Electronic Supplementary Material (ESI) for Environmental Science: Processes & Impacts

SUPPORTING INFORMATION

Jean M.F. Martins*, Samer Majdalani[#], Elsa Vitorge, Aurélien Desaunay, Aline Navel, Véronique Guiné, Jean François Daïan, Erwann Vince, Hervé Denis and Jean Paul Gaudet

Role of macropore flow in the transport of *Escherichia coli* cells in undisturbed cores of a brown leached soil

Laboratoire d'Etudes des Transferts en Hydrologie et Environnement (LTHE, UMR 5564) – CNRS-INSU / Univ. Grenoble I / INPG / IRD – Domaine Universitaire BP53 - 38041 Grenoble Cedex 9, France

* Corresponding author: jean.martins@ujf-grenoble.fr, Tel.: +33476635604

Present address: HydroSciences Montpellier, UMR 5569, Université Montpellier 2, CC MSE, 34095 Montpellier Cedex 5, France.

The supporting information section contains 7 text pages including 3 figures.

MATERIAL AND METHODS

Characterization of bacterial cells properties

Escherichia coli strain K12 MG1655 cells were grown overnight at 37°C in liquid Luria Bertani medium supplemented with ampicillin (100µg mL^{-1}) for cell selection. After growth, the cells were harvested by centrifugation at 9000g and rinsed twice in deionised water (DIW). The bacterial cells were then resuspended in DIW or KBr solution at 8 10^{-3} or 10^{-1} M, for further analysis.

Bacterial size

The size distribution of *E. coli* cells was characterized with a Malvern Mastersizer 2000 Laser Granulometer, equipped with a variable-speed fluid module that measured the size suspended cells through their diffraction of laser light into 52 photodiode detectors. The instrument routinely measures particles size in the range 0.02 to 2000 µm. The technique is nondestructive and rapid, with a sample processing time of 3 to 4 min. (Milfont et al. [1]). The basic operating procedure requires samples to be dispersed in water to form slurry into the fluid module. The analysed samples corresponded to bacterial cells suspended in DIW or KBr solution at 8 10^{-3} or 10^{-1} M. Results on the size distributions (equivalent spherical diameter) of *E. coli* cells, measured by Dynamic Light Scattering (DLS), are presented in Figure S1. The coefficient of uniformity of the cells was 1.96, indicating a quite high uniformity of the size of the injected cells. The d_{50} parameter of the cells determined by DLS was 1.14 µm +/- 0.02, whatever the ionic strength tested (DIW and 8.10^{-3}M or 0.1 M KBr), indicating that no significant cell aggregation occurred, even at the highest ionic strength. This result was confirmed by TEM observations and image analysis (data not shown) that revealed a majority of isolated cells and permitted to determine the average lengths of the major and minor axes of *E. coli* cells: length = 2.1 µm ±0.2 µm and width = 0.6 µm ±0.1 µm.

Bacterial electrophoretic mobility (Zeta potential)

Electrophoretic mobility measurements, also referred to as Zeta potential, ζ, were performed in triplicate in the pH range 2.5 to 9.0 (10 mM sodium salt solutions) with a Nano ZS zetameter (Malvern Instruments). The electrophoretic mobility of bacterial cells is expressed as a potential

(mV) given by the calibrated Zetameter device. These measurements permitted the determination of the cell isoelectric point (pH_{IEP} mV), i.e. the pH of assumed null electrophoretic mobility and consequently null particle charge. At this pH the surface charge and electrical double layer are both neutralized within the limits of experimental significance. Consequently, $pH_{IEP} \sim pH_{PZNPC}$ (Point of Zero Net [no metals] Proton surface Charge). The graphically determined pH_{PZNPC} situates around pH 3.3 (Fig. S2), evidencing that the bacterial cells are negatively charged in the acid-base titration pH range (pH 4 to 10), as already demonstrated for most bacteria (e.g. Guiné et al. [2]).

Bacterial interaction parameters and energy profiles

The measured Zeta potentials of the cells and the soil constituents permitted to calculate DLVO interaction parameters (Table 3) and interaction energy profiles (Figure S3) at the 3 ionic strengths tested using the approach proposed by Redman et al. [3] The Hamaker constant was set to $1.20\ 10^{-20}$ J. The results showed that in both DIW and $8\ 10^{-3}$ M KBr solution, energy barriers exist (Φ_{max}), which limit interactions of the cells with the soil constituents. However, in both conditions a secondary minimum of energy of variable height (Φ_{max}) and depth (Φ_{min2}) exists that may retain bacterial cells in the soil, especially at the intermediate ionic strength. At 0.1 mM KBr, no energy barrier exists indicating the potential occurrence of strong interactions between cells and soil constituents.

