Bioaccessible Porosity in Soil Aggregates and Implications for Biodegradation of High Molecular Weight Petroleum Compounds

ALI AKBARI and SUBHASIS GHOSHAL*

Department of Civil Engineering, McGill University,

Montreal, Quebec H3A 0C3, Canada

This Supporting Information includes 7 pages, and two tables.

* Corresponding Author. Phone: (514) 398-6867; Fax: (514) 398-7361; E-mail: subhasis.ghoshal@mcgill.ca

Materials and Methods

Biodegradation experiments: Bioremediation experiments were performed in pilot scale tanks 1 m × 0.7 m × 0.35 m (L × W × H). Adequate nutrients were added to both RI (250 mg-N/kg) and NWT (95 mg-N/g) soils. Moisture content was adjusted at about 55-60% water holding capacity of the soils. Aeration of soil piles in both cases assured that oxygen concentration in air phase of soil voids were above 80% of ambient oxygen level. Bioremediation end points were determined when the biodegradation patterns reached to the plateau phase. Soil properties are presented in Table S1. Details of experimental conditions have been reported before for RI soils [1] and NWT soils. [2]

Table S1. Physicochemical properties of Resolution Island (RI) and Northwest Territories (NWT) site soils.

	RI	NWT
Soil grain size classification	Gravel: 27% Sand: 71% Silt & clays: 1%	Sand: 33% Silt: 33% Clay: 34%
USDA-SCS classification	Sand	Clay loam
Mineralogy as determined by X-ray diffraction	Quartz: 60% Feldspar: 23% Calcite: 6% Dolomite: 8% Kaolinite: 2% Muscovite: 1%	Quartz: 47% Feldspar: 4% Calcite: 6% Dolomite: 13% Kaolinite: 15% Chlorite: 3% Illite: 12%
Moisture content	11%	17.5%
Initial concentration of nonvolatile petroleum hydrocarbon fraction (>C16-C34)	1019.4±281.5 mg/kg-soil	1068.8±138.0 mg/kg-soil
Total organic carbon (TOC)	2.4%	2.3%
Cation Exchange Capacity	<10 ($cmol^+$/Kg)	19 ($cmol^+$/Kg)

Image analysis procedure: The IK method consists of four major steps starting with partial assignment of pixels based on low and high threshold values (T_0, T_1). The method is quite sensitive to the threshold values. Two different automated methods (entropy method and bi-normal mixture method) have been originally proposed to determine the low and high threshold values[3], however better results were obtained when the threshold values were determined by trial and error.

After initial thresholding, in the second pass, the rest of the population was segmented based on kriging. Based on the following equation the probabilities of the pixel belonging to any of two populations in a circular window with fixed radius of 3 pixels were calculated. In the equation, $P(T_i; x_0 \mid n)$ is the probability of the unknown pixel belonging to the population π_i, i is the indicator variable, λ_α is the weight assigned to pixels in the kriging window, x_o denotes the spatial location of an unclassified pixel and x_α represent the neighboring pixels. The weights were determined by solving a set of constrained equations of ordinary kriging system formed based on semi-variogram model.

$$P(T_i; x_0 \mid n) = \sum_{\alpha=1}^{n} \lambda_\alpha (T_i; x_0) i(T_i; x_0) \qquad i = 0,1 \tag{1}$$

Other than initial thresholding and kriging steps, two further filtering steps were employed as part of IK method for removing uncorrelated isolated noises inside the aggregate pores. These steps known as majority filtering are applied before and after the kriging step to those pixels whose population is assigned in thresholding step. The majority filtering window ignores unclassified pixels and is centered only on previously

thresholded pixels. If the majority of the pixels in the window belong to the opposite population then the assignment of the pixel is reversed. To preserve micro-pores as a characteristic of the fine texture of soil, majority filtering was applied only to those pixels initially assigned as object.

To determine the boundary of soil aggregates, first, each image was thresholded by a single low global threshold value to retain the object pixels as much as possible and assigning the intermediate gray scale values as solid (soil). Then, in order to remove noise from thresholded images, objects smaller than 0.01% of the largest solid (aggregate) component were removed. The value of 0.01% was chosen based on the maximum size of noise (pixels assigned as solid) observed in air surrounding the aggregate body in the thresholded images. Next, the air pixels inside and in between of object components were filled as solid object. Then, the alpha-shape of the aggregate body (alpha value=0.02) was determined. The alpha-shape with alpha value of 0 (radius of infinity), represents the convex-hul of an object.

Table S2. Information of analyzed aggregates from NWT and RI soils.

	Soil	Approximate largest aggregate dimension (mm)	Scanning Resolution (μm)	Bioaccessible porosity (%)
NWT1	NWT, clayey soil	1.0	1.04	25.0
NWT2	NWT, clayey soil	4.3	2.76	23.5
NWT3	NWT, clayey soil	2.2	1.30	26.3
NWT4	NWT, clayey soil	4.2	2.85	28.1
RI1	RI, sandy soil	4.2	3.28	23.4
RI2	RI, sandy soil	2.1	1.04	27.3

N_2 adsorption analysis: N_2 adsorption analysis was performed on air-dried, intact soil aggregates with a Micromeritics TriStar 3000 instrument. Samples were

degassed under nitrogen at 200 °C before analysis and operation conditions were as follow: evacuation rate: 5.0 mmHg/s, evacuation time: 0.10 hours, analysis bath temperature 77.350 K and equilibrium intervals: 5 s.

