

Supporting Information: In-situ detection of subsurface biofilm using low-field NMR—a field study

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Summary: Expanded methods description, additional results and discussion.

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Figures: SI-1. Square of Echoes

Site Preparation

A bleach solution consisting of 2 lb (907 g) sodium dichloro-s-triazinetriene dihydrate ($\text{C}_3\text{H}_4\text{Cl}_2\text{N}_3\text{NaO}_5$) (Spa Guard Chlorinating Concentrate, Bio-Lab Inc., Lawrenceville, GA) was pumped into each well to oxidize any pre-existing biofilm. The solution was mixed in a 55 gal (208 L) drum using enough water to dissolve the granules, approximately 20-30 gallons (75-115 L). The solution was pumped to the target region through a standard garden hose using a 1/3 hp centrifugal pump (AMT Pump Model 3680-975-97 by Gorman Rupp, Royersford, PA) operating at approximately 12 gpm (45.5 L/min). The bleach solution was allowed to react in the wells overnight, and was followed by a 30 min high-flow (12 gpm (45.5 L/min)) flush of groundwater from the test cell to detach EPS and dead cells and purge any remaining bleach solution. Then, the probes were lowered to the bottom of the wells, approximately 18 ft (5.5 m) deep and 15 ft (4.5 m) below the water table in the test cell.

Bacterial Culture

The target organism, *Pseudomonas fluorescens* CPC211A, is the environmental isolate used in the original experiments in the test cell and, therefore, was known to grow successfully in that environment.¹ The bacteria was cultured from cryo-stock using a nutrient broth consisting of 10 g/L molasses (Aunt Patty's Blackstrap Molasses, Eugene, OR), 3 g/L sodium nitrate (SQM industrial grade prills, 98%, SQM North America, Atlanta, GA), 1 g/L yeast extract (Acros Organics, Geel, Belgium), 0.12 g/L potassium phosphate dibasic, and 0.04 g/L potassium phosphate monobasic (Thermo-Fisher Scientific, Waltham, MA). The inoculum was cultured in successively larger volumes at ambient temperature without mixing over seven days to produce a final inoculum volume of 60 L with a viable cell count of 8.5×10^7 colony forming units (cfu)/mL.

The active biofilm growth region of the well-bore environment was designed to be approximately 18 gal (67 L) of pore space, corresponding to the height of the NMR logging device (4.5 ft), a 9 in (22.9 cm) radius and an estimated porosity of 0.3.1 This active region of soil in each well was conditioned with 15 gal (56.7 L) of substrate prior to injection of the inoculum broth. The substrate was pumped at a rate of 1.2 L/min with a peristaltic pump (Masterflex L/S Model 900-1255, Cole-Parmer, Vernon Hills, IL) down each well casing to the sensitive zone of the NMR probe using four lines of 1/8-inch (3.2mm) ID vinyl tubing (Clearflex, McMaster-Carr, Santa Fe Springs, CA). Then 30 L (7.9 gal) of inoculum was injected into each well, followed by 5 gal (18.9L) of fresh substrate to push the bacteria into the soil from the well casing. Finally, water from the test cell was injected for approximately 1-2 minutes to rinse the injection tubing. No attempt was made to maintain a monoculture.

During the biofilm growth phase of the experiment, Days 1-10, the molasses substrate was prepared just prior to use in 50-55 gal plastic drums using groundwater from the lined test cell. Groundwater was pumped from another of the test cell's eleven 4 inch (10.2 cm) wells located 36 ft (11 m) from the nearest experimental well and stored prior to use in a large capacity water storage tank (Ace Roto-Mold, Den Hartog Industries, Hospers, IA). Each well received approximately 100-110 gal (379-416 L) per day of substrate over a 5-6 hour period. This translates to a Darcy velocity at the logging tool's excitation shell of approximately 4.2 cm/hr and an interstitial velocity of 14.4 cm/hr in the LF well during substrate injection, and 7.2 cm/hr and 23.4 cm/hr, respectively, in the HF well. NMR measurements were collected during the pumping of substrate. There was no pumping or flow in the wells for the remainder of each day

following substrate injection. Continuous flow of substrate was not feasible given experimental constraints.

NMR Measurements

T2 relaxation measurements were conducted using a Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence,²⁻³ consisting of a 90° excitation pulse followed by a series of 180° refocusing pulses separated by the echo spacing, t_E . Frequencies and echo spacings for each probe were fixed. The repetition time, T_r , which is the time between 90° rf excitation pulses, was either 1500 ms or 5000 ms for each measurement (Table 2). Faster repetition of the excitation pulse, with a T_r of 1500 ms, allowed for the rapid collection of data from the fastest decaying components of the NMR signal; recording 600 averages improved resolution of the early part of the decay curve.⁴ A longer repetition time of 5000 ms, recorded with 150 averages, allowed signal to be collected from the slower decaying components, such as water in larger pores.

Microbiological and Water Chemical Analysis

Two 10-15 mL samples of water were collected daily in sterile 15 mL Falcon tubes (Becton, Dickinson and Co., Sparks, MD) from each well prior to injection of fresh substrate. Flow on the substrate injection pumps was reversed and allowed to flow for 2-3 minutes in order to collect a water sample from each well's biofilm growth region. The samples were placed on ice in the field and then frozen for later analysis at the conclusion of the field test. The samples were analyzed for pH (VWR symPHony benchtop SB70P pH meter) and by drop plate for heterotrophic plate count (HPC).⁵

Samples were drop plated on Difco tryptic soy agar (Becton, Dickinson and Co., Sparks, MD) plates in duplicate and cultured at room temperature on the benchtop and in anaerobic pouches

(BD GasPak EZPouch, Becton, Dickinson and Co., Sparks, MD). The aerobically grown plates produced higher and more consistent numbers of culturable heterotrophic cells.

Results and Discussion

Another measure of signal attenuation is given by the Square of Echoes (SOE) method which is used to improve the low signal to noise ratio that is typical for a low-field NMR device in natural geologic material. The SOE is the squared value of the mean echo in the signal decay curve. A reduction in the SOE value over the course of the experiment qualitatively indicates that the log mean value of T2 relaxation is also decreasing. When normalized with respect to water content squared, data from the field test shows an SOE decrease of 91% in the LF well and 70% in the HF well during the biofilm growth phase of the experiment. The percent reduction is calculated using the SOE at inoculation (Day 1) and the average of SOE values Day 5-12 when the values were stable (Figure SI-1).

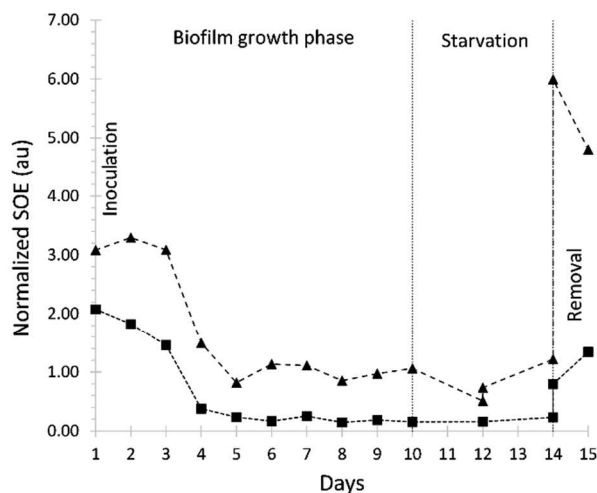


Figure SI-1. Square of Echoes (SOE) data from the HF (triangles) and LF (squares) wells shows a decrease of 70% and 90% respectively.

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