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Biofilm Development in a planar Fracture: Persistance to Starvation and Resistance to Flow Velocity

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Biofilm Development in a Planar Fracture: Persistence to Starvation and Resistance to Flow Velocity

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Abbreviations: ORP: oxidation-reduction potential; EPS: exopolymeric substances; UMB: ultramicrobacteria; K: hydraulic conductivity

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Biofilm Development in a Planar Fracture: Persistence to Starvation and Resistance to Flow Velocity

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ABSTRACT

Biobarriers are a promising approach to control groundwater movement in fractured aquifers. A glass-fracture plane apparatus was designed to monitor a biofilm under 50 days of biostimulation with invertose followed by 45 days of persistence to starvation, and 5 days of resistance to an increase in flow velocity. Direct observations were performed using a light transmittance probe whereas indirect indicators were obtained by enumerating planktonic bacteria, monitoring the oxidation-reduction potential (ORP), and conducting tracer experiments, which included modeling of dimensionless concentrations using an analytical model for two-dimensional advection-dispersion. After 5 days of biostimulation, biofilm clusters appeared downgradient from the biostimulation zone. These clusters developed along the flow lines before extending to a uniform biofilm, which covered 700 cm^2 after 50 days. Starvation conditions led to a bacterial detachment followed by a recolonization of the biofilm. Notwithstanding the large variations in the readings due to interferences, the ORP significantly declined over the three experimental phases. The modeling suggested that an increase in transverse dispersion due to biofilm development only occurs during the biostimulation period, and that no increase in transverse dispersion is imparted by the starved biofilm. Moreover, the measurement of changes in groundwater velocity was in direct observation with the biofilm development, which is key information for a field demonstration of the biobarrier concept. These results suggest that the development of a persistent biofilm, having sufficient hydraulic impedance to significantly alter and diminish groundwater flow in natural fractures in a field setting, is indeed possible.

Formation d'un biofilm dans une fracture plane — Résistance à la privation d'aliments et à l'augmentation de la vitesse d'écoulement

Nathalie Ross*, Kent Novakowski, Suzanne Lesage,
Louise Deschênes et Réjean Samson

RÉSUMÉ

Les biobarrières sont prometteuses comme moyen de contenir les mouvements des eaux souterraines dans les aquifères fracturés. Un montage formant un plan de fracture en verre a été conçu pour surveiller le comportement d'un biofilm pendant 50 jours de biostimulation par approvisionnement en invertose, puis 45 jours de privation d'aliments, 5 jours à une vitesse d'écoulement accrue. On a pu faire des observations directes grâce à une sonde de transmittance de la lumière, et on a utilisé comme méthodes indirectes la numération des bactéries planctoniques, le suivi du potentiel d'oxydo-réduction (POR) et les essais avec traceurs, dont la modélisation de concentrations adimensionnelles grâce à un modèle analytique d'advection-dispersion en deux dimensions. Après 5 jours de biostimulation, des fragments de biofilm sont apparus en aval de la zone de biostimulation, se formant d'abord dans l'axe d'écoulement avant de se rejoindre pour produire un film biologique uniforme qui, au bout de 50 jours, couvrait 700 cm^2. Les conditions de privation ont entraîné un décollement des bactéries puis une recolonisation du biofilm. Compte non tenu des variations importantes dans les mesures à cause des interférences, on a constaté une diminution signification du POR au cours des trois phases expérimentales. La modélisation laissait supposer qu'une augmentation de la dispersion transversale attribuable à la formation du biofilm survenait seulement pendant la période de biostimulation, et que le biofilm privé d'aliments n'avait pas un tel effet. De plus, les fluctuations mesurées dans les vitesses d'écoulement des eaux souterraines étaient en corrélation avec la formation du biofilm observée, ce qui constitue une information cruciale pour la démonstration sur le terrain du concept de biobarrière. Ces résultats semblent indiquer que la formation d'un biofilm persistant, dont l'impédance est suffisante pour modifier et réduire de manière significative l'écoulement des eaux souterraines par des fractures naturelles, sur le terrain, est effectivement possible.

NWRI RESEARCH SUMMARY

Plain language title
Biofilm Development in a Planar Fracture: Persistence to Starvation and Resistance to Flow Velocity

What is the problem and what do sicentists already know about it?
Groundwater flow in bedrock rock aquifers follows complex pathways governed by the arrangement of interconnected fractures. The remediation of contamination in such environments is limited by several technical and financial considerations including the difficulty to reach contamination in micro-fractures, the cost of rock excavation, and the in situ destruction.

Why did NWRI do this study?
Biobarriers are a promising approach to control groundwater movement in fractured aquifers. Although the selective plugging of consolidated media has been studied since the 1980s for the enhancement of oil recovery, the management of groundwater flow at contaminated sites has received only limited study.

What were the results?
After 5 days of biostimulation, biofilm clusters appeared downgradient from the biostimulation zone. Starvation conditions led to a bacterial detachment followed by a recolonization of the biofilm. The modeling suggested that an increase in transverse dispersion due to biofilm development only occurs during the biostimulation period, and that no increase in transverse dispersion is imparted by the starved biofilm.

How will these results be used?
The measurement of changes in groundwater velocity was in direct observation with the biofilm development, which is key information for a field demonstration of the biobarrier concept. These results give the background data necessary to support the scaling-up of the biobarrier at field demonstration.

Who were our main partners in the study?
Queen's University, Dept. of Civil Engineering, Ellis Hall, University Avenue, Kingston, Ontario, K7L 3N6 Canada. École Polytechnique de Montréal, NSERC Industrial Chair in Site Remediation and Management, Dept. of Chemical Engineering, P.O. Box 6079, Station Centre-ville, Montreal, Quebec, H3C 3A7 Canada.

Sommaire des recherches de l'INRE

Titre en langage clair
Formation d'un biofilm dans une fracture plane — Résistance à la privation d'aliments et à l'augmentation de la vitesse d'écoulement

Quel est le problème et que savent les chercheurs à ce sujet?
L'écoulement des eaux souterraines dans les aquifères de substratum rocheux et de roche se fait suivant des modes complexes, déterminés par la disposition relative des fractures communiquant entre elles. La résolution des problèmes de contamination, dans de tels milieux, est limitée par plusieurs considérations d'ordre technique et financier, dont la difficulté à atteindre les microfractures contaminées, le coût de l'excavation du roc et la destruction du site.

Pourquoi l'INRE a-t-il effectué cette étude?
Les biobarrières sont prometteuses comme moyen de contenir les mouvements des eaux souterraines dans les aquifères fracturés. Bien que l'étude de l'obturation sélective des milieux consolidés en vue d'améliorer la récupération des hydrocarbures remonte aux années 1980, la gestion de l'écoulement des eaux souterraines en provenance de sites contaminés ne s'est vu consacrer que peu d'efforts.

Quels sont les résultats?
Après 5 jours de biostimulation, des fragments de biofilm sont apparus en aval de la zone de biostimulation. Les conditions de privation ont entraîné un décollement des bactéries puis une recolonisation du biofilm. La modélisation laissait supposer qu'une augmentation de la dispersion transversale attribuable à la formation du biofilm survenait seulement pendant la période de biostimulation, et que le biofilm privé d'aliments n'avait pas un tel effet.

Comment ces résultats seront-ils utilisés?
Les fluctuations mesurées dans les vitesses d'écoulement des eaux souterraines étaient en corrélation avec la formation du biofilm observée, ce qui constitue une information cruciale pour la démonstration sur le terrain du concept de biobarrière. Ces résultats fournissent les données de base nécessaires pour appuyer la transposition du concept de biobarrière à l'échelle des essais sur le terrain.

Quels étaient nos principaux partenaires dans cette étude?
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INTRODUCTION

Groundwater flow in bedrock rock aquifers follows complex pathways governed by the arrangement of interconnected fractures. The remediation of contamination in such environments is limited by several technical and financial considerations including the difficulty to reach contamination in micro-fractures, the cost of rock excavation, and the *in situ* destruction (Canter and Knox, 1986). Often, pump-and-treat systems are utilized to prevent the migration of contamination away from a source area. Operation of pump-and-treat systems implies long-term cost for the management of contamination at fractured bedrock sites (EPA, 1989).

To control and limit groundwater movement in such geologic formations, the use of a biological barrier has been suggested (Ross and Bickerton, 2002). Biological barriers consist of biofilms that develop after indigenous bacteria or injected bacteria are stimulated to grow, have attached on rock or porous media surfaces, and have secreted exopolymeric substances (EPS) (Bryers, 2000). Once developed, biofilms will clog the fractures and the pores, potentially reducing the effective hydraulic conductivity (K) of the fracture network by several orders of magnitude (Baveye et al., 1998; Ross et al., 2001b).

