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Assessment of artificial substrates for evaluating groundwater microbial quality

Jérémy Voisin[a,b,c,*], Benoit Cournoyer[a,c], Florian Mermillod-Blondin[a,b]

[a] Université de Lyon, France
[b] Univ Lyon, Université Claude Bernard Lyon 1, CNRS, ENTPE, UMR5023, Ecologie des Hydrosystèmes Naturels et Anthropisés, Villeurbanne, F-69622, France
[c] Univ Lyon, Université Claude Bernard Lyon 1, VetAgro Sup, CNRS, UMR5557, INRA UMR1418, Ecologie Microbienne, Research Team "Bacterial Opportunistic Pathogens and Environment", Marcy L'Etoile, F-69280, France

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ABSTRACT

Protection of groundwater resources requires the development of reliable ecological indicators. Microorganisms involved in ecological services or being associated with particular hosts or habitats could be used for this purpose. Nevertheless, their tracking remains limited because of sampling issues, and a lack of devices for their long term monitoring. In the present study, three artificial substrates (glass and clay beads, and gravel particles) were tested in terms of efficacy at favoring bacterial growth, and at capturing bacterial diversity of waters (i.e., groundwater, surface water and wastewater). Total proteins, total carbohydrates, dehydrogenase and hydrolytic activities were used to monitor biofilm development on these artificial substrates. Fingerprinting analyses based on *rrs* (16S rRNA) – *rrl* (23S rRNA) spacer analyses (ARISA) and next generation sequencing (NGS) of partial *rrs* DNA segments (V5-V6) were used to compare operating taxonomic units (OTUs), and infer bacterial genera trapped on these substrates. Glass beads were found less efficient than the other two artificial substrates at increasing protein contents and microbial activities (hydrolytic and dehydrogenase activities). ARISA showed a discrimination of bacterial communities developing on artificial substrates that was matching water types. An incubation period of 7 days allowed a reliable assessment of bacterial diversity. From this incubation period, around 75% of water genera with more than four V5-V6 *rrs* DNA sequences detected in a water type were recovered from biofilms growing on artificial substrates. Based on relative abundances of genera, clay beads and gravel particles were more efficient than glass beads to capture and obtain bacterial communities matching those of the initial waters. Between 45–67% of similarities were found for these artificial substrates while it was between 36 and 43% for glass beads. This study demonstrated clay beads and gravel particles as being efficient tools for capturing bacterial diversity and monitoring bacterial growth. Overall, clay beads appeared the best choice for field monitoring because of the ease of their size standardization in comparison with gravel particles.

1. Introduction

Groundwater is a major source of drinking and irrigation waters (Gibert, 2001) but is under threat due to intensive agriculture, industrial activities and urbanization (Griebler and Avramov, 2015). Protection of groundwater resources is recognized as a major challenge in industrial countries (EU Groundwater Directive 2006/118/EC). The development of a coherent and efficient protection strategy requires an extensive evaluation of groundwater quality (Danielopol et al., 2004; Griebler et al., 2010). To date, the assessment of groundwater resources has been based exclusively on chemical and hydrogeological parameters (e.g., Wendland et al., 2005) but several environmental agencies recognize the need for biological and ecological indicators of ecosystem quality attributes (e.g., ecosystem services: e.g., Korbel and Hose, 2011; Schurig et al., 2014). In this context, microorganisms are recognized as pertinent indicators of groundwater ecosystem health and functioning (Burns and Ryder, 2001; Griebler and Lueders, 2009; Iribar et al., 2015; Paerl et al., 2003). For instance, several studies (Cho and Kim, 2000; Foulquier et al., 2011; Goldscheider et al., 2006; Griebler et al., 2006) demonstrated that microbial analyses (biomass, activity and diversity) could be efficient to detect organic matter enrichments in groundwater ecosystems.

* Corresponding author at: Université Lyon 1, CNRS, UMR5023, ENTPE, Ecologie des Hydrosystèmes Naturels et Anthropisés, Villeurbanne, F-69622, France.
E-mail address: jeremy.voisin@univ-lyon1.fr (J. Voisin).

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Monitoring of microbial change can be a pertinent ecological indication of the quality of groundwater ecosystems (Griebler and Lueders 2009) but sampling issues have hindered its implementation. Indeed, in groundwater systems, bacterial biomass and activities have been shown to be related to bacterial communities attached to surfaces rather than free-living bacteria (Alfreider et al., 1997; Goldscheider et al., 2006; Griebler et al., 2002; Iribar et al., 2008). Thus, the sampling of groundwater and associated free-living bacteria is not considered reliable for groundwater ecosystem assessment. In addition, analyses of aquifer sediments obtained by pumping or coring are technically difficult and expensive, and remain a challenging task in deep alluvial aquifers (i.e. when the vadose zone thickness exceeds 10 m (Chapelle, 2001)). To circumvent these methodological issues, a growing number of studies used artificial substrates such as glass beads or gravel particles to assess microbial biomass, activities and diversity in groundwater aquifers (Claret, 1998; Iribar et al., 2015; Mermillod-Blondin et al., 2013; Williamson et al., 2012; Yu et al., 2014). Analysis of microorganisms attached to these artificial substrates gave promising hints on trophic conditions (e.g., Williamson et al., 2012) and water purification processes (e.g., Iribar et al., 2015). Despite these successful uses, the substrates (e.g., glass, sand, gravel) were deployed in the field without strong knowledge on their efficacy at trapping microorganisms from these systems. Also, it is expected that significant differences in trapping efficacies among artificial substrates exist. For example, glass and natural sediment surfaces have contrasting properties such as roughness which can greatly impact the dynamics of microbial development at their surface (Gharechahi et al., 2012; Whitehead and Verran, 2009).

