Bacterial deposition in a parallel plate and a stagnation point flow chamber: microbial adhesion mechanisms depend on the mass transport conditions

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Deposition onto glass in a parallel plate (PP) and in a stagnation point (SP) flow chamber of Marinobacter hydrocarbonoclasticus, Psychrobacter sp. and Halomonas pacifica, suspended in artificial seawater, was compared in order to determine the influence of methodology on bacterial adhesion mechanisms. The three strains had different cell surface hydrophobicities, with water contact angles on bacterial lawns ranging from 18 to 85 degrees. Bacterial zeta potentials in artificial seawater were essentially zero. The three strains showed different adhesion kinetics and the hydrophilic bacterium H. pacifica had the greatest affinity for hydrophilic glass. On average, initial deposition rates were two- to threefold higher in the SP than in the PP flow chamber, possibly due to the convective fluid flow toward the substratum surface in the SP flow chamber causing more intimate contact between a substratum and a bacterial cell surface than the gentle collisions in the PP flow chamber. The ratios between the experimental deposition rates and theoretically calculated deposition rates based on mass transport equations not only differed among the strains, but were also different for the two flow chambers, indicating different mechanisms under the two modes of mass transport. The efficiencies of deposition were higher in the SP flow chamber than in the PP flow chamber: 62±4 and 114±28% respectively. Experiments in the SP flow chamber were more reproducible than those in the PP flow chamber, with standard deviations over triplicate runs of 8% in the SP and 23% in the PP flow chamber. This is probably due to better-controlled convective mass transport in the SP flow chamber, as compared with the diffusion-controlled mass transport in the PP flow chamber. In conclusion, this study shows that bacterial adhesion mechanisms depend on the prevailing mass transport conditions in the experimental set-up used, which makes it essential in the design of experiments that a methodology is chosen with mass transport conditions resembling the problem under investigation.

Keywords: biofilms, adhesion mechanisms, deposition efficiency, cell surface hydrophobicity, marine bacteria

INTRODUCTION

Adhesion of marine organisms, such as bacteria, diatoms, protozoa and microalgae, occurs on all materials immersed under seawater for prolonged periods and is the first stage of the formation of a marine biofilm finally covered by invertebrates and seaweed. Marine biofilms pose a serious problem for the ship industry because they add drag, which increases the fuel consumption; for an average US Naval vessel the increase is approximately 20%, costing US$400 more per hour at a speed of 26 knots (48 km h⁻¹) (Cooksey & Wigglesworth-Cooksey, 1995). The biofilm can even damage the surface of the
ship hull by the production of corroding agents that have the ability to penetrate ship coatings (Little et al., 1999).

Numerous studies have been conducted to determine the influence of the physical (Tsibouklis et al., 1999) and toxic (Dempsey, 1981) properties of coatings on adhesion of marine bacteria, but it has seldom been realized that bacterial adhesion to surfaces depends in part on the type of system used to study adhesion in situ (Elimelech, 1994). Both under laboratory conditions (‘slight rinsing’ and ‘dipping’ to remove the notoriously undefined ‘loosely bound’ organisms) and in real life (waves), surfaces with adhering bacteria are exposed to liquid–air interfaces and other hydrodynamic detachment forces. It has been shown theoretically (Leenars & O’Brien, 1989) and experimentally (Gomez-Suarez et al., 2001) that a passing liquid–air interface can remove up to 100% of all bacteria adhering to a substratum surface, depending on the surface properties of the bacterial strain and the substratum. For this reason, several devices have been developed (Adamczyk & Van de Ven, 1984; Adamczyk, 1989), allowing a well-controlled flow and enabling in situ observation of adhering bacteria. However, an extensive comparison of bacterial deposition in different flow devices and whether bacterial adhesion proceeds according to the same mechanisms under different modes of mass transport has not been made.

Theoretical comparisons of different flow devices are possible by solving the so-called convective-diffusion equation (Adamczyk & Van de Ven, 1981; Peters, 1990) describing bacterial mass transport to a surface under flow. Exact analytical solutions of the convective-diffusion equation are often too difficult to obtain, but several approximate solutions exist. In the Von Smoluchowski–Levich approximation attractive Lifshitz–van der Waals forces between a bacterium and a substratum surface are thought to be counterbalanced by the hydrodynamic drag which a bacterium experiences when approaching a substratum surface, while electrostatic interactions are neglected (Brenner, 1961). Consequently, bacterial deposition rates obtained from the Von Smoluchowski–Levich approximation are considered as an upper limit for bacterial mass transport in a given device, although Sjollema and coworkers (Sjollema et al., 1990a) have presented bacterial deposition rates in excess of this theoretical upper limit. Bacterial strains showing such excessively high deposition rates could be identified as fibrillated strains and the conclusion was drawn that fibrils assist bacterial adhesion in a way not accounted for in the Von Smoluchowski–Levich approximation.

