Bacteria in sediments: pore size effects

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Field data show that cell counts decrease with sediment depth. Suggested limiting factors include age, high temperature, low porosity and a lack of water, nutrients or energy. This letter shows that pore size distribution, rather than porosity, can properly account for the reduction in population with depth. Clay mineralogy controls the constitutive parameters that define pore size; therefore, the sediment mineralogy must be properly considered in the analysis of bioactivity in sediments.

INTRODUCTION

Specimens recovered from deep boreholes show the presence of life at depths in excess of 800 m. However, cell counts decrease with depth (Parkes *et al.*, 1994; Wellsbury *et al.*, 2002; Schippers *et al.*, 2005). Limiting factors suggested to explain this trend include age, high temperature, lack of water, limited nutrients or energy, and low porosity (Parkes *et al.*, 2000; Mitchell & Santamarina, 2005; Jorgensen & D'Hondt, 2006). Pore and pore-throat sizes restrict habitable pore space and traversable interconnected porosity, and sediment-cell interaction may cause puncture or tensile failure of the cell membrane. Analyses and experimental data show that bacteria size relative to sediment pore size, and granular forces relative to the organism's puncture resistance, define three distinct regions in a particle size versus sediment depth plot, where bacteria (1) remain 'active and motile', (2) are 'trapped' or (3) suffer 'membrane puncture' (Rebata-Landa & Santamarina, 2006).

Pore size is not uniform in sediments. Therefore, the viability of life in sediments is related to the probability of finding pores large enough to house micro-organisms. This letter shows that pore size distribution, rather than porosity, can account for changes in bioactivity as a function of grain size and depth.

ANALYSIS

Macroscale porosity versus depth

The porosity n of a sediment decreases with depth due to an increase in vertical effective stress σ'. In terms of the void ratio $e=n/(1-n)$, the sedimentation compression curve can be expressed as (Skempton, 1970; Burland, 1990)

$$e = e_{100} - C_c \log\left(\frac{\sigma'}{100\,\text{kPa}}\right) \text{ at depth } z \qquad (1)$$

where e_{100} is the void ratio at $\sigma'=100$ kPa and C_c is the sediment compressibility inferred from the in situ sedimentation compression curve. These two constitutive parameters e_{100} and C_c correlate with the sediment plasticity or specific surface S, as shown in Table 1: high specific surface

montmorillonitic formations have higher e_{100} and C_c values than low specific surface kaolinite and silts. Therefore, the porosity of sediments under equal effective stress decreases with decreasing specific surface, from montmorillonite (highest porosity), to illite, kaolinite and silt (smallest porosity).

Mean pore size

The mean pore size can be estimated from the void ratio e, the specific surface S and the mass density of the mineral that makes the grains ρ

$$\mu_d = k\frac{e}{S\rho} \text{ at depth } z \qquad (2)$$

where the structure factor k depends on the grain shape and the sediment structure (i.e. geometrical arrangement of the grains (see supplementary information)). For platy clay particles, the structure factor varies between $k=2$ (parallel stacking) and $k \approx 6$ (cubic arrangement), and the 'effective' specific surface may reflect less exposed surface in face-to-face aggregated platelets. The mean pore size predicted using equations (1) and (2) for dispersed fabrics is in reverse order compared with the porosity: silt (largest mean pore size), kaolinite, illite and montmorillonite (smallest pores). Note that pore fluid pH and ionic concentration govern fabric formation and affect both e_{100} and C_c and the effective specific surface S.

Variance in pore size

Published mercury intrusion porosimetry data are analysed to determine standard statistical parameters. The results plotted in Fig. 1 show that the standard deviation σ_d increases with mean pore size μ_d so that $\sigma_d/\mu_d \approx 0.4$ (bounds 0.2 and ~1). Measured distributions, the inherently positive nature of pore size and the need to properly capture the tails of the distribution support the use of a lognormal function for general analysis. Statistics for $\ln(d)$ can be computed from linear statistics σ_d and μ_d as follows

$$\mu_{ln} = \ln(\mu_d) - 0.5\left(\frac{\sigma_d}{\mu_d}\right)^2$$

and

$$\sigma_{ln}^2 = \ln\left[1 + \left(\frac{\sigma_d}{\mu_d}\right)^2\right]$$

Table 1. Constitutive parameters for selected sediments - nominal values (Lambe & Whitman, 1969; Skempton, 1970; Burland, 1990; Santamarina et al., 2001; Mitchell & Soga, 2005)

Sediment	e_{100}	C_c	S: m^2/g
Silt	0·6–0·8	0·02–0·09	0·045–1·000
Kaolinite	0·9–1·1	0·2–0·4	10–20
Illite	2–3	0·5–1·1	65–100
Montmorillonite	2·5–4·0	1–2	300–780

which simplifies to

$$\sigma_{\ln}^2 \approx \left(\frac{\sigma_d}{\mu_d}\right)^2$$

for low σ_d/μ_d values (Ang & Tang, 1975). These relationships are corroborated by fitting porosimetry data using the log-normal distribution.