References

1. M.L. Milfont, J.M.F. Martins, A.C.D. Antonino, E. Gouveia, V. Guiné, H. Mas, A.M. Netto, M.B. S. Freire. *J. Environ. Qual*. 2008, **37**, 90-97.
2. V. Guiné, L. Spadini, M. Muris, G. Sarret, C. Delolme, J.P. Gaudet and J.M.F. Martins. *Environ. Sci. Technol*. 2006, ***40***, 1806-1813.
3. J. A. Redman, S. L. Walker and M. Elimelech, *Environ. Sci. Technol.* 2004, ***38***, 1777-1785.

FIGURE CAPTIONS

Figure S1

Size distribution of *Escherichia coli* DH5α cells measured in deinonized water and in 0.008 and 0.1M KBr solutions. Measurements were done in triplicate (error bars corresponding to standard deviations are in symbol size range) with a Mastersizer 2000 Granulometer (Malvern LTD).

Figure S2

Evolution of the zeta potential (ζ) of *Escherichia coli* cells with pH measured in de-ionized water. The square and triangle symbols are the zeta potential of the cells measured in 0.008 and 0.1 M KBr solutions, respectively. Error bars correspond to the standard deviation calculated with triplicates. The measurements were done with a Nano ZS zetameter (Malvern Instruments).

Figure S3

Calculated DLVO energy profiles of interactions between *E. coli* cells and LCSA soil constituents using a Hamaker constant of 1.20 10^{-20} J. [3] The corresponding parameters are summarized in Table 3

