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Analysis of the contribution of sedimentation to bacterial mass transport in a parallel plate flow chamber

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ABSTRACT

In order to investigate bacterium–substratum interactions, understanding of bacterial mass transport is necessary. Comparisons of experimentally observed initial deposition rates with mass transport rates in parallel-plate-flow-chambers (PPFC) predicted by convective-diffusion yielded deposition efficiencies above unity, despite electrostatic repulsion. It is hypothesized that sedimentation is the major mass transport mechanism in a PPFC. The contribution of sedimentation to the mass transport in a PPFC was experimentally investigated by introducing a novel microscopy-based method. First, height-dependent bacterial concentrations were measured at different times and flow rates and used to calculate bacterial sedimentation velocities. For *Staphylococcus aureus* ATCC 12600, a sedimentation velocity of 240 $\mu m\,h^{-1}$ was obtained. Therewith, sedimentation appeared as the predominant contribution to mass transport in a PPFC. Also in the current study, deposition efficiencies of *S. aureus* ATCC 12600 with respect to the Smoluchowski–Levich solution of the convective-diffusion equation were four-to-five fold higher than unity. However, calculation of deposition efficiencies with respect to sedimentation were below unity and decreased from 0.78 to 0.36 when flow rates increased from 0.017 to 0.33 $cm^3\,s^{-1}$. The proposed analysis of bacterial mass transport processes is simple, does not require additional equipment and yields a more reasonable interpretation of bacterial deposition in a PPFC.

1. Introduction

Bacterial adhesion occurs to virtually all surfaces exposed to an aqueous environment and leads to the subsequent growth of a biofilm. Biofilms can be found nearly everywhere, such as in groundwater, pipelines in waterworks, wastewater treatment plants, on the surfaces of heat exchanger plates and biomedical devices, and on ship hulls [1–5]. Flow displacement systems are widely used to study bacterial adhesion to surfaces [6–10] as they allow control of hydrodynamic conditions, including shear rates and mass transport.

Bacterial mass transport in flow displacement systems is generally established by a combination of convection, diffusion and sedimentation. Previous studies on bacterial mass transport in a parallel plate flow chamber (PPFC) have adopted the Smoluchowski–Levich (SL) approximation of the two-dimensional convective-diffusion equation to predict mass transport in the absence of gravitational, colloidal and hydrodynamic interactions [11] to either the top or bottom plate of a chamber [12–14]. In the SL approximation, it is assumed that the substratum surface acts as a perfect sink (i.e. every particle or bacterium that arrives at the surface adheres irreversibly). Accordingly, the ratio between the experimentally observed initial deposition rate, j_0, and the bacterial mass transport rate calculated by SL approximation, $j_0{}^*$, indicates the fraction of bacteria arriving at the surface that manages to adhere successfully under the influence of the interaction forces between arriving bacteria and the substratum surface. For this reason, the ratio $j_0/j_0{}^*$ is also referred to as "deposition efficiency".

Electrostatic repulsion usually exists between bacterial cell surfaces and most substratum surfaces [15,16], which negates the assumption made in the SL approximation that the substratum surface should act as a perfect sink. Hence, deposition efficiencies with respect to the SL approximation should be smaller than unity. However, values in excess of unity have been reported for different bacterial strains and species. *Streptococcus cricetus* showed a high deposition efficiency of almost 2 to the bottom polymethylmethacrylate plate of a PPFC [12], while also *Streptococcus thermophlius* B to the bottom glass plate of a PPFC had a deposition efficiency in excess of unity [13]. Deposition efficiencies of six coagulase-negative staphylococcal strains to negatively

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charged acrylate bottom plates were as high as 1.4 [14]. Deposition efficiencies in excess of unity have been largely attributed to the complex structural and chemical nature of bacterial cell surfaces and it has even been envisaged that nanoscopic surface appendages protruding from bacterial cell surfaces penetrate the energy barrier between two negatively charged surfaces to give strong primary minimum adhesion. However, these explanations are largely hypothetical, while a much more simple explanation might be that the mass transport rate as derived from the SL approach with respect to which the deposition efficiency is calculated, underestimates the actual mass transport rate towards the substratum.

Therefore, in order to gain a more comprehensive understanding of bacterial mass transport in a PPFC we present a new microscopy-based experimental method to determine the contribution of sedimentation to bacterial mass transport. The method is illustrated for a staphylococcus strain, but is equally applicable to other bacterial strains as well as to inert colloidal particles and does not require additional equipment.

2. Materials and methods

2.1. Bacterial strain and culture conditions

S. aureus ATCC 12600 used in this study was physico-chemically characterized previously [17] and cultured aerobically from a blood agar plate in 10 mL Tryptone Soya Broth (OXOID, Basingstoke, England) at 37 °C. After 24 h, several colonies were used to inoculate 200 mL Tryptone Soya Broth for 16 h at 37 °C, and harvested for use. Bacteria were pelleted by centrifugation (Beckman J2-MC centrifuge, Beckman Coulter, Inc., Brea, CA, USA) for 5 min at 6500 rpm via a JA14 rotor (7000 × *g*) and resuspended in 10 mL PBS (5 mM K_2HPO_4, 5 mM KH_2PO_4, 0.15 M NaCl, pH 7.0). Centrifugation was done twice in order to remove all traces of growth medium. To break bacterial aggregates, sonication at 30 W (Vibra Cell Model 375, Sonics and Materials Inc., Danbury, CT, USA) was applied (3 times 10 s), while cooling in an ice/water bath. Finally, bacteria were resuspended in PBS to a concentration of 3×10^8 mL^{-1}, as determined in a Bürker-Türk counting chamber.