The development of the biobarrier concept is more advanced in porous media where pilot-scale and site applications have been conducted (Hiebert et al., 2001; James and Hiebert, 2001). Although the selective plugging of consolidated media has been studied since the 1980s for the enhancement of oil recovery (Cusack et al., 1992; Davey et al., 1998; Lappin-Scott et al., 1988), the management of groundwater flow at contaminated sites has received only limited study. The capacity to secrete copious

amount of EPS (accumulation of 800 mg L^{-1} after 8 days) (Ross et al., 1998) and to develop thick biofilms (up to 1100 μm) (Ross et al., 2001a) through the biostimulation of indigenous groundwater bacteria were demonstrated in static microcosms using ceramic coupons to mimic a rock fracture surface. The dominant bacterial species in the groundwater used for these studies were affiliated with *Cytophaga* spp., *Arcobacter* spp., and *Rhizobium* spp.; bacteria that are known to secrete EPS and form biofilms (Ross et al., 2001b). During the biostimulation of this specific groundwater microbial population in a single limestone fracture (section 2.5 mm^2, 50 cm long), a decrease in K was measured at 0.8 % of its initial value. This apparatus was also used to monitor the effect of a 6-month starvation phase following a 1.5-month biostimulation phase. The biostimulation was conducted with 2.2 mg $\cdot$ m^{-2} $\cdot$ min^{-1} molasses and led to a decrease in K of three orders of magnitude. Under starvation conditions, K appeared to continue decreasing steadily for an additional 0.5 order of magnitude over 50 days and then fluctuated, which was attributed to cycles of biofilm detachment and fracture clogging within the 50-cm single fracture (unpublished data).

The development of a biobarrier in a large fracture plane, a geological feature which is likely to be found at field scale (e.g. (Lapcevic et al., 1999a), has yet to be demonstrated. At a small laboratory scale (21 cm wide by 28 cm long glass parallel plate fracture roughened by sandblasting), Hill and Sleep (2002) have bioclogged 72 % of a 500 μm fracture, which translated into a decrease in K by a factor of 0.033, after 140 hours of biostimulation. The biostimulation was conducted using glucose, oxygen, and nutrient in a continuous supply at room temperature. Throughout the development of the biofilm, Hill and Sleep (2002) quantified the changes in the flow and transport via tracer

experiments. Their findings confirmed that macrodispersion (geometrical dispersion due to variations in the fracture aperture) increased logarithmically with a decrease in K, although flow conditions in the parallel plates prior to the biofilm growth were dominated by Taylorian dispersion.

In natural or controlled environments, the direct, real time, and non invasive monitoring of biofilm development is challenging (Dupin and McCarty, 2000; Tamachkiarowa and Flemming, 1996). Flow cells and other small-scale laboratory apparatus in conjunction with microscopic observations have allowed an important development in understanding the biofilm structure, assessing the community dynamics, and improving mathematical models (Bryers, 2000; Ritcher et al., 1999; Wimpenny et al., 2000; Wimpenny and Colasanti, 1997). However, the assessment of bioclogging at large-scale as well as *in situ* has yet to be conducted. As a promising approach, Tamachkiarowa and Flemming (1996) have correlated biofilm thickness with changes in optical density using a glass fiber sensor. Although primary colonization could not be detected with this device, the changes in the intensity of reflected light, as the biofilm was developing on the 1-mm diameter sensor, were similar to a typical biofilm accumulation curve showing initial, logarithmic, and stationary phases. Light microscopy was used to quantify biofilm accumulation in a rectangular duct biofilm reactor (Bakke et al., 2001). The changes in optical density were translated into physical biofilm thickness, and this approach allowed for the detection of biofilms as thin as 35 μm. Using a more sophisticated approach, Okkerse et al. (2000) have developed a laser triangulation sensor, which distinguished differences in biofilm roughness within a wetted-wall column.

Indirect indications of *in situ* biofilm development and the resulting bioclogging of geological features can be obtained by monitoring bacterial activities involved in the process. As such, bacterial density (Batchelor et al., 1997; Lehman et al., 2001; O'Toole et al., 2000), tracer experiments (Hill and Sleep, 2002; Taylor and Jaffé, 1990), and monitoring of geochemical conditions, via oxidation-reduction potential (ORP) probes (Appelo and Postma, 1993; Bishop and Yu, 1999), have been reported. Again, little information exists on the application of such techniques in fractured media.

A parallel study to that reported herein is focused on a field demonstration of biofilm development in a flat-lying, thinly bedded shale (average fracture aperture of 165 μm and groundwater velocity of 5 m day^{-1}) located in Mississauga (Ontario, Canada). This site and the primary fracture planes have been well characterized (Lapcevic et al., 1999a; Novakowski, 1992; Novakowski and Lapcevic, 1994; Novakowski et al., 1995). The need, however, to investigate potential monitoring tools for this type of demonstration requires laboratory-scale studies. (Ross and Bickerton, 2002)

In the present study, a large fracture plane (2-m long and 0.6-m wide) apparatus was fashioned out of glass, and natural groundwater was used at a controlled temperature (10 °C) to develop a biofilm under biostimulation. Following to the development of the biofilm, the persistence under starvation and the resistance to increasing groundwater velocity were examined. The persistence of biofilms under starvation conditions represents a key element in the design of the full-scale biobarrier, whereas the groundwater velocity is a major environmental factor, which is likely to affect the bioclogging in the field. Specifically, the study focused on 1) conducting direct observations of biofilm development, persistence, and resistance via visual testing and

light transmittance monitoring and 2) measuring the indirect effects of the bioclogging using the density of planktonic cells, the measurements of ORP, and the results of tracer experiments including the fitting of an analytical model for two-dimensional advection-dispersion.

MATERIALS AND METHODS

The Fracture Plane Apparatus

The fracture plane apparatus consists of two glass-sheets 0.02-m thick, 0.6-m wide, and 2-m long separated by a 1.5-mm wire along each edge (Fig. 1). This creates a fracture plane having an aperture of 1.5 mm approximately. The fracture is intercepted by a Plexiglas borehole, 76 mm in diameter, equipped with: a pneumatic, double packer system for the isolation of the fracture (Cherry et al., 1993), a nitrogen line for the inflation of the packer (40 psi), two lines for the injection of nutrients and tracers, and a propeller (60 rpm) for mixing in the isolated zone (Fig. 1-A). Fifteen Teflon ports were installed on the upper glass-sheet for groundwater sampling and ORP measurement (Fig. 1-B, Table 1). Groundwater flow was established by generating a difference in hydraulic head between the inlet and outlet reservoirs.

Groundwater and Experimental Conditions

Groundwater, collected weekly in a fracture shale located 10 m below the surface (Lapcevic et al., 1990), was pumped into the fracture plane model to maintain a mean flow velocity, v, of 5 $m \cdot d^{-1}$ using a two-head peristaltic pump (Masterflex Model 7014-52, Cole Parmer Instrument Company, Illinois, USA) (Table 2). The study, conducted in

a 10-°C cold room to reproduce groundwater conditions, was divided in three phases over 100 days: biostimulation, persistence, and resistance.

The biostimulation of the groundwater microbial population was conducted by injection of invertose (50 g · L^{-1} pumped at 0.3 mL · min^{-1}; Casco, Etobicoke, Ontario) into the fracture through the borehole (mixing zone ≡ 225 mL) over 50 days, which allowed for the development of mature biofilm. Invertose syrup was selected because of its transparency, thus facilitating the visual observation of the biofilm development within the glass fracture. At the end of this phase, the nutrient injection was terminated, and the biofilm was starved over a 45-day period to assess the persistence of the biofilm. Then, the resistance of the biofilm was tested by tripling <v> for 2 days (to <v> = 15 m · d^{-1}), then doubling <v> again for 3 more days (to <v> = 30 m · d^{-1}).

Direct Observations of the Biofilm

The glass-fracture plane allowed for direct visual observation of the biofilm development, which was documented throughout the three phases of the experiment. For a non-invasive assessment of the biofilm surface area, the changes in light transmittance were measured every other day at 100 locations on the fracture plane. To do so, fluorescent light bulbs, located underneath the 2 glass-sheets, were illuminated while the lights in the cold room were doused, and the light transmittance was recorded manually using a hand-held probe that transformed the readings into millivolts (Fig. 2). The results were expressed as the difference between the present light transmittance and the initial light transmittance at that location, and t-Tests were performed to compare changes over

time ($\alpha = 0.05$). The mapping of the readings allowed for the estimation of the biofilm surface area.

Indirect Measurements of the Fracture Bioclogging

To obtain indirect measurements of bioclogging, the density of planktonic bacteria (bacteria in suspension), the changes in ORP, and the transport of tracers were monitored from different ports during the experiment. Several results of the tracer experiments were modeled using an analytical model for two-dimensional advection-dispersion. These indirect measurements of bioclogging were assessed as techniques that could be transferred to the field application.

