.d.	n.d.
48,69	173,38	4	6,70	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
48,84	166,53	4	6,97	GVG-13-192	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0,48	0,09	94,12	n.d.	n.d.	n.d.	n.d.	n.d.

n.d.: not determined. b.d.l.: below detection limit. n.a.: not available.

Table E8. Flow-through experiment: vertical profiles analytical data.

profile	time (hours)	time after previous injection (h)	sample	sample point	column height (m)	NO_3^- (mg/l)	NO_3^- (mmol/l)	NO_2^- (mg/l)	NO_2^- (mmol/l)	SO_4^{2-} (mg/l)	SO_4^{2-} (mmol/l)	ethanol (mg/l)	$\delta^{13}C$-ethanol
VP1	891.17	24.5	GVG-13-VP1-E	inflow	0	109.07	1.76	b.d.l.	b.d.l.	127.56	1.33	n.d.	n.d.
			GVG-13-VP1-1	1	0.06	105.47	1.70	0.18	0.00	125.71	1.31	n.d.	n.d.
			GVG-13-VP1-2	2 (injection)	0.16	89.44	1.44	2.33	0.05	126.80	1.32	n.d.	n.d.
			GVG-13-VP1-3	3	0.26	11.22	0.18	0.31	0.01	126.54	1.32	n.d.	n.d.
			GVG-13-VP1-4	4	0.36	5.25	0.08	0.17	0.00	126.24	1.31	n.d.	n.d.
			GVG-13-VP1-5	5	0.46	50.40	0.81	0.58	0.01	127.44	1.33	n.d.	n.d.
			GVG-13-VP1-6	6	0.56	73.88	1.19	0.69	0.01	128.96	1.34	n.d.	n.d.
VP2	1.082.17	48.5	GVG-13-VP2-E	inflow	0	97.81	1.58	6.03	0.13	120.71	1.26	n.d.	n.d.
			GVG-13-VP2-1	1	0.06	101.63	1.64	5.27	0.11	123.27	1.28	n.d.	n.d.
			GVG-13-VP2-2	2 (injection)	0.16	95.99	1.55	8.20	0.18	118.70	1.24	n.d.	n.d.
			GVG-13-VP2-3	3	0.26	3.81	0.06	b.d.l.	b.d.l.	122.56	1.28	n.d.	n.d.
			GVG-13-VP2-4	4	0.36	1.22	0.02	b.d.l.	b.d.l.	124.62	1.30	n.d.	n.d.
			GVG-13-VP2-5	5	0.46	b.d.l.	b.d.l.	b.d.l.	b.d.l.	125.35	1.31	n.d.	n.d.
			GVG-13-VP2-6	6	0.56	14.56	0.23	b.d.l.	b.d.l.	123.23	1.28	n.d.	n.d.
VP3	1.155.17	121.50	GVG-13-VP3-E	inflow	0	102.79	1.66	0.23	0.01	121.26	1.26	n.d.	n.d.
			GVG-13-VP3-1	1	0.06	108.09	1.74	b.d.l.	b.d.l.	118.80	1.24	n.d.	n.d.
			GVG-13-VP3-2	2 (injection)	0.16	99.25	1.60	0.55	0.01	122.80	1.28	n.d.	n.d.
			GVG-13-VP3-3	3	0.26	88.30	1.42	0.59	0.01	120.07	1.25	n.d.	n.d.
			GVG-13-VP3-4	4	0.36	77.38	1.25	1.08	0.02	120.18	1.25	n.d.	n.d.
			GVG-13-VP3-5	5	0.46	55.33	0.89	1.37	0.03	118.57	1.24	n.d.	n.d.
			GVG-13-VP3-6	6	0.56	9.79	0.16	0.14	0.00	124.34	1.30	n.d.	n.d.
VP4	2.331.33	124.50	GVG-13-VP4-E	inflow	0	97.01	1.56	b.d.l.	b.d.l.	113.50	1.18	n.d.	n.d.
			GVG-13-VP4-1	1	0.06	86.71	1.40	b.d.l.	b.d.l.	112.70	1.17	n.d.	n.d.
			GVG-13-VP4-2	2 (injection)	0.16	82.44	1.33	0.21	0.00	112.38	1.17	n.d.	n.d.
			GVG-13-VP4-3	3	0.26	38.09	0.61	0.14	0.00	92.74	0.97	n.d.	n.d.
			GVG-13-VP4-4	4	0.36	39.44	0.64	b.d.l.	b.d.l.	111.66	1.16	n.d.	n.d.
			GVG-13-VP4-5	5	0.46	11.63	0.19	b.d.l.	b.d.l.	117.09	1.22	n.d.	n.d.
			GVG-13-VP4-6	6	0.56	2.47	0.04	b.d.l.	b.d.l.	109.11	1.14	n.d.	n.d.
VP5	2.760.50	21.75	GVG-13-VP5-E	inflow	0	82.32	1.33	b.d.l.	b.d.l.	108.40	1.13	n.d.	n.d.