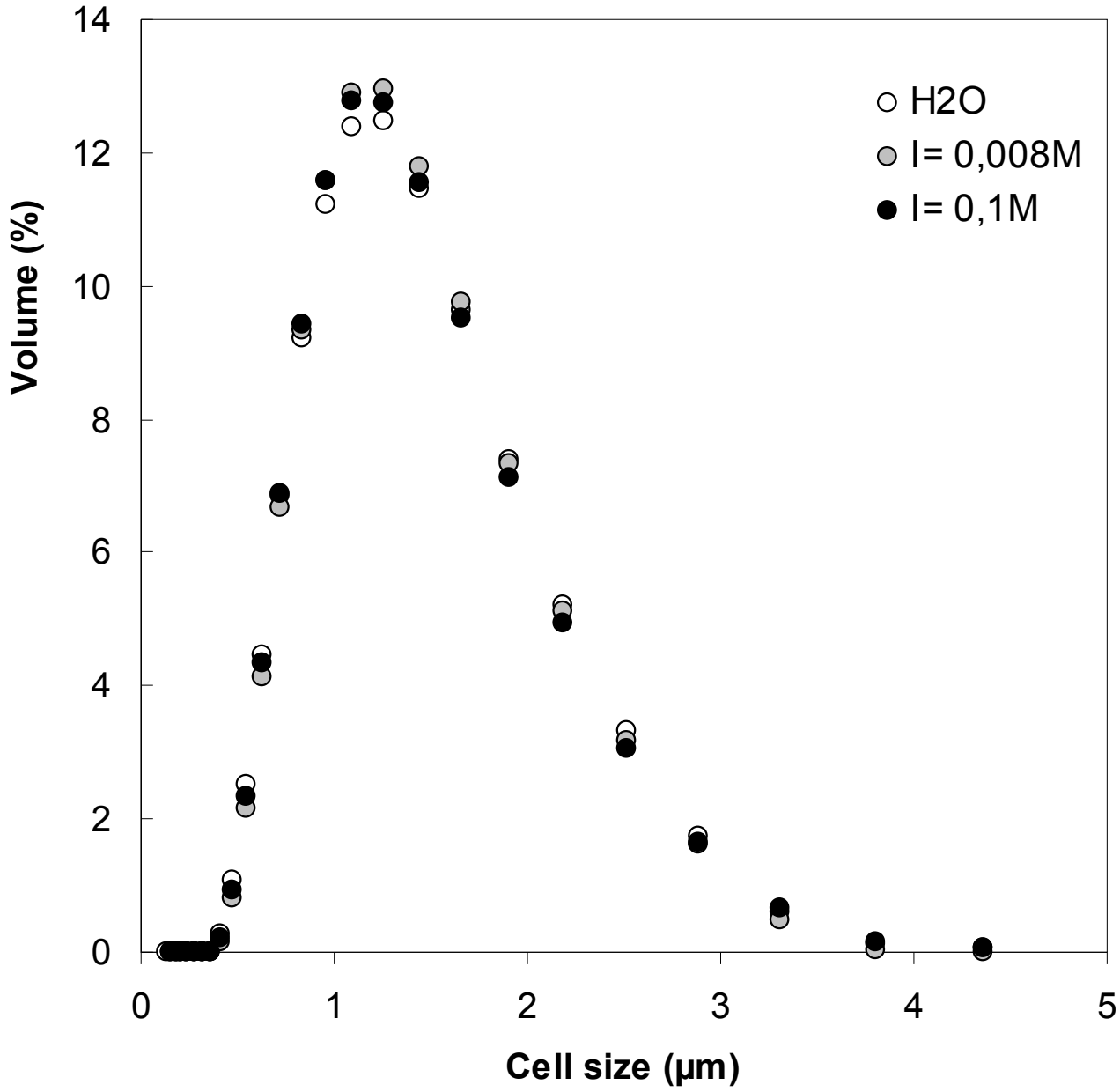

Figure S1

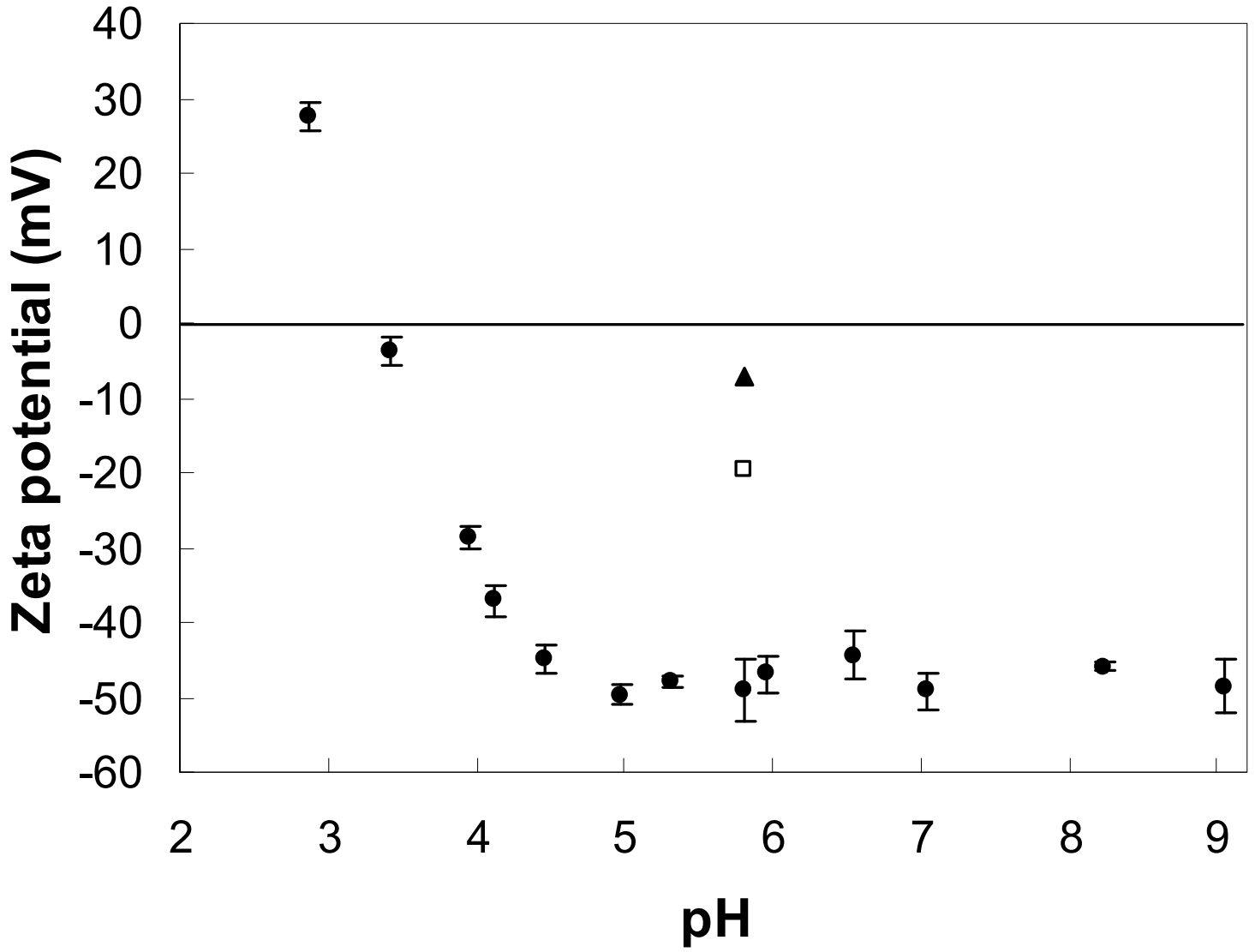