Planktonic Bacterial Density. Planktonic bacteria sampled from six ports (0, 1, 2, 4, 5, and 13) were enumerated once a week using the LIVE/DEAD® BacLight™ Viability Kit (L-7012, Molecular Probes, Eugene, OR, USA) as described in Ross et al. (2001a). Port 0, located upgradient from the biostimulation zone, was used as the control. Ports 1 and 4 were chosen to assess the lateral variations in planktonic bacterial density whereas ports 2, 5, and 13 allowed for measuring the longitudinal changes. The bacterial density from each port was compared statistically using t-Tests ($\alpha = 0.05$).

ORP Monitoring. Fourteen platinum (Pt) ORP electrodes and one reference electrode (DriRef-2SH, World Precision Instruments, Sarasota, Florida, USA) were installed in the fracture plane, and the readings were recorded on-line at 30-min intervals (Delta-T Logger PC Software, Delta-T Devices Ltd, UK). The Pt electrodes (D = 500 μm) were designed and calibrated as described in Swerhone et al. (1999) with minor modifications in their configuration. A Pt wire (2.5 cm) was soldered to a copper wire (1

m), and the electrodes were introduced through the ports in a septum ensuring that their tips were centered into the 1.5-mm fracture. To separate the significant differences in ORP amongst the 14 locations in the fracture plane, the large data file was processed statistically and classified hierarchically using Euclidean distance (Branham, 1990).

Tracer Experiments. Lissamine FF and KBr were selected as tracers since they are considered biologically stable (Davis et al., 1985) and non ecotoxic (Smart, 1984). These tracers have been extensively used in fractured bedrock due to their resistance to adsorption losses and their facility to be detected (Lapcevic et al., 1999b; Novakowski et al., 1985). Tracer experiments were performed every other week by injecting Lissamine FF (250 μL of 1 g · L^{-1}) and KBr (5 mL of 1 g · L^{-1}) (C_0 = 1.02 and 22.04 mg · L^{-1} respectively) in the borehole, and monitoring the subsequent migration via measurement in ports 5, 12, and 13 over 16 hours. Port 5 was selected for its central location whereas ports 12 and 13 allowed for the maximum transport assessment in the fracture plane. Lissamine concentration was measured by fluorometry (Model 111 Fluorometer, G. K. Turner Associates, Palo Alto, CA, USA), and Br^- was quantified by HPLC (Waters 501 HPLC pump, Waters 712 WISP, Waters 340 Conductivity Detector, Alltech 335 Suppressol Module and Solid Phase Suppressor – Water Chromatography, Milford, Mass, USA) equipped with an IC-Pak Anion HC column using sodium bicarbonate and sodium carbonate as eluents (0.0017 M $NaHCO_3$ and 0.0018 M Na_2CO_3).

Modeling. To quantify the impact of the biofilm on the groundwater velocity as well as longitudinal and transverse hydrodynamic dispersion, the results of several of the tracer experiments were modeled. Because of the uniformity of the velocity field and relatively simple boundary conditions, an analytical model for two-dimensional

advection-dispersion (Cleary and Ungs, 1978) was used for the simulations. The governing equation is given by:

$$\frac{\partial c}{\partial t} + v\frac{\partial c}{\partial x} - D_x\frac{\partial^2 c}{\partial x^2} - D_y\frac{\partial^2 c}{\partial y^2} = 0 \qquad 0 \le x \le \infty; 0 \le y \le L$$

where v is groundwater velocity, c is tracer concentration, and D_x and D_y are the hydrodynamic dispersion coefficients in the x and y directions, respectively. The dispersion coefficients are related to longitudinal and transverse dispersivity (α_x and α_y) via $D = \alpha v$. In this case, the dispersivity values should represent the spreading of the solute due to Taylorian dispersion in the longitudinal direction and due to the channeling effects of the biofilm in the transverse direction. Reflection boundaries are used in the y direction (finite fracture width), and an exponentially decaying source was used to accommodate the mixing in the borehole at the inlet boundary. The mixing process is described by the point dilution equation:

$$V\frac{dc(t)}{dt} = -Avc(t)$$

where V is the volume of the mixing zone (i.e. the volume between the packers), and A is the effective cross-sectional area through which the tracer enters the fracture. Accounting for the presence of the open borehole, A is equal to twice the diameter of the borehole times the fracture aperture. Solution of the point dilution equation yields:

$$c = c_0 \exp\{-\gamma t\}$$

where γ is equal to $(A/V)\cdot v$. This expression is used in conjunction with an equation of continuity between the solute in the borehole and the solute in the fracture to formulate the final boundary value problem. The solution is presented (Cleary and Ungs, 1978) in terms of Exponential and Complimentary Error Functions that require numerical

integration. The integration was conducted with Gauss Quadrature using 104 Gauss points.

RESULTS AND DISCUSSION

Direct Observations of the Biofilm

Biofilm macrostructure. The visual observations of the biofilm development in the glass planar fracture revealed the formation of 1 to 2-mm clusters located downgradient from the nutrient injection borehole approximately 5 days after the initiation of biostimulation. The clusters are a typical structural attachment of microbial communities that develop under laminar flow conditions (Davey and O'Toole, 2000). These clusters increased in number and assumed the shape of the flow lines after 15 days (data not shown). This biofilm macrostructure shows similarity to observations made using microscopy and computer investigations reported by Ritcher et al. (1999). Studying biofilm development patterns on a glass surface, their findings showed chains, branches, and tree-like structures with no side growth and relatively small direction changes compared to the development on other surfaces, such as silicon and stainless steel. In the present study, the relatively low roughness and low hydrophobicity of the glass surface as well as the flow pattern give an explanation for the flow lines-like biofilm macrostructure observed in the first few weeks of the biostimulation (Mueller et al., 1992; Rijnaarts et al., 1996). In a similar experiment, Hill and Sleep (2002) observed clusters of up to 0.05 m diameter after 4 days. These clusters developed to full connection after 7 days under constant glucose injection (5 mg/L). In the present work, the macrostructure extended to a uniform biofilm visible after 30 days.

During the persistence phase (starvation between days 50 to 95), the biofilm darkened from a light beige to medium brown, which can be an indication of an accumulation of silts, precipitation of ferrous oxides, and/or changes in bacterial activities (Characklis and Marshall, 1990). During the resistance phase (increase of groundwater velocity by 6 times over 5 days), no noticeable changes in the biofilm surface were observed with the unaided eye. A uniform biofilm and an increase in surface coverage are typical of a biofilm structure developed in a high nutrient concentration. (Wimpenny and Colasanti, 1997)

Light Transmittance Probe. To date, the development of non-destructive methods for the quantification of biofilm accumulation remains a challenge (Okkerse et al., 2000; Tamachkiarowa and Flemming, 1996). The light transmittance probe used in the present study allowed assessment in real time, inexpensively, and in a non-invasive way, of the changes in biofilm surface area, which covered approximately 70 cm^2 and 700 cm^2 after 38 and 50 days of biostimulation respectively (Fig. 3). Over the 50-day biostimulation phase, the highest light transmittance reduction reached a steady-state at a 25-mV signal. Assuming that this steady-state value is governed by the fracture thickness (1.5 mm), and that a direct relation exists between light transmittance and biofilm thickness, it could be inferred that the maximum thickness, obtained directly downgradient the nutrient injection borehole, reached 780 μm and 1.5 mm after 38 and 50 days respectively (Fig. 3). This assumption is supported by the demonstration reported by Bakke et al. (2001) and Characklis and Marshall (1990) in which a linear correlation between biofilm optical density and biofilm mass obtained in a reactor designed specifically to accommodate optical measurement.

Indirect Measurements of the Fracture Bioclogging

Planktonic Bacterial Density. The groundwater pumped weekly from the Mississauga site had a bacterial density varying from 5.7×10^4 to 2.4×10^5 total bact. $\cdot$ mL^{-1}. The bacterial enumeration conducted in port 4 during the experiment was not significantly different from this initial density, which confirmed the location of port 4 to be outside the biostimulation zone ($p > 0.1$) (Fig. 4).

In the other ports, the 50-day biostimulation promoted an increase in the density of planktonic bacteria at first, followed by a decrease between 0.82 to 1.09 orders of magnitude in total bact. $\cdot$ mL^{-1}. This was observed to be more significant in the neighborhood of the injection borehole (ports 1 and 2, $p < 0.1$) (Fig. 4). This decrease in planktonic bacteria suggested an increase in sessile bacteria (attached bacteria), which is in accordance with the visual assessment of the biofilm development showing an accelerated surface covering after 30 days. The partitioning of attached and unattached bacteria in aquifers has been shown to be affected by the organic carbon concentration (Lehman et al., 2001). In the initial stages of the biofilm development, it is possible that the cell attachment was promoted by an increase in both planktonic cell concentration and cell hydrophobicity due to the high nutrient content (Fletcher, 1977; Mueller et al., 1992). It is known that high nutrient content stimulates copious production of EPS by groundwater micro-organisms (Ross et al., 2001a; Ross et al., 1998), which likely promoted bacterial attachment in the fracture plane.