Table E8. Flow-through experiment: vertical profiles analytical data.

profile	time (hours)	time after previous injection (h)	sample	sample point	column height (m)	NO_3^- (mg/l)	NO_3^- (mmol/l)	NO_2^- (mg/l)	NO_2^- (mmol/l)	SO_4^{2-} (mg/l)	SO_4^{2-} (mmol/l)	ethanol (mg/l)	$\delta^{13}C$-ethanol
			GVG-13-VP5-1	1	0.06	76.40	1.23	b.d.l.	b.d.l.	107.07	1.12	n.d.	n.d.
			GVG-13-VP5-2	2 (injection)	0.16	42.15	0.68	b.d.l.	b.d.l.	108.87	1.13	n.d.	n.d.
			GVG-13-VP5-3	3	0.26	1.99	0.03	b.d.l.	b.d.l.	106.59	1.11	n.d.	n.d.
			GVG-13-VP5-4	4	0.36	0.76	0.01	b.d.l.	b.d.l.	106.87	1.11	n.d.	n.d.
			GVG-13-VP5-5	5	0.46	1.15	0.02	b.d.l.	b.d.l.	108.29	1.13	n.d.	n.d.
			GVG-13-VP5-6	6	0.56	0.65	0.01	b.d.l.	b.d.l.	110.34	1.15	n.d.	n.d.
			GVG-13-VP6-E	inflow	0	79.21	1.28	b.d.l.	b.d.l.	103.91	1.08	n.d.	n.d.
			GVG-13-VP6-1	1	0.06	75.86	1.22	b.d.l.	b.d.l.	105.68	1.10	n.d.	n.d.
			GVG-13-VP6-2	2 (injection)	0.16	11.60	0.19	0.32	0.01	107.09	1.12	n.d.	n.d.
VP6	3.090.08	21.60	GVG-13-VP6-3	3	0.26	3.00	0.05	b.d.l.	b.d.l.	103.78	1.08	n.d.	n.d.
			GVG-13-VP6-4	4	0.36	2.13	0.03	b.d.l.	b.d.l.	105.99	1.10	n.d.	n.d.
			GVG-13-VP6-5	5	0.46	1.07	0.02	b.d.l.	b.d.l.	103.62	1.08	n.d.	n.d.
			GVG-13-VP6-6	6	0.56	2.07	0.03	b.d.l.	b.d.l.	108.79	1.13	n.d.	n.d.
			GVG-13-VP7-E	inflow	0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			GVG-13-VP7-1	1	0.06	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			GVG-13-VP7-2	2 (injection)	0.16	21.99	0.35	b.d.l.	b.d.l.	101.44	1.06	48.6	-29.4
VP7	4.393.25	4.00	GVG-13-VP7-3	3	0.26	2.34	0.04	0.29	0.01	100.60	1.05	12.0	-29.8
			GVG-13-VP7-4	4	0.36	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			GVG-13-VP7-5	5	0.46	3.50	0.06	0.31	0.01	99.72	1.04	4.0	-30.8
			GVG-13-VP7-6	6	0.56	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			GVG-13-VP8-E	inflow	0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			GVG-13-VP8-1	1	0.06	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			GVG-13-VP8-2	2 (injection)	0.16	60.46	0.98	b.d.l.	b.d.l.	102.64	1.07	n.d.	n.d.
VP8	4.610.25	3.00	GVG-13-VP8-3	3	0.26	5.44	0.09	0.30	0.01	101.93	1.06	<l.d.	n.d.
			GVG-13-VP8-4	4	0.36	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			GVG-13-VP8-5	5	0.46	1.71	0.03	0.21	0.00	103.59	1.08	<l.d.	n.d.
			GVG-13-VP8-6	6	0.56	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			GVG-13-VP9-E	inflow	0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
VP9	4.754.83	3.5	GVG-13-VP9-1	1	0.06	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			GVG-13-VP9-2	2 (injection)	0.16	20.30	0.33	b.d.l.	b.d.l.	104.10	1.08	85.4	-29.6

Table E8. Flow-through experiment: vertical profiles analytical data.