Theoretical comparisons of bacterial mass transport in a parallel plate (PP) (Adamczyk, 1989; Sjollema et al., 1989) and a stagnation point (SP) (Dabros & Van de Ven, 1987) flow chamber have demonstrated that mass transport in the PP flow chamber is slow, because convective flow is parallel to the substratum surface, while slow diffusional mass transport brings flowing bacteria toward the substratum surface; in contrast, convective flow is toward the substratum surface in the SP flow chamber as illustrated in Fig. 1. By expressing experimental deposition rates relative to the upper limit for mass transport in the Von Smoluchowski–Levich approximation, a so-called deposition efficiency can be obtained that should theoretically account for the differences between different devices. However, it can also be anticipated that different adhesion mechanisms are operative under the different mass transport conditions. It is becoming increasingly apparent that microbial cell surfaces must be considered as possessing a soft surface layer (Morisaki et al., 1999; Ohshima, 1995; Van der Mei et al., 2000) that may be somewhat compressed during head-on collisions with a substratum surface as in a SP flow chamber, allowing inner regions to interact. In the PP flow chamber, collisions are much gentler and only the outside of a microbial soft surface layer is anticipated to interact, which may yield a different adhesion mechanism.

The aim of this paper is to compare the adhesion in a PP and a SP flow chamber for three marine bacterial strains with different cell surface hydrophobicities on an experimental basis and to compare deposition efficiencies calculated from the Von Smoluchowski–Levich approximation for the two devices.
Bacterial strains and culture conditions. Three marine bacterial strains were selected with different cell surface hydrophobicities, namely the motile rod-shaped bacterium 

**Marinobacter hydrocarbonoclasticus** ATCC 27132, the non-motile spherical bacterium 

**Psychrobacter** sp. SW5H (kindly provided by H. M. Dalton, University of New South Wales, Kensington, Australia) and the non-motile spherical bacterium Halomonas pacifica ATCC 27122. 

**Psychrobacter** sp. SW5H was isolated from surfboard wax after exposure to seawater off Wanda Beach, Sydney (Dalton *et al.*, 2000). To preserve the strains, cultures were transferred to beads (TSC) and vials were stored at −80 °C, from which new stock cultures were periodically established. For each adhesion experiment, bacteria were precultured from marine agar plates (Difco Marine agar 2216) in batch culture in artificial seawater (Sigma Sea Salts, 38.5 g l⁻¹), supplemented with 5 g l⁻¹ bacteriological peptone (Oxoid) and 1 g l⁻¹ yeast extract (Oxoid) for 24 h at 30 °C, with vigorous shaking (180 r.p.m.) for oxygenation and to avoid bacterial aggregates. These precultures were used to inoculate second cultures which were grown for 16 h. Bacterial cell suspensions were fixed on sample holder plates with double-sided sticky tape and resuspended in artificial seawater to a density of 3 × 10⁸ bacteria ml⁻¹.

**Cell surface characterization.** Bacterial cell surfaces were characterized by their cell surface hydrophobicities, as determined by water contact angles, and by their zeta potentials (James, 1991), bacteria were washed twice with artificial seawater off Wanda Beach, Sydney (Dalton *et al.*, 2000). To preserve the strains, cultures were transferred to beads (TSC) and vials were stored at −80 °C, from which new stock cultures were periodically established. For each adhesion experiment, bacteria were precultured from marine agar plates (Difco Marine agar 2216) in batch culture in artificial seawater (Sigma Sea Salts, 38.5 g l⁻¹), supplemented with 5 g l⁻¹ bacteriological peptone (Oxoid) and 1 g l⁻¹ yeast extract (Oxoid) for 24 h at 30 °C, with vigorous shaking (180 r.p.m.) for oxygenation and to avoid bacterial aggregates. These precultures were used to inoculate second cultures which were grown for 16 h. Bacterial cell suspensions were fixed on sample holder plates with double-sided sticky tape and resuspended in artificial seawater to a density of 3 × 10⁸ bacteria ml⁻¹.