After the cessation of the nutrients supply, the density of planktonic bacteria increased by 0.4 order of magnitude then significantly decreased to average 1.1×10^5

bact. · mL^{-1} in ports 1, 2, 5, and 13 after 45 days of starvation (Fig. 4). Bacterial detachment from the biofilm is a possible explanation for the increase in planktonic bacteria in the first few days of starvation conditions. This result has been attributed to enzymatic activities in biofilm when exposed to starvation conditions after being supplied with nutrients in studies by Batchelor et al. (1997) and O'Toole et al. (2000). The bacterial detachment in the fracture plane was followed by an important decline in suspended cells, which can be hypothesized as a recolonization of the biofilm by fresh groundwater bacteria.

The augmentation of the groundwater velocity, between days 95 and 100, led to an augmentation in the density of planktonic cells (Fig. 4). This might be the result of the detachment of more loosely attached bacteria. Indeed, it is known that the shear stress has an important effect on bacterial detachment until a pseudosteady state (cycles of attachment and detachment) is reached especially in high carbon loading rates. (Characklis and Marshall, 1990; Wanner et al., 1995)

ORP Monitoring. As a prospective tool for monitoring the bioclogging in a future field application, a series of 14 probes were installed in the fracture plane recording changes in ORP every 30 minutes over 100 days (Fig. 5). The overall decrease in the ORP suggested geochemical changes due to microbial activities (Appelo and Postma, 1993). However, the large variation in the readings complicated the interpretation of the results.

Three significant differences were revealed by the Euclidean hierarchical classification (Fig. 6). First, ORP decreased on average from ≅ 370 to 230 mV (Fig. 5–A: ports 2 and 9), second ORP diminished on average from ≅ 225 to 140 mV (Fig. 5–B:

ports 1, 3, 4, 5, and 6), and third ORP decreased on average from ≅ 185 to 50 mV (Fig. 5-C: ports 7, 8, 10, 11, 12, 13, and 14) (p = 95 %). With the exception of port 9, this classification showed that the changes in ORP were related to the location of the probes versus the biostimulation zone and the biofilm development zone.

Close to the borehole, but in the plume of nutrients (port 2), the supply of oxygen contained in the nutrient media (7.5 mg $O_2 \cdot L^{-1}$) and the mixing action contributed to maintain an ORP as high as 370 mV at the beginning of the stimulation phase (Fig. 5-A). Such a high ORP was expected during the first stages of the biofilm development under a high oxygen supply (Appelo and Postma, 1993; Bishop and Yu, 1999). As the bioclogging occurred, ORP diminished to reach ≅ 230 mV at the end the biostimulation phase (t = 50 d). The starvation phase (between days 50 and 95) accelerated the decrease in ORP bringing conditions closer to anoxic. This decrease in ORP was likely due to the consumption of O_2 for the oxidation of invertose as well as the decrease in diffusion of O_2 in the biofilm.

Previous work on diffusivities in biofilm have shown oxygen diffusion coefficient ranging from 70 % to 95 % of the corresponding value in water (Characklis and Marshall, 1990). Using microelectrodes in biofilms, Bishop and Yu (1999) have reported stratified structures with depth, which corresponded to different redox potentials. These stratifications led to a decrease in the redox potential from +375 mV (relative to the potential of hydrogen electrode) at the surface to -174 mV near the bottom of the biofilm. In the present work, the 500-µm diameter probes were positioned in the middle of the 1.5 mm fracture. Assuming that the biofilm thickened from the surface of the glass towards

the center of the fracture, it is likely that the probes were surrounded with the EPS matrix once a mature biofilm was established.

Outside the nutrient plume around the borehole (ports 1, 3, and 4), the ORP decreased moderately (from 225 to 140) over 100 days (Fig. 5-B). The same effect was measured for the ORP monitored in ports 5 and 6, an area where a biofilm was measured after 32 days of biostimulation (Fig. 3). The lag time for the biofilm to develop at 24 cm downgradient the nutrient injection, as well as the dispersion created by the upgradient biofilm, might explain the relatively small decrease in the ORP in ports 5 and 6 over time. As the groundwater flowed in the fracture, the ORP dropped towards the exit more importantly after the biostimulation phase (days > 50) (Fig. 5-C: ports 7, 8, 10, 11, 12, 13, and 14). Between days 50 and 100, the release of by-products, namely soluble microbial products (SMPs) (Ross et al., 1998) and biofilm sloughing (Characklis and Marshall, 1990), in addition to the depletion of the available electron acceptors, are believed to explain this change in the redox balance.

The presence of a high concentration of organic matter and the injection of bromide used to conduct tracer experiments are elements that were reported to interfere with the redox capacity of the probes and may explain the large variability in the ORP readings (American Public Health Association et al., 1995). When observing closely the curves, erratic ORP readings, ranging up to 400 mV, can be observed when conducting tracer experiments (days 2, 18, 34, 50, 59, and 78) (Fig. 5). Nevertheless, the decreasing trend observed throughout the bioclogging of the fracture plane is assumed to be attributable to the geochemical changes due to the bacterial activities as well as the decreasing the diffusion of O_2 in the biofilm.

Tracer Experiments and Modeling. The effects of the fracture bioclogging on groundwater flow were assessed using tracer experiments. These are reported for the initial stage of the biofilm development (t = 2 d), during the biostimulation phase (t = 50 d), and under starvation conditions (t = 78 d). Tracer concentrations were measured in ports 5, 12, and 13 (Fig. 7). Throughout the experiment, the similarity of Br^- and Lissamine breakthrough curves suggested no loss of Lissamine due to adsorption on the biofilm matrix (data not shown). Although low to no adsorptive loss of Lissamine was reported in fractured rock (Lapcevic et al., 1999b; Novakowski et al., 1985) and soil (Trudgill, 1987), little information was available on the potential adsorption of dyes on biofilms (Hill and Sleep, 2002). Mainly composed of EPS, biofilms offer a plethora of sorption sites, and electrostatic interactions between Lissamine and charged EPS could have been significant. (Flemming, 1995; Shirahama et al.,)

The bioclogging impacted the transport of Lissamine and Br^- in the fracture resulting first in a lag time in tracers' breakthrough, which reached 1.15 hours at 1.2 m downgradient from the injection point, independent of whether the biofilm was under stimulation or starvation (Fig. 7, port 13). Second, the bioclogging led to a minor amount of lateral dispersion in the fracture as revealed by the weak presence of tracers in port 12 (Fig. 7), which is located outside the tracer plume in pre-clogging conditions (Hoag, 1995).

The model fits obtained using dimensionless concentrations from port 13 at 2, 50, and 78 days are illustrated on Fig. 8 -A, -B, and -C. The fits were obtained visually by manipulating velocity, and longitudinal and transverse dispersivity. The fits are believed to be unique as velocity was found to position the peak in time, α_x to determine the slope

of the backside of the peak, and α_y to determine the overall height of the curve. The fitting process was found to be very sensitive to α_y. The quality of the fit is relatively good in the peak area, although later-time concentrations are overestimated by the model in all cases. The fits to all three data sets were obtained with longitudinal dispersivities of 0.5 to 1 mm. The values of α_x are consistent with what might be expected for transport in a smooth parallel-plate fracture dominated by Taylorian dispersion. This suggests that despite the observation of complex biofilm the impact on dispersion of the conservative solutes in the direction of flow is not observed.

The values of transverse dispersivity range from 5 mm for Fig. 8 -A and -C to 25 mm for Fig. 8-B. The highest transverse dispersion was observed at the end of the biostimulation period when the biofilm was likely to be most densely populated. The transverse dispersion observed in the starved biofilm is no different than that observed in the absence of the biofilm. Thus, during the period of detachment observed to follow the onset of starvation, the clumps of weakly attached clusters that were likely responsible for the transverse dispersion at the end of the biostimulation period were removed. Because no increase in velocity was observed between the tracer experiments (see following discussion), the presence of the weakly attached clusters did not impede flow. Based on the results of the tracer experiments, the velocity in the absence of the biofilm was 7.5 m $\cdot$ day^{-1} and in the presence of the biofilm was 6.2 m $\cdot$ day^{-1} (under both biostimulation and starvation conditions). Thus, the presence of the biofilm can be attributed to a reduction in the effective fracture aperture to approximately 1.36 mm. This calculation was conducted using the cubic law where the velocity in a fracture is proportional to the square of the fracture aperture. Because only a minor decline in

effective fracture aperture is observed, it can be surmised that the biofilm, even during the time at which the population should have been most dense, was quite porous and of a thickness much less then that predicted by the light transmittance probes. Based on these results, it is suggested that measurements of groundwater velocity are a reliable means by which to directly measure the development and performance of a biofilm.