profile	time (hours)	time after previous injection (h)	sample	sample point	column height (m)	NO_3^- (mg/l)	NO_3^- (mmol/l)	NO_2^- (mg/l)	NO_2^- (mmol/l)	SO_4^{2-} (mg/l)	SO_4^{2-} (mmol/l)	ethanol (mg/l)	$\delta^{13}C$-ethanol
			GVG-13-VP9-3	3	0.26	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			GVG-13-VP9-4	4	0.36	1.10	0.02	b.d.l.	b.d.l.	77.99	0.81	<l.d.	n.d.
			GVG-13-VP9-5	5	0.46	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			GVG-13-VP9-6	6	0.56	1.31	0.02	b.d.l.	b.d.l.	80.98	0.84	<l.d.	n.d.
			GVG-13-VP11-2	2 (injection)	0.16	37.71	0.61	b.d.l.	b.d.l.	77.97	0.81	5.0	-29.9
VP11	5.734.25	21.00	GVG-13-VP11-4	4	0.36	b.d.l.	b.d.l.	b.d.l.	b.d.l.	92.68	0.97	<l.d.	n.d.
			GVG-13-VP11-6	4	0.56	1.38	0.02	b.d.l.	b.d.l.	89.88	0.94	<l.d.	n.d.
			GVG-13-VP12-E	inflow	0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			GVG-13-VP12-1	1	0.06	74.61	1.20	b.d.l.	b.d.l.	105.77	1.10	n.d.	n.d.
			GVG-13-VP12-2	2 (injection)	0.16	66.37	1.07	b.d.l.	b.d.l.	107.41	1.12	n.d.	n.d.
VP12	5.975.50	23.00	GVG-13-VP12-3	3	0.26	b.d.l.	b.d.l.	b.d.l.	b.d.l.	94.30	0.98	n.d.	n.d.
			GVG-13-VP12-4	4	0.36	0.76	0.01	b.d.l.	b.d.l.	92.55	0.96	n.d.	n.d.
			GVG-13-VP12-5	5	0.46	0.84	0.01	b.d.l.	b.d.l.	92.54	0.96	n.d.	n.d.
			GVG-13-VP12-6	6	0.56	0.86	0.01	b.d.l.	b.d.l.	92.82	0.97	n.d.	n.d.
			GVG-13-VP13-E	inflow	0	87.25	1.41	b.d.l.	b.d.l.	105.24	1.10	n.d.	n.d.
			GVG-13-VP13-1	1	0.06	76.48	1.23	b.d.l.	b.d.l.	102.83	1.07	n.d.	n.d.
			GVG-13-VP13-2	2 (injection)	0.16	39.39	0.64	b.d.l.	b.d.l.	98.92	1.03	n.d.	n.d.
VP13	6.411.50	27.40	GVG-13-VP13-3	3	0.26	4.30	0.07	b.d.l.	b.d.l.	84.93	0.88	n.d.	n.d.
			GVG-13-VP13-4	4	0.36	4.67	0.08	b.d.l.	b.d.l.	99.49	1.04	n.d.	n.d.
			GVG-13-VP13-5	5	0.46	1.38	0.02	b.d.l.	b.d.l.	97.33	1.01	n.d.	n.d.
			GVG-13-VP13-6	6	0.56	3.54	0.06	b.d.l.	b.d.l.	107.46	1.12	n.d.	n.d.
			GVG-13-VP14-E	inflow	0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			GVG-13-VP14-1	1	0.06	45.82	0.74	b.d.l.	b.d.l.	99.47	1.04	n.d.	n.d.
			GVG-13-VP14-2	2 (injection)	0.16	82.20	1.33	0.25	0.01	108.05	1.13	n.d.	n.d.
VP14	7.990.10	23.40	GVG-13-VP14-3	3	0.26	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			GVG-13-VP14-4	4	0.36	4.96	0.08	b.d.l.	b.d.l.	94.45	0.98	n.d.	n.d.
			GVG-13-VP14-5	5	0.46	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
			GVG-13-VP14-6	6	0.56	1.63	0.03	b.d.l.	b.d.l.	88.01	0.92	n.d.	n.d.

n.d.: not determined. b.d.l.: below detection limit .