Bioreactor experiments: The *Dietzia maris*; CA160 strain contains *alkB* gene and is capable of using hexadecane as a sole carbon source. It was isolated from weathered petroleum hydrocarbon contaminated soils from Northwest Territories (Canada). Enrichment culturing and single colony isolation techniques was repeatedly used to isolate hydrocarbon degrader, salt tolerant species from the soil microbial community. Cultures were prepared by adding soil sample to Bushnell-Hass (BH) media (magnesium sulfate 0.2 g/L, calcium chloride 0.02 g/L, monopotassium phosphate 1.0 g/L, dipotassium phosphate 1.0 g/L, ammonium nitrate 1.0 g/L, ferric chloride 0.05 g/L)[4] amended with 1.6% (v/v) Arctic diesel and were incubated for 4 weeks. The *Dietzia maris* cells were nonmotile as examined in the test tubes containing nutrient broth, 0.2% agar and 5 mL of 1% solution of triphenyltetrazolium chloride (TTC).[5] Bacterial culture was grown at 25 ˚C in a 150-mL flask containing 75 mL mineral medium of BH and 1% v/v n-hexadecane as carbon source. After four days, the bacterial solution was centrifuged at 5000 g for 10 min. The precipitated cells were washed twice with the same BH mineral solution to remove hexadecane droplets before introducing to the cells.

Microbial adhesion to hydrocarbon (MATH) test is commonly used to determine the hydrophobicity of bacterial cell surfaces.[6] The optical density (OD at 600 nm) of bacterial solution was first determined. Then, after mixing with hexadecane and vortexing, the solution was kept for 15 min. without mixing to allow cells to adhere to the hexadecane layer. The OD of bulk solution was measured again and percent of adhered cells were

determined. Significant attachment to oil layer and no settling of cells were visually observed.

The pore densities of the membranes with 12 μm, 8 μm, 3.0 μm and 0.4 μm pore diameters were 1.1×10^5, 1.0×10^5, 2×10^6, and 1.01×10^8 pores/cm^2, respectively. The membrane thickness were approximately 9 μm. The contact angle of the membrane was imaged and measured using a goniometer (VCA optima XE, AST products Inc., MA, USA). Measurements were made on two opposite sides of 1 μL sessile water droplets. The results are reported as average of three equilibrium contact angles.

An inoculum of washed cells (1.5×10^8 CFU/mL) in 13 mL BH mineral medium was introduced into the lower chamber of bioreactor through the side arm. After inoculation, 20 μL of radiolabeled hexadecane was introduced at the air-water interface of the top chamber of the bioreactor. The added hexadecane partially covered the aqueous phase-air interface. Sterile techniques were used in handling the bioreactors and experiments were repeated three times.

The biodegradation kinetics of hexadecane was determined by monitoring $^{14}CO_2$ production rate. The basic NaOH solution from traps was sampled at sampling days, was mixed thoroughly and then three subsamples were taken and were transferred to scintillation vials containing *Ultimagold* scintillation cocktail (Perkinelmer), and finally activation was measured using a LS3600 scintillation counter (Beckman Coulter).

Scanning Electron Microscopy: For environmental scanning electron microscopy (ESEM), a droplet of bacterial suspension sampled from bottom chamber of the bioreactors was manually placed on the nuclepore membrane and were immediately

imaged to minimize the dehydration of sample during imaging on a FEI ESEM Quanta 450 FEG at low vacuum mode.

For conventional SEM, bacterial solution sample was first rinsed with PBS buffer solution (0.1x), and then the solution of 4% formaldehyde in PBS was added to the sample for fixation. The sample was then placed on poly-L-lysine-coated glass cover slips. After fixation, samples were dehydrated and dried by rinsing with graded anhydrous ethyl alcohol bath and critical point drying (CPD, Leica EM CPD 030). The dried sample was coated with 3 nm platinum in sputter coater (Leica EM ACE 600). Samples were imaged using FEI Inspect F50 at 5 kV.

Literature Cited

1. Chang, W.; Dyen, M.; Spagnuolo, L.; Simon, P.; Whyte, L.; Ghoshal, S. Biodegradation of semi- and non-volatile petroleum hydrocarbons in aged, contaminated soils from a sub-Arctic site: Laboratory pilot-scale experiments at site temperatures. *Chemosphere* **2010,** *80* (3), 319-326.

2. Akbari, A.; Ghoshal, S. Pilot-scale bioremediation of a petroleum hydrocarbon-contaminated clayey soil from a sub-Arctic site. *J. Hazard. Mater.* **2014,** *280* (0), 595-602.

3. Oh, W.; Lindquist, W. B. Image thresholding by indicator kriging. *Pattern Analysis and Machine Intelligence, IEEE Transactions on* **1999,** *21* (7), 590-602.

4. Bushnell, L. D.; Haas, H. F. The Utilization of Certain Hydrocarbons by Microorganisms. *J. Bacteriol.* **1941,** *41* (5), 653-673.

5. Reller, L. B.; Mirrett, S. Motility-indole-lysine medium for presumptive identification of enteric pathogens of Enterobacteriaceae. *J. Clin. Microbiol.* **1975,** *2* (3), 247-252.

6. Abbasnezhad, H.; Gray, M.; Foght, J. Influence of adhesion on aerobic biodegradation and bioremediation of liquid hydrocarbons. *Appl. Microbiol. Biotechnol.* **2011,** *92* (4), 653-675.