CONCLUSIONS

A large fracture plane apparatus having a 1.5-mm aperture was designed for monitoring the development of a biofilm, its persistence to starvation, and its resistance to groundwater velocity as key steps and environmental factors likely to affect the biobarrier development in a field application. This apparatus was effective to evaluate the potential of monitoring tools to be transferred to a site demonstration. A light transmittance probe, a non intrusive, real-time, and inexpensive device, was effectively used to assess the biofilm surface area through the glass-fracture plane. It was shown that, under biostimulation with invertose, a uniform biofilm developed after 35 days in the 1.5-mm fracture, and that the surface area increased by 1 order of magnitude in the following 15 days. These results are valuable for the scaling-up of the biobarrier concept (e.g. injection rate and spacing of boreholes).

As indirect measurements of the fracture bioclogging, the enumeration of planktonic bacteria, the monitoring of ORP, and the transport of tracers gave insightful information on the development of the biofilm as well as on the changes due to starvation and to an increase in groundwater velocity. Phases of bacterial attachment, of detachment followed by recolonization, and of significant re-detachment, that matched respectively

with the biostimulation, the starvation, and the increase of groundwater velocity, suggested the planktonic bacteria should be monitored closely during the field demonstration. The monitoring of planktonic EPS concurrently with planktonic bacteria would complete the information on biofilm sloughing, especially under starvation when it is known that bacteria usually detach with a large section of the biofilm. The interpretation of the ORP readings was made difficult due to the large variations. It is believed that these probes will be less affected by the interferences in a real bedrock situation due to the higher electrolyte interactions with the rock surface.

Monitoring the tracer transport in the fracture, and furthermore modeling several results, gave fundamental information on changes in the flow during the three phases of the experiment. The unique fitting of an analytical model for two-dimensional advection-dispersion confirmed the transverse dispersion to be more substantial with a densely populated biofilm, i.e. at the end of the biostimulation phase, and shown the starved biofilm to have no effect on the transverse dispersion. More importantly for the design of monitoring tools in support of a field application, a direct relation was established between the changes in groundwater velocity and the development of the biofilm. These results give the background data necessary to support the scaling-up of the biobarrier at field demonstration.

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REFERENCES

American Public Health Association, A.W.W. Association, and W.E. Federation. 1995. Standard Methods for the Examination of Water and Wastewater. 18th ed. American Public Health Association, Washington, DC.

Appelo, C.A.J., and D. Postma. 1993. Geochemistry, Groundwater and Pollution A. A. Balkema, Brookfield.

Bakke, R., R. Kommedal, and S. Kalvenes. 2001. Quantification of biofilm accumulation by an optical approach. Journal of Microbiological Methods 44:13-26.

Batchelor, S.E., M. Cooper, S.R. Chhabra, L.A. Glover, G.S.A.B. Stewart, P. Williams, and J.I. Prosser. 1997. Cell Density-Regulated Recovery of Starved Biofilm Populations of Ammonia-Oxidizing Bacteria. Applied and Environmental Microbiology 63:2281-2286.

Baveye, P., P. Vandevivere, B.L. Hoyle, P.C. DeLeo, and D. Sanchez de Lozada. 1998. Environmental Impact and Mechanisms of the Biological Clogging of Saturated Soils and Aquifer Materials. Critical Reviews in Environmental Science and Technology 28:123-191.

Bishop, P.L., and T. Yu. 1999. A microelectrode study of redox potential change in biofilms. Water Science and Technology 39:179-185.

Branham, R.L.J. 1990. Scientific Data Analysis - An Introduction to Overdetermined Systems Springer-Verlag.

Bryers, J.D., (ed.) 2000. Biofilms II: Process Analysis and Applications, pp. 1-432. John Wiley and Sons, Inc., New York.

Canter, L.W., and R.C. Knox. 1986. Groundwater Pollution Control Lewis Publishers, Inc., Chelsea.

Characklis, W.G., and K.C. Marshall. 1990. Biofilms John Wiley & Sons, Inc., New York.

Cherry, J.A., E.A. Sudicky, C.W. Mase, K.S. Novakowski, and P.A. Lapcevic. 1993. Measuring groundwater velocity and hydrodynamic dispersion in a single fracture in fractured shale Grant No. 393G. Ministry of Environment, Ontario.

Cleary, R.W., and M.J. Ungs. 1978. Analytical models for groundwater pollution and hydrology Report 78-WR-15. Princeton University, Princeton.

Cusack, F. (ed.) 1992. SPE Internaltional Meeting on Petroleum Engineering, Beijing, China. 24-27 March.

Davey, M.E., and G.A. O'Toole. 2000. Microbial biofilms: from ecology to molecular genetics. Microbiology and Molecular Biology Reviews 64:847-867.

Davey, M.E., D. Gevertz, W.A. Wood, J.B. Clark, and G.E. Jenneman. 1998. Microbial selective plugging of sandstone through simulation of indigenous bacteria in a hypersaline oil reservoir. Geomicrobiology 15:335-352.

Davis, S.N., D.J. Campbell, H.W. Bentley, and T.J. Flynn. 1985. Ground Water Tracers U.S. Environmental Protection Agency, Oklahoma.

Dupin, H.J., and P.L. McCarty. 2000. Impact of colony morphologies and disinfection on biological clogging in porous media. Envionmental Science and Technology 34:1513-1520.

EPA, U.S. 1989. Evaluation of ground-water extraction remedies: Volume 1, Summary report EPA/540/2-89/054, NTIS PB90-183583. Office of Solid Waste and Emergency Response, Washington, DC.

Flemming, H.-C. 1995. Sorption Sites in Biofilms. Water Science and Technology 32:27-33.

Fletcher, M. 1977. The Effects of Culture Concentration and Age, Time, and Temperature on Bacterial Attachment to Polystyrene. Canadian Journal of Microbiology 23:1-6.

Hiebert, R., R. Sharp, A. Cunningham, and G. James. 2001. Development and demonstration of subsurface biofilm barriers using starved bacterial cultures. Contaminated Soil, Sediment, and Water August.

Hill, D.H., and B.E. Sleep. 2002. Effects of biofilm growth on flow and transport through a glass parallel plate fracture. Journal of Contaminant Hydrology 56:227-246.

Hoag, R.S. 1995. Interpretation of Point-dilution Experiments for Determining Groundwater Velocity in a Single Fracture. University of Waterloo, Faculty of Engineering, Waterloo.

A. Leeson, et al. (ed.) 2001. The Sixth International In Situ and On-Site Bioremediation Symposium, San Diego, California. June 4-7. Battelle Press.

Lapcevic, P.A., K.S. Novakowski, and J.A. Cherry. 1990. The Characterization of Two Discrete Horizontal Fractures in Shale Contribution 90-96. National Water Research Institute, Burlington.

Lapcevic, P.A., K.S. Novakowski, and E.A. Sudicky. 1999a. The interpretation of a tracer experiment conducted in a single fracture under conditions of natural groundwater flow. Water Resources Research 35:2301-2312.

Lapcevic, P.A., K.S. Novakowski, and E.A. Sudicky. 1999b. Groundwater flow and solute transport in fractured media, p. 17-1 - 17-39, *In* J. W. Delleur, ed. The Handbook of Groundwater Engineering. CRC Press, Boca Raton.

Lappin-Scott, H.M., F. Cusack, and J.W. Costerton. 1988. Nutrient resuscitation and growth of starved cells in sandstone cores: a novel approach to enhance oil recovery. Applied and Environmental Microbiology 54:1373-1382.

Lehman, R.M., F.S. Colwell, and G.A. Bala. 2001. Attached and unattached microbial communities in a simulated basalt aquifer under fracture- and porous-flow conditions. Applied and Environmental Microbiology 67:2799-2809.

Mueller, R.F., W.G. Characklis, W.L. Jones, and J.T. Sears. 1992. Characterization of Initial Events in Bacterial Surface Colonization by Two *Pseudomonas* Species Using Image Analysis. Biotechnology and Bioengineering 39:1161-1170.

Novakowski, K.S. 1992. The analysis of tracer experiments conducted in divergent radial flow field. Water Resources Research 28:3215-3225.

Novakowski, K.S., and P.A. Lapcevic. 1994. Field measurement of radial solute transport in fractured rock. Water Resources Research 30:37-44.