Table E9. Flow-through experiment: microbial counts at the outflow.

sample	time (weeks)	feeding strategy	total heterotrophs (mpn/l)	denitrifiers (mpn/l)	denit./het. (%)
GVG-13-1	0.13	1	2.81E+02	3.75E+01	13.3%
GVG-13-8	1.29	1	3.33E+05	2.30E+03	0.7%
GVG-13-19	3.14	1	3.26E+04	1.84E+04	56.4%
GVG-13-24	4.01	1	4.90E+04	3.76E+04	76.7%
GVG-13-30	5	1	7.75E+03	9.60E+03	123.9%
GVG-13-54	9.29	1	1.38E+06	9.60E+05	69.4%
GVG-13-68	11.96	1	1.22E+06	9.60E+05	78.8%
GVG-13-114	20.12	2	2.10E+06	1.85E+06	88.0%
GVG-13-130	29.7	3	2.60E+06	2.34E+06	90.0%
GVG-13-150	35.57	3	7.60E+05	9.30E+05	122.4%
GVG-13-180	44.2	no feeding	5.15E+04	1.56E+05	302.1%
GVG-13-192	48.84	4	1.63E+05	3.34E+04	20.5%

Table E10. Flow-through experiment: vertical profiles of microbial counts.

profile	time (weeks)	sample	sample point	column height (m)	total heterotrophs (mpn/l)
VP(i)	11.96	GVG-13-VP(i)-E	inflow	0	1,10E+05
		GVG-13-VP(i)-1	1	0,06	n.d.
		GVG-13-VP(i)-2	2 (injection)	0,16	6,35E+06
		GVG-13-VP(i)-3	3	0,26	n.d.
		GVG-13-VP(i)-4	4	0,36	5,73E+06
		GVG-13-VP(i)-5	5	0,46	n.d.
		GVG-13-VP(i)-6	6	0,56	1,43E+06
VP(ii)	20.12	GVG-13-VP(ii)-E	inflow	0	3,55E+06
		GVG-13-VP(ii)-1	1	0,06	n.d.
		GVG-13-VP(ii)-2	2 (injection)	0,16	2,33E+07
		GVG-13-VP(ii)-3	3	0,26	n.d.
		GVG-13-VP(ii)-4	4	0,36	n.d.
		GVG-13-VP(ii)-5	5	0,46	2,33E+06
		GVG-13-VP(ii)-6	6	0,56	n.d.

n.d.: not determined.

AGRAÏMENTS

Fet. Ja ho tenim enllestit. Una tesi acabada. L'enèsima tesi de la Universitat de Barcelona, l'enèsima i encara més del recull de la recerca científica, i la primera de BIOREM i la meva, certament. Una tesi amb resultats interessants, alguns defectes i desencisos, i encara més preguntes pendents. Però, amb tot, crec que una tesi útil.

És una tesi que duu el meu nom, però que engloba molts esforços i aportacions. Els dels meus directors, a voltes esporàdics però sovint brillants; els dels meus companys de projectes, especialment el Raül, la Laia, i l'Albert, i també la Paula i la Neus, de vegades participants forçats, de vegades necessaris –i escoltats- alliçonadors; els dels serveis d'anàlisi, particularment de la Mercè, al lab de MAiMA, i dels Centres Científics i Tecnològics de la Universitat de Barcelona, imprescindibles col·laboradors amb qui dansem a ritme de mostres i protocols. I els dels membres del Departament de Cristal·lografia (...), de la Comissió de Doctorat, i de la Secretaria de la Facultat que ens acullen i organitzen i ens fan seguir el (necessari) camí.

És una tesi costosa, com totes suposo. De temps, recursos i energia. No hauria estat possible sense l'Agència Catalana de l'Aigua, agent promotor d'innovació en gestió de l'aigua, ni, principalment, de BIOREM, agent promotor, finançador i suportador. El meu més sincer agraïment a les meves sòcies i germanes; m'agradaria pensar que n'hem tret quelcom de positiu. Al finançament rebut per a la recerca també li mostro el meu agraïment i confio oferir-hi un bon retorn.

Acabo, ja en tinc prou i no cal allargar-nos massa. Afegeixo però un bon record per tots els companys MAiMONS (i extensions), per ser cadascú com sou i aportar eixelebrats punts de vista. Un reconegut agraïment al meu amic Eric, per les seves correccions i companyonia. Una salutació volguda a tots els amics, ambientòlegs, tiramissuns, inigualables pares i germans (i associats), que sempre sou al voltant i en sortiu sovint esquitxats (de la tesi i d'altres coses); n'hi ha que em vau haver d'acompanyar en algunes visites nocturnes a la Facultat, que de nit fa por. I també una abraçada a aquells que heu decidit marxar recentment; records a l'àvia i la iaia si us les trobeu.

Com diu un bon amic meu, *tot succeeix per una bona raó...a favor nostre*.

Georgina, Martina, Peronella.

Gracias a la vida que me ha dado tanto
Me ha dado la risa y me ha dado el llanto
Así yo distingo dicha de quebranto
Los dos materiales que forman mi canto
Y el canto de ustedes que es el mismo canto
Y el canto de todos que es mi propio canto.

Violeta Parra, 1966.

PD: Anna Maria, finalment he estat l'última. La teva última doctorand. Confio haver estat també prou digna.