**METHODS**

**Bacterial deposition in flow chambers.** Bacterial deposition was observed in the centre of the bottom plate with a charge-coupled-device camera (CCD-MXR/5010, High Technology) mounted on a phase-contrast microscope (Olympus BH-2), equipped with a ×40 ultra-long working distance objective (Olympus UPLWD-CD Plan 40 PL). For the SP flow chamber, deposition in the area 1-1 mm away from the stagnation point was observed with a CCD-LDH camera (Philips) mounted on a dark-field microscope (Leitz) equipped with a ×50 objective (Leitz Wetzlar). Live images were acquired with a PC-Vision® frame grabber (Imaging Technology) and sharp filtered. For both systems, deposited bacteria were discriminated from the background by single grey value thresholding. This yielded binary black and white images which were stored on disk for later analysis. Experiments were carried out at a flow rate of 0.050 ml s⁻¹ for the PP and 0.0088 ml s⁻¹ for the SP flow chamber at 20 °C, corresponding to a common wall shear rate of 22.5 s⁻¹ and a common Pelet number of 1.05 × 10⁻³. The Reynolds numbers for the PP and SP flow chambers were 1.3 and 2.6 respectively, proving a laminar flow in the flow chamber. Before each experiment the flow chamber and the collector surfaces were washed with artificial seawater for 15 min to condition the surface. All experiments were performed at 20 °C in triplicate, with separately cultured strains.

The total number of adhering bacteria per unit area \( n(t) \) was recorded as a function of time by image sequence analysis for at least 5 h. After a stationary end-point had been reached, an air bubble was passed through the flow chamber in order to obtain an indication of the adhesion force of attached bacteria, i.e. their retention capacity.

**Data analysis.** The number of adhering bacteria in the stationary end-point and a characteristic adhesion time were determined by fitting the bacterial adhesion kinetics to

\[
 n(t) = n_a \left(1 - e^{-\frac{t}{\tau}}\right)
\]

(Elimelech *et al.*, 1995), in which \( n_a \) is the number of adhering bacteria in the stationary end-point, \( t \) is the time in seconds and \( \tau \) is a characteristic adhesion time.

The initial deposition rate, \( j_0 \), was calculated directly by linear regression analysis from the initial increase of the numbers of adhering bacteria as a function of time, after which the entire adhesion kinetics over the full duration of an experiment was used to obtain the characteristic adhesion time \( \tau \). The characteristic adhesion time is composed of two components due to desorption and blocking according to

\[
 1/\tau = \beta + j_0 A_1
\]

(Elimelech, 1994), in which \( \beta \) is the desorption rate and \( A_1 \) is the area blocked by an adhering particle. The blocked area \( A \) can be derived from the radial pair distribution \( g(r) \) as can be calculated from the spatial distribution of the adhering bacteria. Radial pair distribution functions describe the relative density of adhering bacteria around a given centre bacterium as a function of their separation distance (Kamiti & Van de Ven, 1995)

\[
 g(r) = \rho(r,dr)/\rho_0
\]

where \( \rho(r,dr) \) is the density of adhering particles in a shell with width \( dr \) at a distance \( r \) from a centre particle. Each adhering particle is taken once as a centre particle. As an example, the spatial arrangement of adhering bacteria and the resulting distribution function \( g(r) \) are given in Fig. 2. The blocked area was calculated from the region for which \( g(r) < 1 \) (Spollena *et al.*, 1990b). Small blocked areas with a high relative density of adhering bacteria have been associated with a positive cooperativity for oral streptococci (Doyle *et al.*, 1982), while large blocked areas are indicative of repulsion between adhering bacteria.
The Von Smoluchowski–Levich approximate solution was used to calculate the theoretical upper limit for the deposition rate in both flow chambers under the experimental conditions employed. The equations necessary to calculate the deposition rate differ not only among the strains, but also between the PP and SP flow chamber.

**Table 1. Equations for mass transport in the PP and SP flow chambers**

<table>
<thead>
<tr>
<th></th>
<th>$\text{Pe}$</th>
<th>$\text{j}_b^*$</th>
<th>$\sigma$</th>
<th>$\text{Re}$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PP</strong></td>
<td>$\frac{3V_m a^2}{2bD_w^*} = \frac{3Q_{pp}}{4na^2}$</td>
<td>$D_w^* \left( \frac{2}{b} \text{Pe} \right)^{1/3}$</td>
<td>$\frac{3}{2} \frac{Q_{pp}}{b^2} \frac{\rho}{\mu}$</td>
<td>$\frac{\rho Q_{pp}}{(\mu + 2b)\nu}$</td>
</tr>
</tbody>
</table>
| **SP**  | $\frac{2a^2}{D_w} \approx 1 + \frac{3}{2} \frac{Q_{sp}}{V^*} - \frac{R^*/a^2}{2}$ | $0.616 \frac{cD_w^*}{a} \text{Pe}^{1/3}$ | $\frac{\sigma_s r}{\pi\nu}$ | $\frac{\rho Q_{sp}}{\pi
u}$ |