Novakowski, K.S., G.V. Evans, D.A. Lever, and K.G. Raven. 1985. A field example of measuring hydrodynamic dispersion in a single fracture. Water Resources Research 21:1165-1174.

Novakowski, K.S., P.A. Lapcevic, J.W. Voralek, and G.S. Bickerton. 1995. Preliminary interpretation of tracer experiments conducted in a discrete fracture under conditions of natural flow. Geophysical Research Letters 22:1417-1420.

Okkerse, W.J.H., S.P.P. Ottengraf, and B. Osinga-Kuipers. 2000. Biofilm thickness variability investigated with a laser triangulation sensor. Biotechnology and Bioengineering 70:619-629.

O'Toole, G., H.B. Kaplan, and R. Kolter. 2000. Biofilm formation as microbial development. Annual Review in Microbiology 54:49-79.

Rijnaarts, H.H.M., W. Norde, E.J. Bouwer, J. Lyklema, and A.J.B. Zehnder. 1996. Bacterial Deposition in Porous Media: Effects of Cell-Coating, Substratum Hydrophobicity, and Electrolyte Concentration. Environmental Science and Technology 30:2877-2883.

Ritcher, A., R. Smith, R. Ries, and H. Lenz. 1999. Growth of biological films - microscopal investigations and computer simulations. Material Science and Engineering C 8-9:451-462.

Ross, N., and G. Bickerton. 2002. Application of biobarriers for groundwater containment at fractured bedrock sites. Remediation 12:5-21.

Ross, N., R. Villemur, É. Marcandella, and L. Deschênes. 2001a. Assessment of changes in biodiversity when a community of ultramicrobacteria isolated from groundwater is stimulated to form a biofilm. Microbial Ecology 42:56-68.

Ross, N., R. Villemur, L. Deschênes, and R. Samson. 2001b. Clogging of a limestone fracture by stimulating groundwater microbes. Water Research 35:2029-2037.

Ross, N., L. Deschenes, J. Bureau, B. Clément, Y. Comeau, and R. Samson. 1998. Ecotoxicological assessment and effects of physicochemical factors on biofilm development in groundwater conditions. Environmental Science and Technology 32:1105-1111.

Shirahama, K., S. Sato, L. Yang, and N. Takisawa. The interaction of amphiphiles with polysaccharides.

Smart, P.L. 1984. A review of the toxicity of twelve fluorescent dyes used for water tracing. NSS Bulletin 46:21-33.

Swerhone, G.D.W., J.R. Lawrence, J.G. Richard, and M.J. Hendry. 1999. Construction and testing of a durable platinum wire Eh electrode for in situ redox measurments in the subsurface. Ground Water Monitoring and Remediation Spring:132-136.

Tamachkiarowa, A., and H.-C. Flemming. 1996. Glass fiber sensor for biofouling monitoring. DECHEMA Monographs 133:31-36.

Taylor, S.W., and P.R. Jaffé. 1990. Biofilm growth and the related changes in the physical properties of a porous medium 3. Dispersivity and model verification. Water Resources Research 26:2171-2180.

Trudgill, S.T. 1987. Soil water dye tracing, with special reference to the use of rhodamine WT, lissamine FF and amino G acid. Hydrological Processes 1:149-170.

Wanner, O., A.B. Cunningham, and R. Lundman. 1995. Modeling Biofilm Accumulation and Mass Transport in a Porous Media Under High Substrate Loading. Biotechnology and Bioengineering 47:703-712.

Wimpenny, J., W. Manz, and U. Szewzyk. 2000. Heterogeneity in biofilms. FEMS Microbiology Reviews 24:661-671.

Wimpenny, J.W.T., and R. Colasanti. 1997. A unifying hypothesis for the structure of microbial biofilms based on cellular automaton model. FEMS Microbiology Ecology 22:1-16.

Table 1. Location of the ports on the upper glass-sheet for groundwater sampling and ORP reading

Port	Location from the center of the borehole x (cm)	y (cm)
0	-45.0	0
1	8.8	10
2	8.8	0
3	8.8	-20
4	23.8	20
5	23.8	0
6	23.8	-10
7	43.8	0
8	63.8	10
9	63.8	0
10	63.8	-20
11	93.8	0
12	123.8	20
13	123.8	0
14	123.8	-10

1

2 Table 2. Constituents of the groundwater pumped from an isolated fracture 10 m below

3 surface in shale formation, Mississauga, Ontario

4

Constituent[†]	Concentration[¶] (mg L^{-1})	Standard Deviation (mg L^{-1})
Cl^-	268.6	31.6
Na^+	240.0	44.9
SO_4^{2-}	117.4	11.2
Ca^{2+}	11.36	3.52
K^+	11.18	0.48
SiO_2	7.63	0.32
Mg^{2+}	2.885	0.955
TKN[‡]	1.54	0.20
NH_3^+	1.28	0.32
Fe total	0.010	0.015
Mn	0.006	0.008
NO_3-NO_2	0.005	0.008
SRP[§]	0.003	0.001

5 [†]: Al, Cd, Co, Cr, Cu, Ni, Pb, and Zn were below detection limits; [‡]: Total

6 Kjeldahl Nitrogen; [§]: Soluble Reactive Phosphorus; [¶]: Averaged over the

7 100-day experiment.

1

2 **CAPTIONS**

3 Fig. 1. The fracture plane model. A: side view, B: top view. Not to scale.

4

5 Fig. 2. Schematic of the light transmittance monitor device for the assessment of the
6 biofilm development.

7

8 Fig. 3. Contour-lines of the decrease in light transmittance assessing the biofilm surface
9 area; the 10-mV contour line matched the visible biofilm. A: after 35 days of
10 biostimulation and B: after 50 days of biostimulation.

11

12 Fig. 4. Changes in the density of planktonic bacteria sampled at six ports on the fracture
13 plane model during A- biostimulation phase, B- the persistence phase, and C- the
14 resistance phase. Results not significantly different were grouped ($\alpha = 0.05$).

15

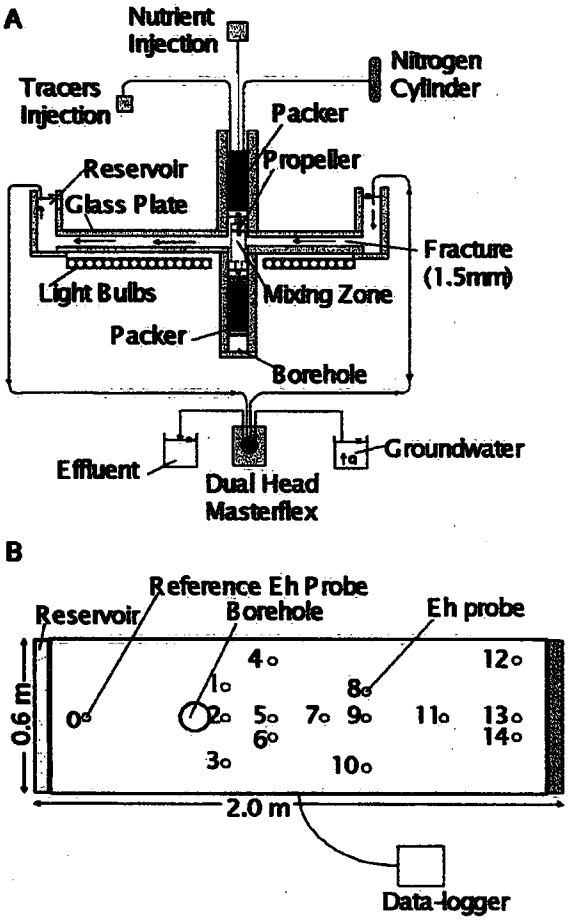
16 Fig. 5. Changes in ORP during the biostimulation phase, the persistence phase, and the
17 resistance phase. Ports showing no significant difference were grouped. A: ports 2 and 9;
18 B: ports 1, 3, 4, 5, and 6; C: ports 7, 8, 10, 11, 12, 13, and 14 ($p = 95$ %).

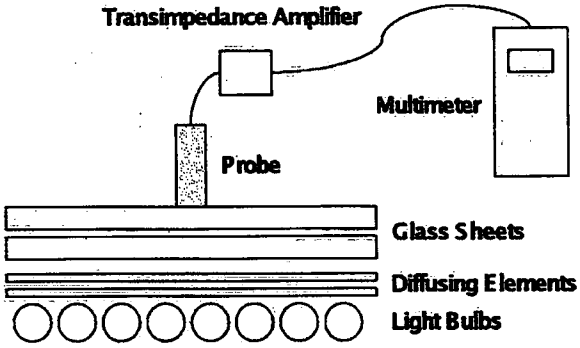
19

20 Fig. 6. Hierarchical classification of the ORP responses from 14 probes monitored over
21 100 days at 30 minutes interval ($\alpha = 95$ %).