2.2. Substratum surface and parallel plate flow chamber

Glass slides (7.6 cm × 2.6 cm × 0.1 cm, Menzel-Glaser, Menzel GmbH & Co KG, Germany) constituted the top and bottom plates of the PPFC. Prior to use, slides were sonicated for 3 min in 2% RBS35 (Omnilabo International BV, The Netherlands) followed by thorough rinsing with tap water, demineralized water, methanol, tap water and finally demineralized water again to obtain a hydrophilic surface with a 0° water contact angle and a zeta potential by streaming potentials in PBS of −8 mV. The top and bottom glass plates of the chamber on which bacterial adhesion was observed, were placed in the middle of a stainless steel frame (17.5 cm × 4 cm) and are separated by a Teflon spacer creating a flat channel with a length of 17.5 cm, a height of 0.058 cm and a width of 1.70 cm and a gradually diverging and converging (62°) inlet and outlet region [10,13]. In this configuration, an established flow develops within 2–3 cm from the inlet [10] under laminar conditions. No microscopic flow disturbances were observed due to the steel–glass junction, as judged from the absence of irregular bacterial movements along the surface.

Prior to use, the flow chamber was washed with 2% Extran (Merck, Germany) and rinsed thoroughly with tap water and demineralized water before mounting clean glass slides in the chamber. The flow chamber was positioned between two communicating vessels, and the system was filled with PBS while care was taken to remove all air bubbles. In- and outlet vessels of PBS and bacterial suspension were placed at different heights to create a pulse-free flow by hydrostatic pressure. The difference in fluid level between the vessels was maintained by a roller pump between the vessels to ensure a circulating pulse free flow throughout the duration of the experiment.

2.3. Bacterial deposition

Deposition of bacteria was monitored with a phase-contrast microscope (Olympus BH-2) equipped with a 40× ultra long working distance objective (Olympus ULWD-CD Plan 40 PL), connected to a CCD-MXRi camera (Basler A101F, Basler AG, Germany). In order to enhance the signal-to-noise ratio and eliminate moving bacteria from the analysis, 15 images were taken with 0.25 s time intervals, and added to yield a single-time point average. Image analysis was done using proprietary software based on the Matlab Image Processing Toolkit (The Math Works, MA, USA).

The bacterial suspension was allowed to flow through the flow chamber during 1 h at flow rates of 0.017, 0.033, 0.083, 0.17 or 0.33 mL s^{-1}, corresponding with Reynolds numbers (Re), calculated on basis of the hydraulic radius of the channel [10], of 0.95, 1.9, 4.7, 9.5 and 19, respectively. Adhesion was monitored on both top and bottom plates at a distance of 6.2, 8.2 and 11.2 cm from the end of the cylindrical inlet of the stainless steel frame containing the glass slides.

For each flow rate, the number of bacteria adhering per unit area was recorded as a function of time. Adhesion kinetics were expressed in terms of the initial deposition rate j_0 (cm^{-2} s^{-1}), as determined by linear regression analysis of the initial increase in numbers of adhering bacteria per cm^2 with time. In addition, bacterial deposition assays were carried out under stagnant conditions; i.e. the adhering bacteria were monitored when the silicon tubings, connecting the inlet and outlet of the PPFC, were both closed after 20 min of recirculation (0.17 mL s^{-1}) of a bacterial suspension. All experiments were conducted in six-fold, with at least three separately grown bacterial cultures, i.e. two experiments were carried out with one culture.

The initial deposition rate, j_0, was related to the convective-diffusion controlled deposition in a PPFC, calculated using the SL approximation as a solution of the two-dimensional convective diffusion equation, according to [11]:

$$j_0* = \frac{D_\infty c_0}{0.89r}\left[\frac{2}{9}\cdot\frac{bPe}{x}\right]^{1/3} \quad (1)$$

in which D_∞ is the bacterial diffusion coefficient at large distances from the surface, c_0 is the original number concentration of bacteria in the prepared suspension, r is the bacterial radius, x is the longitudinal distance from the flow chamber entrance, b is the half height of PPFC, and Pe is the dimensionless Peclet number defined as

$$Pe = \frac{3Qr^3}{4wb^3D_\infty} \quad (2)$$

in which Q is the applied volumetric flow rate.

2.4. Bacterial sedimentation rates

In this paper, we present a simple, microscopy-based method to determine the gravitational contribution to bacterial mass transport in a PPFC on the basis of bacterial concentration over the height of the flow channel. First, bacterial concentrations in suspension are measured at different heights in the flow chamber. As a necessary step, the volume of the field of view (FOV) is experimentally determined. Subsequently, the bacterial sedimentation velocity is evaluated under stagnant conditions from the rate of change of