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Temporal and spatial dynamics of blocking and ripening effects on bacterial transport through a porous system: A possible explanation for CFT deviation

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Abstract

The studies on transport of particles across porous systems are based on the Colloid Filtration Theory (CFT). According to CFT, the collision efficiency is constant along the system length [J.N. Ryan, M. Elimelech, Colloids Surf. A: Physicochem. Eng. Aspects 107 (1996) 1–56]. Decreasing values of collision efficiency have been reported, a phenomenon that has been interpreted as a deviation from the CFT [X. Li, T.D. Scheibe, W.P. Johnson, Environ. Sci. Technol. 38 (2004) 5616–5625; N. Tufenkji, J.A. Redman, M. Elimelech, Environ. Sci. Technol. 37 (2003) 616–623; N. Tufenkji, M. Elimelech, Langmuir 20 (2004) 10818–10828; N. Tufenkji, M. Elimelech, Langmuir 21 (2005) 841–852]. This paper presents data on transport of *Bacillus megaterium* spores through quartz sand columns. The occurrence of consecutive phases of increase and decrease of the values of C/C_0, the effluent spore concentration expressed as a fraction of the influent spore concentration, is reported. These patterns of change in C/C_0 were interpreted as the result of the concomitant occurrence of blocking and ripening, the prevalence of these phenomena in different moments of the experiment, and the spatial distribution of the prevalence of blocking and ripening effects along the porous system. It is argued that this spatial distribution in the predominance of blocking and ripening, what leads to the intensification of ripening at the entrance of the porous system, might be a possible explanation for the reported deviation from the CFT for experimental conditions where ripening and blocking take place.

Keywords: Adhesion; Bacteria; Transport; *Bacillus megaterium*; Quartz sand

1. Introduction

According to the Colloid Filtration Theory (CFT), the efficiency of colloid collision (α) is constant throughout the length of a porous system [1]. This is only valid under favorable adhesion conditions, such as in experiments with colloids suspended in high ionic strength solutions [2,5].

Under unfavorable adhesion conditions, the collision efficiency diminishes along the porous system, a phenomenon interpreted as a deviation from the CFT [2–5]. In experiments involving the transport of bacterial cells, various reasons for the deviation from the CFT have been discussed at present, such as differences of adhesion among the cells in a population and the occurrence of ripening [2–5].

Bacterial cells may adhere not only to inert particles in a porous system, but also to each other. This phenomenon, known as ripening, affects cell transport through a porous medium [6]. Ripening occurs when attached particles, the cells, act as additional collecting particles for attachment of cells moving through the porous system [6]. Ripening can be seen as a progressive decrease in cell concentration in the effluent [6].

Another phenomenon observed during the transport of bacterial cells through porous media – blocking – occurs after some cells adhere to the surface of the inert particles that compose the porous medium [7–11]. Once a cell attaches to the surface of a particle, it blocks the attachment of other cells to the surface of the particle by an area greater than its projected area [7]. Blocking is evidenced by an increase in cell concentration in the effluent [7–11].

Collision efficiency (α) can be measured using either values of C/C_0 (ratio of cell concentration in the effluent to the cell concentration in the influent) or the number of cells retained in the porous system after the passage of a cell suspension [7,10].

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The numbers of cells retained in different parts of a porous system can be used to calculate α_i, or the collision efficiency for a specific section of the system. Decreasing α_i values along porous media have been reported [10]. Camesano and Logan [10] found a decrease of α_i along the porous system. This is interpreted as the occurrence of ripening. At the same experiment, those authors observed that α was inversely related to the cell concentration injected in different porous systems, an evidence of blocking. However, those authors expected a direct relationship between α and C_0, taking in account the observed decrease of α_i along each porous system. Those authors considered these data inconsistent and could not explain the phenomenon.

The consecutive occurrence of a period of increase, followed by a period of decrease and another one of increase in C/C_0 values was observed [7]. However, the mathematical model used by the authors to explain the data [7] did not take in account the alternation of increase and decrease of C/C_0 values with time.

In this study, we present data supporting a model which considers the simultaneous occurrence of blocking and ripening in the same porous system, under unfavorable adhesion conditions. We propose that either blocking or ripening predominate at different moments, when the porous system is considered as a whole. However, considering the different regions of the system, both phenomena predominate simultaneously, albeit in different sections. An increased ripening at the entrance of the system is proposed to explain CFT deviation for experimental conditions where ripening and blocking take place.