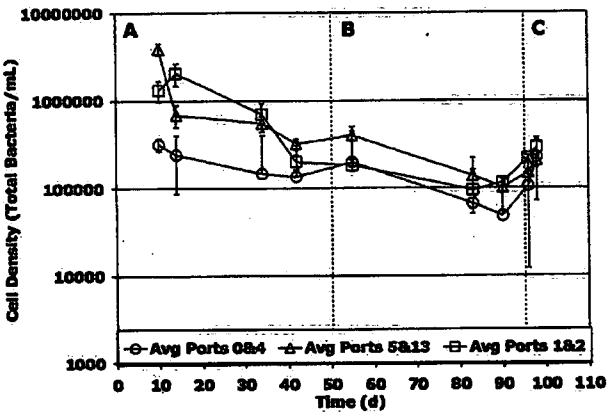
Fig. 7. Breakthrough of Br^- sampled from three ports comparing the transport at initial stage, through a mature biofilm, and in a biofilm under starvation conditions.

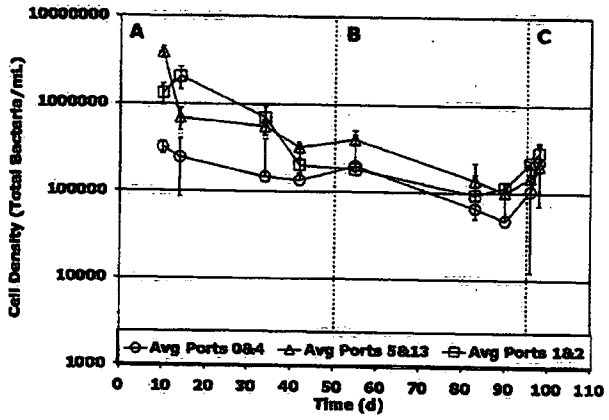
Fig. 8. Fitting of the dimensionless Br^- concentrations from port 13. A: t = 2 d, B: t = 50 d, and C: t = 78 d. A velocity of 7.5 m/day was obtained for A and 6.2 m/day was obtained for B and C respectively. A transverse dispersivity of 5 mm was used for A and C, and a transverse dispersivity of 25 mm was used for B. A longitudinal dispersivity of 1 mm was used for A and 0.5 mm used for B and C.

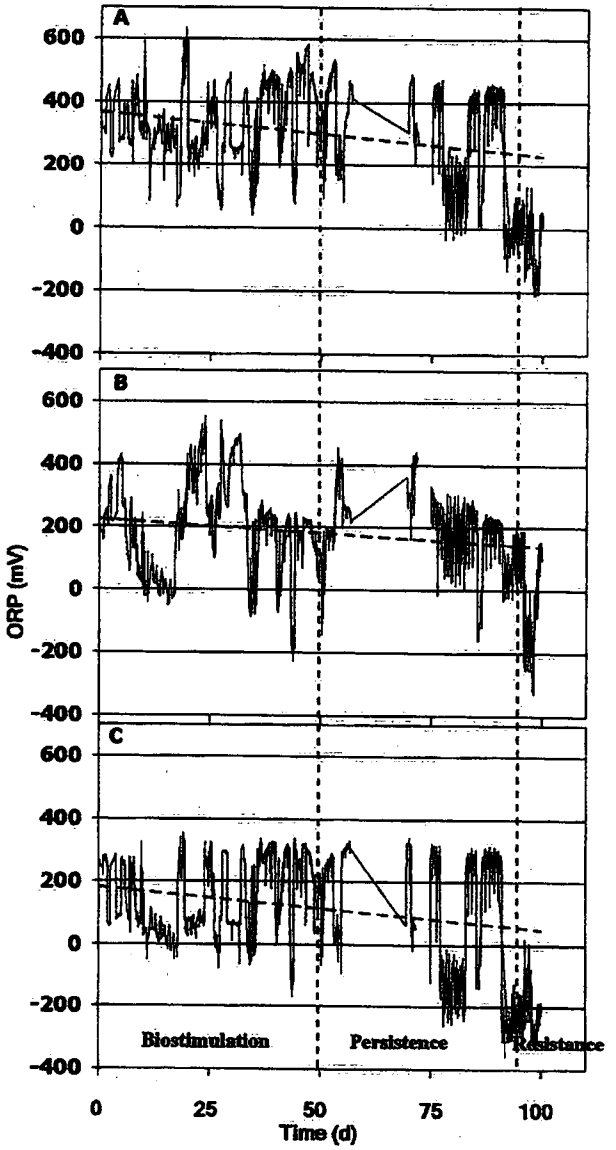

A
Nutrient Injection
Nitrogen Cylinder
Tracers Injection
Packer
Propeller
Reservoir
Glass Plate
Fracture (1.5mm)
Light Bulbs
Mixing Zone
Packer
Borehole
Effluent
Groundwater
Dual Head Masterflex
B
Reference Eh Probe
Reservoir
Borehole
Eh probe
0.6 m
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
2.0 m
Data-logger

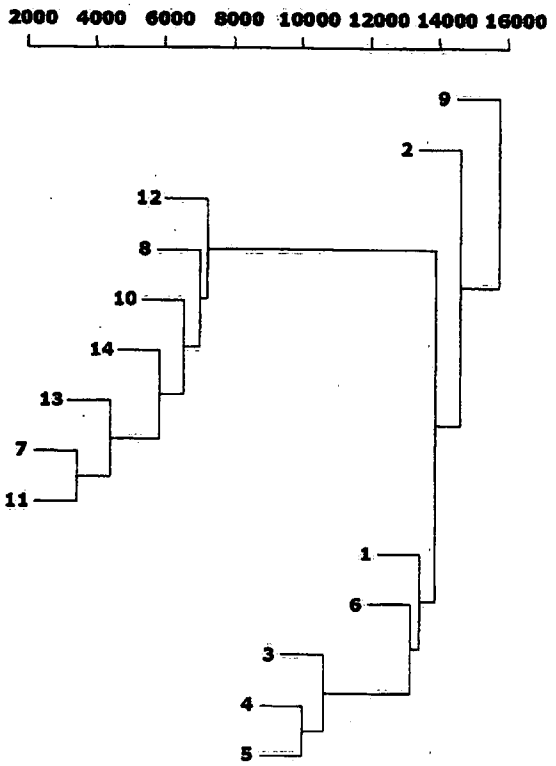

Transimpedance Amplifier
Multimeter
Probe
Glass Sheets
Diffusing Elements
Light Bulbs

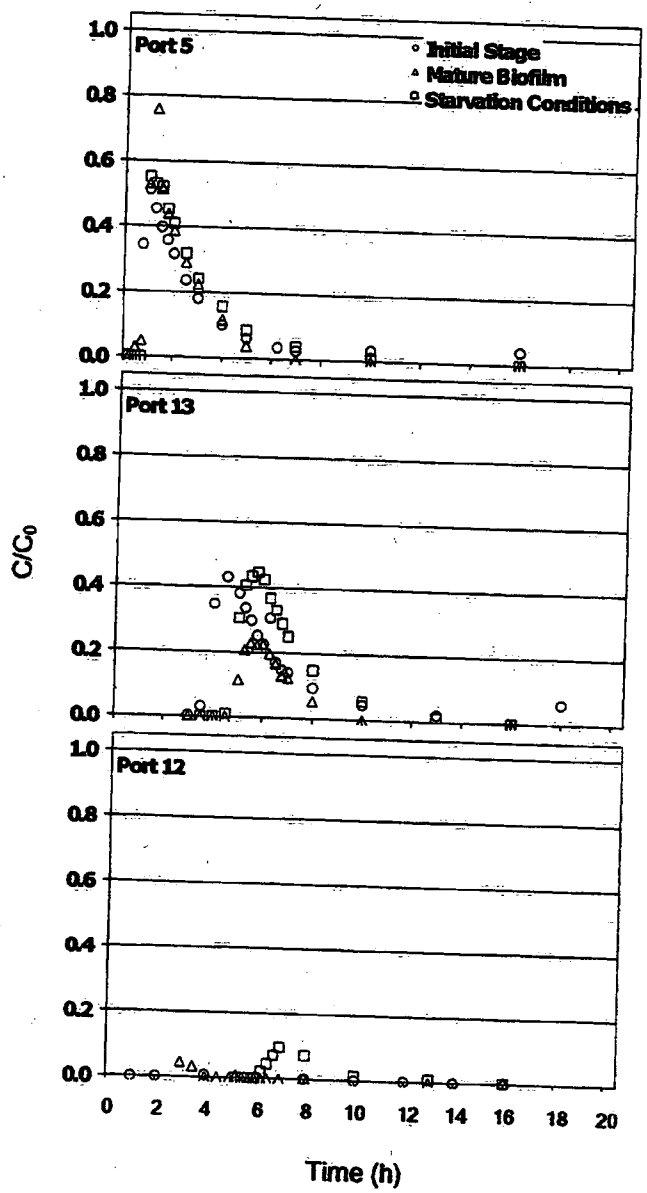
Y-distance from the borehole (cm)
20
15
10
5
0
-5
-10
-15
-20
B
10
15
20
25
30
35
40
X-distance from the borehole (cm)
0
5
10
15
20
25