Probability of large voids
The probability of finding voids equal to or larger than the size of micro-organisms $d \geqslant b$ is computed assuming the log-normal distribution

$$P(d \geq b) = \frac{\mu_d}{\sigma_d} \frac{1}{(2\pi)^{1/2}} \int_{\ln(b)}^{\infty} \exp\left\{-\frac{1}{2}\left[\frac{\mu_d}{\sigma_d}\ln\left(\frac{d}{\mu_d}\right) + \frac{1}{2}\frac{\sigma_d}{\mu_d}\right]^2\right\} dd \quad (3a)$$

For $\sigma_d/\mu_d \approx 0\cdot 4$, this simplifies to

$$P(d \geq b) \cong \int_{\ln(b)}^{\infty} \exp\left\{-\left[\frac{2.5}{4}\ln\left(\frac{d}{\mu_d}\right) + 0.05\right]^2\right\} dd \quad (3b)$$

These equations allow us to estimate the probability of voids being equal to or larger than the micro-organisms' size $b = 1\,\mu m$ at depths between 0·1 m and 1000 m: $P(d \geqslant 1\,\mu m) \approx 1\cdot 0$ in silt, decreases from 10^{-2} to 10^{-5} in kaolinite, from 10^{-3} to 10^{-10} in illite and from 10^{-5} to 10^{-12} in montmorillonite.

Cell count per unit volume of sediment
The total cell count in the sediment c_{sed} (cells/ml of sediment) is restricted by the cell concentration in the fluid c_{fl} that fills pores larger than size b and by the sediment porosity n
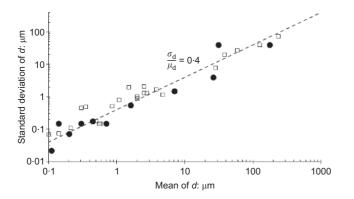

Fig. 1. Mercury porosimetry data: pore size mean and standard deviation. Squares, remoulded specimens; circles, natural sediments. Data compiled from Garcia-Bengochea et al. (1979), Juang & Holtz (1986), Griffiths & Joshi (1990), Simms & Yanful (2001), Tanaka et al. (2003), Jones et al. (2007), Ninjgarav et al. (2007), Angeli et al. (2008), Monroy et al. (2009) and Delage (2010)

$$c_{sed} = c_{fl}nP(d \geq b) = c_{fl}\frac{e}{1+e}P(d \geq b) \quad \text{at depth } z \quad (4)$$

Cell count versus depth
Equations (1)–(4) allow us to compute the variation in cell count with depth for different sediments. Figure 2 presents the results computed by assuming a constant cell count in the pore fluid equal to $c_{fl} = 10^{10\cdot 5}$ cells/ml and sediment constitutive parameters in agreement with those listed in Table 1. The shaded band represents the data reported by Parkes et al. (1994, 2000).

CLOSING COMMENTS
The analysis and the trends in Fig. 2 highlight the importance of pore size rather than porosity on the viability of life in
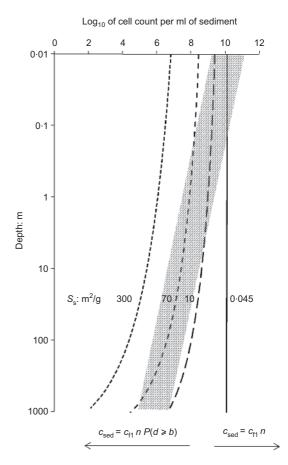

Fig. 2. Cell count in sediments c_{sed} as a function of depth for different sediment mineralogies. Assumed parameters: standard deviation $\sigma_{\ln} = 0\cdot 4$ (for all deposits; refer to Fig. 1), cell counts in pore fluid $c_{fl} = 10^{10\cdot 5}$ cells/ml. Sediments: coarse silt-like ($S = 0\cdot 045$ m^2/g, $e_{100} = 0\cdot 7$, $C_c = 0\cdot 02$); low-plasticity clay (e.g. kaolinite, $S = 10$ m^2/g, $e_{100} = 1\cdot 0$, $C_c = 0\cdot 35$); high-plasticity clay (e.g. illite, $S = 70$ m^2/g, $e_{100} = 2\cdot 4$, $C_c = 0\cdot 91$); very high plasticity clay (e.g. montmorillonite, $S = 300$ m^2/g, $e_{100} = 3\cdot 25$, $C_c = 1\cdot 26$). The shaded area represents data reported by Parkes et al. (1994, 2000)

sediments. Anomalous high cell counts reported by Parkes *et al.* (1994) may correspond to silt and sand seams in which pore size is not the limiting factor or to sediments with dual-porosity fabric (i.e. high pore size variability (not reported in Fig. 1)).

If high pore size variability is assumed, such as the upper bound in Fig. 1, $\sigma_d/\mu_d \approx 1$, the computed cell counts exceed those found in situ. In those cases, pore size is not the limiting factor and other factors will control bacterial activity (e.g. water, nutrients, energy, temperature).

For a given pore fluid chemistry, clay mineralogy determines the three parameters that define pore size at a given depth: e_{100}, C_c and S in fine-grained sediments (equations (1) and (2)). On the other hand, a small fraction of fines is sufficient to fill the porosity in silts and sands, and limit bioactivity in these soils. Let us define the 'critical fines content' FC* as the ratio of the weight of fines w_f (packed at void ratio e_f) to the weight of coarse grains w_c (at void ratio e_c) when the fine sediment fills the voids in the coarse-grain structure

$$\text{FC} * = \frac{w_f}{w_c} = \frac{e_c}{1+e_f} \tag{5}$$

Assuming $e_c = 0.6$ and e_f equal to the void ratio at the liquid limit, the critical fines content ranges from FC* $\sim$ 20% for kaolinite to less than 8% for montmorillonite. Consequently, fines content and mineralogy/plasticity must be considered in analyses of bioactivity in both fine- and coarse-grained sediments.

Acknowledgements

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