**RESULTS**

**Bacterial cell surface characterization**

The zeta potentials of the bacteria in artificial seawater were all close to zero (ranging from −1 to 0 mV), because surface charge is effectively shielded by counterions. Water contact angles, however, differed greatly amongst the strains used: *M. hydrocarbonoclasticus* was the most hydrophobic, with a water contact angle of 85 degrees, while *Psychrobacter* sp. had an intermediate hydrophobicity (water contact angle 54 degrees) and *H. pacifica* was the most hydrophilic strain (water contact angle 18 degrees).

**Deposition experiments**

Fig. 3 presents the deposition kinetics for *M. hydrocarbonoclasticus*, *Psychrobacter* sp. and *H. pacifica* to a glass surface from artificial seawater in the PP flow and SP flow chamber. As can be seen, the deposition kinetics differ not only among the strains, but also between the
two flow chambers. In the SP flow chamber a stationary end-point is reached faster than in the PP flow chamber, while the final surface coverage reached in the two flow chambers was similar, at least for *H. pacifica*.

The initial deposition rates $j_0$, numbers of bacteria adhering at the stationary end-point $n_\infty$, blocked areas $A_b$, reciprocal adhesion times $1/\tau$, and desorption rates $\beta$ are summarized in Table 2. The initial deposition rates are two- to threefold higher on average in the SP flow chamber than in the PP flow chamber for all strains. At the stationary end-point, however, similar numbers of *M. hydrocarbonoclasticus* and *H. pacifica* adhered in both flow chambers (Table 2), but *Psychrobacter* sp. cells adhered in slightly lower numbers in the SP chamber than in the PP flow chamber. Note that the most hydrophilic strain, *H. pacifica*, adheres in the highest numbers to the hydrophilic glass surface. Blocked areas in the SP flow chamber are two to three times fewer than in the PP flow chamber, except for *H. pacifica*. Desorption is two- to fourfold higher in the SP flow chamber than in the PP flow chamber, and correspondingly the passage of an air bubble through the flow chamber yields a higher detachment rate of bacteria adhering in the SP than in the PP flow chamber. *M. hydrocarbonoclasticus*, the most hydrophobic strain, was more easily detached from the hydrophilic glass surface than the other two strains.

The Von Smoluchowski–Levich theoretical initial deposition rates $j_0^\text{vsl}$ are 4.2 times higher in the SP flow chamber (3472 cm$^{-2}$ s$^{-1}$) than in the PP flow chamber (826 cm$^{-2}$ s$^{-1}$), but the ratio between the experimental deposition rates amounts to only 2.5, indicating that experimentally there is less difference between the two flow chambers than theoretically expected. The deposition efficiency (see also Table 2) exceeds 100% in the PP flow chamber for *Psychrobacter* sp. and *H. pacifica*, while *M. hydrocarbonoclasticus* has the lowest deposition efficiency in both flow chambers. Generally, deposition efficiencies differ significantly in the two flow chambers, indicating that different adhesion mechanisms are operative.

Finally, with regard to the comparison of the flow chambers, Table 2 also shows that the experimental reproducibility is better in the SP than in the PP flow chamber.