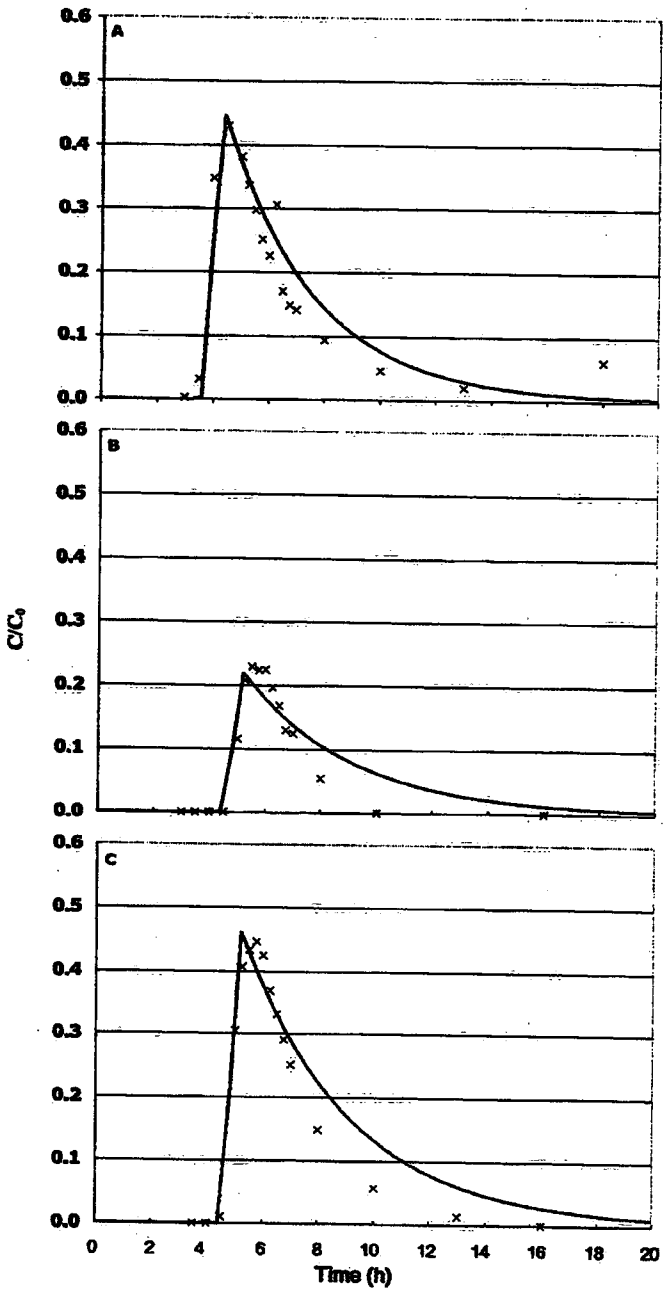

10000000
1000000
100000
10000
1000
A
B
C
Cell Density (Total Bacteria/mL)
Avg Ports 0&4
Avg Ports 5&13
Avg Ports 1&2
0
10
20
30
40
50
60
70
80
90
100
110
Time (d)


10000000
1000000
100000
10000
1000
A
B
C
Cell Density (Total Bacteria/mL)
Avg Ports 08.4
Avg Ports 5&13
Avg Ports 1&2
0
10
20
30
40
50
60
70
80
90
100
110
Time (d)


A
B
C
600
400
200
0
-200
-400
ORP (mV)
Biostimulation
Persistence
Resistance
0
25
50
75
100
Time (d)


2000 4000 6000 8000 10000 12000 14000 16000
9
2
12
8
10
14
13
7
11
1
6
3
4
5


Port 5
Initial Stage
Mature Biofilm
Starvation Conditions
Port 13
Port 12
C/C0
1.0
0.8
0.6
0.4
0.2
0.0
0
2
4
6
8
10
12
14
16
18
20
Time (h)


A
B
C
C/C0
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
2
4
6
8
10
12
14
16
18
20
Time (h)

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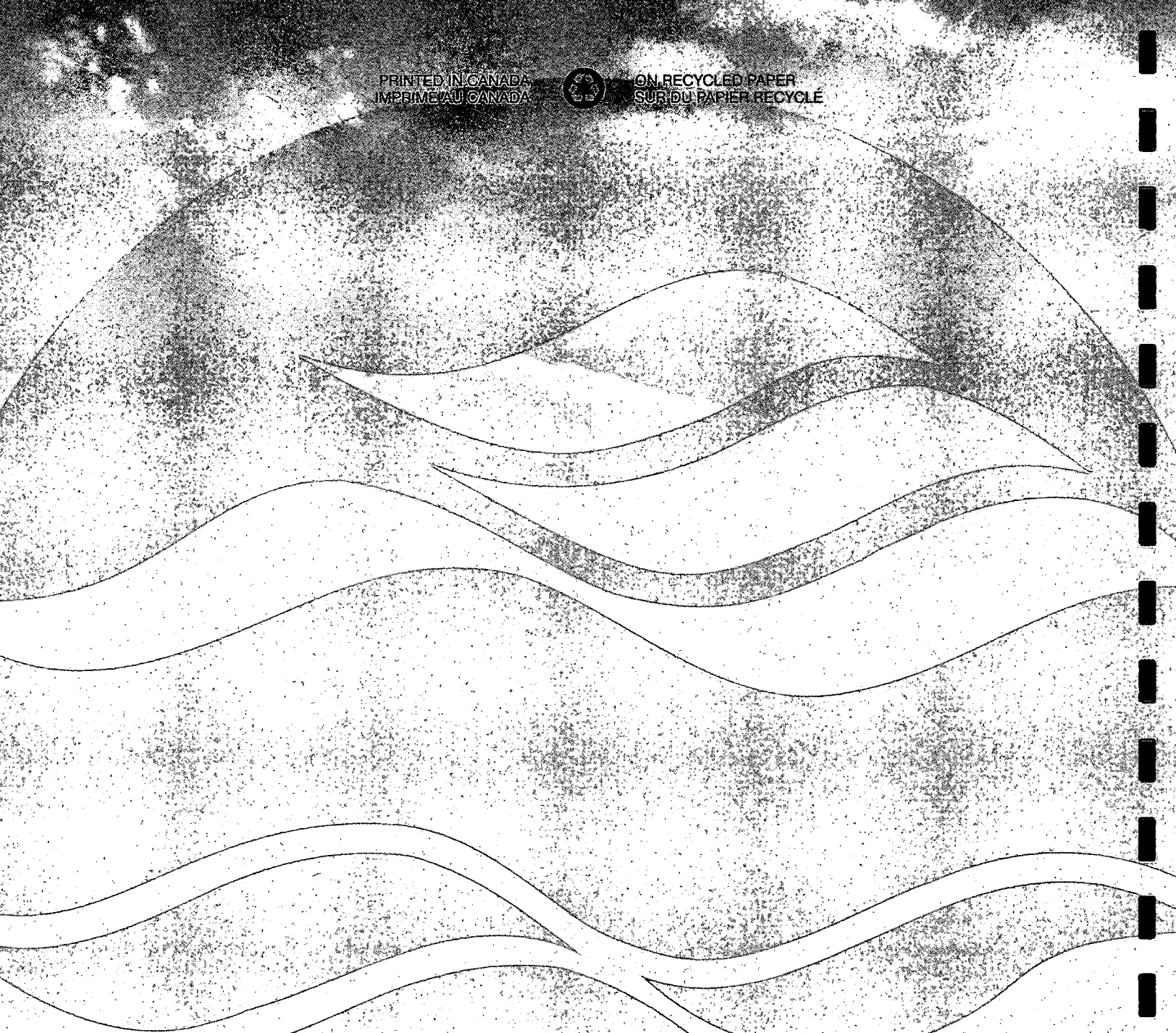

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Institut national de recherche sur les eaux
Environnement Canada
Centre canadien des eaux intérieures
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867, chemin Lakeshore
Burlington, Ontario
L7R 4A6 Canada

Centre national de recherche en hydrologie
11, boul. Innovation
Saskatoon, Saskatchewan
S7N 3H5 Canada

Environment Canada Environnement Canada

Canada