Figure S2

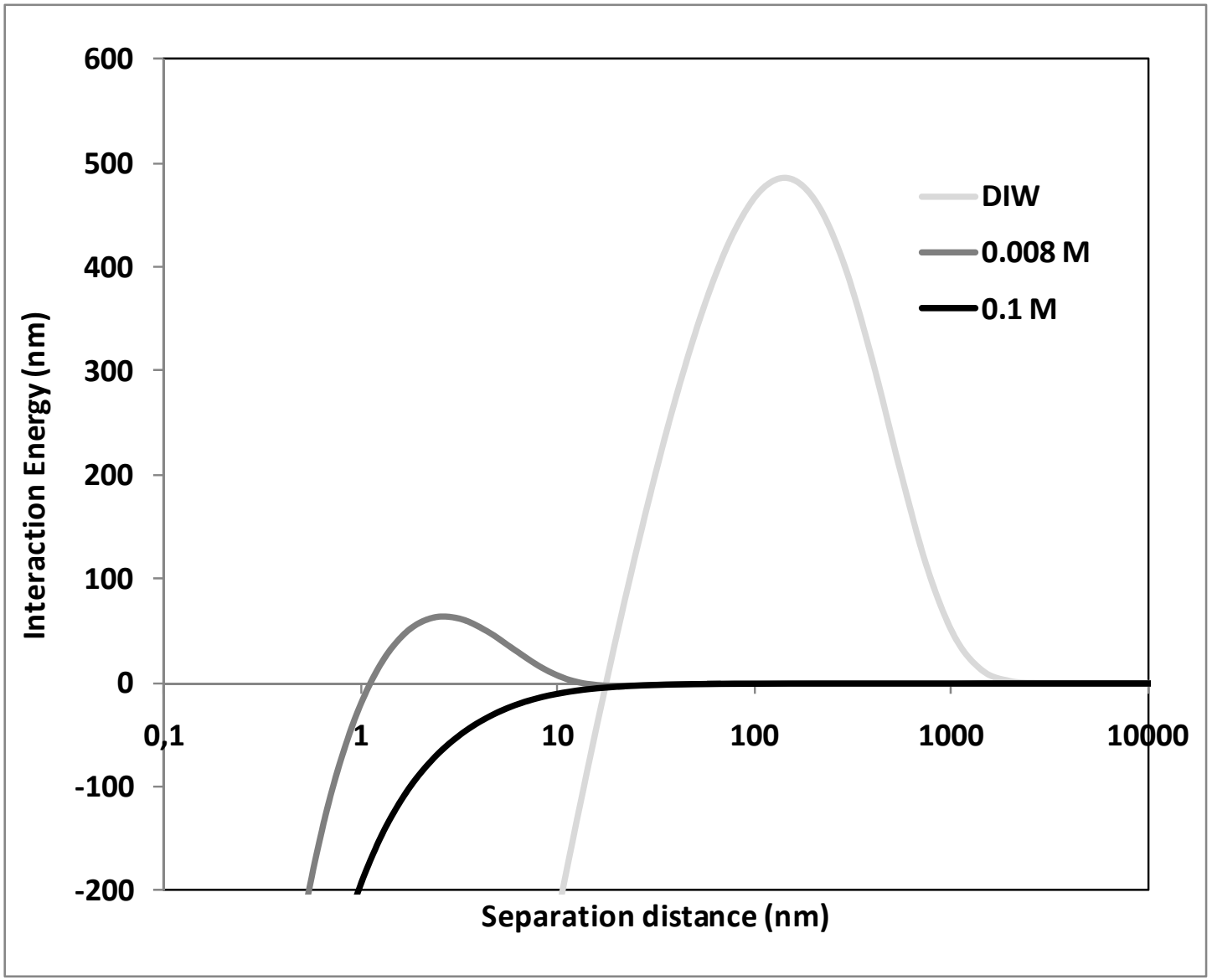

Figure S3