2. Material and methods

Bacillus megaterium LBBMA65 was grown in R2A medium [12], harvested at mid log-growth phase, and washed three times by centrifugation at 2000 × *g* in 0.85% saline. Finally, the cells were re-suspended in the same solution. An aliquot was transferred to 100 mL of minimal medium lacking $(NH_4)_2SO_4$, to a $DO_{600\,nm}$ of 0.02. The cell suspension was incubated at 20 °C and 150 rpm in a rotary shaker for 5 days, to obtain endospores. These were harvested by centrifugation at 2000 × *g* and re-suspended in sterile Phosphate buffered saline (PBS) at pH 7.1. For the different treatments (T1 and T2), the spore concentration was 1.2×10^6 CFU mL^{-1} and 1.6×10^6 CFU mL^{-1}.

The porous medium consisted of quartz sand (150–200 μm), dry packed into 150 mm × 25 mm glass tubes. These were capped with metallic caps and attached to 1 mm inner diameter tygon tubes. The columns were conditioned with 2 pore volumes (PV) of PBS prior to each experiment. Five PV of spore suspension in PBS, followed by 2 PV of PBS, were injected into the porous system in an upward flow at 0.1 mL min^{-1}. Effluent was collected in sterile glass vials every 30 min. Three 10 μL aliquots of suitable dilutions of the effluent fractions were plated on Nutrient Agar added of 20 g L^{-1} NaCl, and incubated at 28 °C until colonies could be visible and counted.

The cell concentration (C), measured as CFU mL^{-1}, in different samples of the effluent was divided by the cell concentration in the injected suspension (C_0). The mean values of C/C_0, with standard deviations, were plotted against time, expressed as PV.

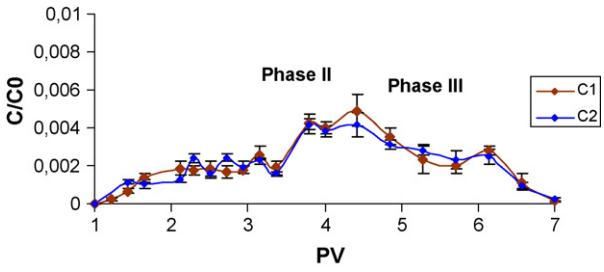

Fig. 1. *B. megaterium* LBBMA65 spore transport through quartz sand columns in phosphate buffered saline (PBS), plotted as C/C_0. Curves represent treatments with different cell concentrations: T1 (1.2×10^6 CFU mL^{-1}) and T2 (1.6×10^6 CFU mL^{-1}). Five PV of spore suspensions, followed by 2 PV of PBS were injected, and transported spore concentration (C) was measured and recorded as a fraction of the initial spore concentration (C_0). Phases II and III refer to those of "blocking predominance", and "ripening predominance", respectively, as discussed in the text.

3. Results and discussion

The transport of *Bacillus megaterium* LBBMA65 endospores through a porous medium composed of quartz sand is characterized by a period of stabilization in C/C_0, followed by a period of increase and finally by a period of decrease in C/C_0 (Fig. 1). The hereby proposed theoretical interpretation of these data is schematically represented in Fig. 2 and is composed of four assumptions: (i) the simultaneous occurrence of blocking and ripening; (ii) the existence of a liquid effect of the predominance of the interaction between endospores and clean quartz surface, followed by the predominance of blocking and finally of ripening on C/C_0 values; (iii) the predominance of these phenomena at specific sections of the porous system in different moments during the transport; and (iv) simultaneous predominance of both blocking and ripening in the same column, but in different sections.

Considering that bacterial cells or spores may be involved in blocking and ripening, it is plausible to expect that both phenomena may happen simultaneously under certain conditions.

The observed pattern of a phase of increase (Phase II, Fig. 1) followed by a phase of decrease of C/C_0 (Phase III, Fig. 1) supports an interpretation by which blocking and ripening have predominant effects on C/C_0 values at different moments, under our experimental conditions. In Fig. 2, three phases are schematically drawn. We propose an interpretation of the C/C_0 data (Fig. 1) based on the occurrence of these three phases, as follows:

Phase I: Clean bed system, during which α depends on the interactions between the cells and the porous system surfaces (Fig. 2, Phase I). It cannot be concluded that the phase of constant C/C_0 values on Fig. 1 is related to a clean-bed filtration, since the data suggest removal greater than 99%. Theoretically, it is necessary to consider that a phase of clean-bed filtration is the first one to occur, even if it is transient and cannot be detected in the C/C_0 values, since the first cells flowing on a system encounter the majority of the surfaces clean. Considering this theoretical assumption, this phase will be referred as Phase I.