The aim of the present study was to evaluate and compare the abilities of three artificial substrates to act as efficient trapping and growing surfaces for aquatic microorganisms. Accordingly, two materials (i.e. glass and gravel), that had been used in previous studies in alluvial aquifers (Hartland et al., 2011; Iribar et al., 2015; Mermillod-Blondin et al., 2013; Williamson et al., 2012; Yu et al., 2014), and one material (i.e., clay), commonly applied for sampling stream microbial communities (e.g., Murdock and Dodds, 2007; Risse-Buhl et al., 2012; Tuchman and Stevenson, 1980), were tested. As local environmental conditions in groundwater generate different patterns of colonization and bacterial community structures (Chapelle, 2001; Ludvigsen et al., 1999; Shen et al., 2015), three artificial substrates (glass beads, clay beads and gravel particles) were exposed to three water types (groundwater, wastewater, and surface water) characterized by contrasting chemical (nutrient concentrations) and microbial assemblages. Biofilm development was then monitored by measuring microbial biomass and activity through time. Trapping efficiencies of the three artificial substrates and bacterial diversity analyses were performed by ARISA (automated ribosomal intergenic spacer analyses: Fisher and Triplett, 1999) and *rrs* (16 S rDNA) meta-taxogenomics.

2. Materials and methods

2.1. Experimental design

A factorial experimental design was used to evaluate the abilities of the three artificial substrates (glass beads, clay beads and gravel particles) to capture microbial biomass and bacterial diversity from three tested waters (i.e. wastewater, surface water and groundwater). Glass beads (soda-lime glass, Carl Roth, Karlsruhe, Germany), gravel particles (natural carbonated Rhone river sediment obtained by manual sieving) and clay beads (expanded clay aggregates, Botanic®, Saint-Julien en Genevois, France) were selected to be spherical with a diameter of 8 mm and a calculated surface area of 50.27 mm^2. This size was selected on the basis of previous experiments using glass beads (Voisin et al., 2015). The selection of a diameter of 8 mm was verified for the two other artificial substrates (gravel particles and clay beads). Analyses of biofilm total proteins were performed on gravel particles and clay beads of four diameters (4, 6, 8 and 10 mm) and incubated in surface water during 28 days. These investigations confirmed biofilm development to be better on 8 mm spheres than smaller ones (Fig. S1). Similar to a previous study (Mermillod-Blondin et al., 2013), the glass beads used in this study were depolished to increase microbial adherence. All artificial substrates (glass beads, clay beads and gravel particles) were heated to 550 °C to eliminate organic matter traces. In addition, the substrates were autoclaved at 120 °C for 20 min directly before incubation.

Incubations of the beads and gravel particles were performed over 28 days at 15 °C and in the dark. Nine aquaria (length × width × height: 500 × 150 × 500 mm) were used for the experiment with three replicated aquaria per water type (wastewater, surface water, groundwater). Each aquarium was filled with 20 L of tested water oxygenated by air bubbling and constantly renewed using a peristaltic pump to obtain a renewal time of one week for each aquarium. Artificial substrates were incubated in aquaria using small polypropylene bags (75 × 40 mm) with a mesh size of 3.4 mm, previously treated with ethanol (70%). A total of 20 glass beads, clay beads and gravel particles were inserted per bag, and 5 bags per substrate were incubated per aquarium, for a total of 135 bags for the whole experiment (5 bags × 3 substrates × 9 aquaria). One bag per substrate was collected per aquarium to evaluate microbial dynamics depending on artificial substrate and water type five times during incubation (0, 7, 14, 21 and 28 days).

2.2. SEM observations

Beads and gravel particles were recovered for scanning electron microscopy (SEM) analyses after 7 days of incubation in the three waters. SEM analyses were realized at the Centre Technologique des Microstructures of University Lyon 1 (Villeurbanne, France) according to standard protocols (Pottu-Boumendil, 1989). Samples of artificial substrates were fixed with 2% glutaraldehyde at a pH value of 8, washed in a buffer, and post-fixed with 0.5% osmium tetroxide. Fixed samples were then washed in distilled water and dehydrated progressively in acetone. Critical-point drying was then performed with CO_2 (Boyde and Franc, 1981). The samples were finally coated with gold-platinum. Observations were carried out on a FEG Hitachi S-800 SEM at an acceleration voltage of 10 kV. Comparisons were performed with sterile artificial substrates.

2.3. Roughness index of artificial substrates

The wetted layer technique developed by Harrod and Hall (1962) was used to calculate a roughness index for the three substrates. For each artificial substrate, 6 beads or gravel particles were dried (60 °C for 48 h) and individually weighed. They were then individually dipped into a detergent solution (Enzypin) and weighed again to obtain the weight of the adhering detergent solution (for details on weighing procedure see Bergey, 2006). The weight of the adhering detergent solution on the beads and gravel particles was converted to area using a linear relationship between the weight of the detergent solution and the surface area of smooth tungsten beads. The index of roughness for each artificial substrate was then calculated as the ratio between the surface area of adhering detergent solution and the artificial substrate area (calculated by considering beads and gravel particles as spheres of 8 mm in diameter).