### Table 2. Experimental data from deposition experiments

<table>
<thead>
<tr>
<th></th>
<th><em>M. hydrocarbonoclasticus</em> ATCC 27132</th>
<th><em>Psychrobacter</em> sp. SW5H</th>
<th><em>H. pacifica</em> ATCC 27122</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PP</td>
<td>SP</td>
<td>PP</td>
</tr>
<tr>
<td>$j_0$ (cm$^{-2}$ s$^{-1}$)</td>
<td>486 ± 84</td>
<td>1648 ± 117†</td>
<td>1263 ± 381</td>
</tr>
<tr>
<td>$n_\infty$ (10$^6$ cm$^{-2}$)</td>
<td>5.1 ± 0.7</td>
<td>5.5 ± 1.2</td>
<td>5.0 ± 0.7</td>
</tr>
<tr>
<td>$1/\tau$ (10$^{-5}$ s$^{-1}$)</td>
<td>9.5 ± 1.4</td>
<td>23.5 ± 5.7†</td>
<td>25.0 ± 5.1</td>
</tr>
<tr>
<td>$A_b$ (µm$^2$)</td>
<td>1.6 ± 0.2</td>
<td>0.5 ± 0.3</td>
<td>2.4 ± 0.9</td>
</tr>
<tr>
<td>$\beta$ (10$^{-5}$ s$^{-1}$)</td>
<td>8.8 ± 1.4</td>
<td>22.7 ± 5.5†</td>
<td>21.9 ± 4.2</td>
</tr>
<tr>
<td>Percentage detached by air bubble</td>
<td>69 ± 12</td>
<td>100 ± 0†</td>
<td>9 ± 9</td>
</tr>
<tr>
<td>Percentage of $j_0^\text{vsl}$</td>
<td>59 ± 10</td>
<td>48 ± 3†</td>
<td>153 ± 46</td>
</tr>
</tbody>
</table>

† Indicates that the values for this parameter differ significantly in the PP and SP flow chamber for this strain.
chamber. Initial deposition rates in the SP chamber are nearly four times more reproducibly measured than in the PP chamber, while stationary end-point numbers are obtained with the same reproducibility.

**DISCUSSION**

In this paper, two devices to measure bacterial adhesion of three marine strains to glass under flow have been compared on an experimental and theoretical basis. The measurements were done at a common Péclet number and shear rate. Although one of the strains involved, *M. hydrocarbonoclasticus*, is motile this is unlikely to have an impact on the adhesion kinetics, because the flow speed is higher than the bacterial movement.

All three strains showed different adhesion kinetics. The hydrophilic bacterium *H. pacifica* had the greatest affinity for the hydrophilic glass surface, in line with the thermodynamically predicted preference of hydrophilic affinity for the hydrophilic glass surface, in line with the hydrophilic bacterium *H. pacifica*. All three strains showed different adhesion kinetics. The hydrophilic bacterium *H. pacifica* had the greatest affinity for the hydrophilic glass surface, in line with the thermodynamically predicted preference of hydrophilic strains for hydrophilic substrata (Absolom et al., 1983). Similarly, the hydrophobic *M. hydrocarbonoclasticus* was detached most easily from hydrophilic glass by a passing air bubble, also in line with the thermodynamically predicted dislike of hydrophobic strains for hydrophilic substrata, expressing the fact that the free energy required to detach hydrophobic bacteria from a hydrophilic surface is smaller than the energy needed to detach hydrophilic bacteria.

The initial deposition rates observed are higher in the SP flow chamber than in the PP flow chamber. In the PP flow chamber mass transport by convection is parallel to the substratum surface and bacteria in the flowing suspension have to bridge a relatively great distance by ‘slow’ diffusion in order to reach the surface. On the other hand, in the SP flow chamber suspended bacteria are transported almost perpendicularly toward the surface by convection, and diffusion plays only a minor role. In most microbial habitats, including the marine environment, both modes of mass transport occur and the different deposition efficiencies observed for a given strain in the two flow chambers clearly indicate that different adhesion mechanisms are at work.

The deposition efficiencies are generally smaller in the SP flow chamber, as compared with the PP flow chamber, indicating a relatively high number of arrivals of bacteria at the substratum that do not result in eventual adhesion. This may indicate that the outermost cell surfaces gently interacting with the substratum surfaces are more adhesive than the inner surfaces of the bacteria after compression. On the basis of the Von Smoluchowski–Levich approach, a factor of 4.2 between initial deposition rates in both flow chamber devices is expected, which is larger than the experimental ratio of 2.5. The reason that deposition in the SP flow chamber does not exceed the theoretical maximum and the ratio is smaller might be that the theoretical initial deposition rate was calculated for the stagnation point, while measurements were done adjacent to the stagnation point. The shear rate at the stagnation point is 0 and increases to 22.5 s⁻¹ at the measured point. For Psychrobacter sp. and *H. pacifica* in the PP flow chamber, the initial deposition rates exceed the theoretical maximum, possibly due to structural cell surface components assisting adhesion (Sjollema et al., 1990a).

Higher ionic strength solutions, like seawater, lead to smaller blocked areas (Yang et al., 1999), due to decreased electrostatic repulsion between adhering and flowing particles. The slightly larger blocked areas as found in the PP flow chamber probably result from a disturbance of the flow lines behind an adhering bacterium yielding a local depletion of the suspension and a ‘shadow’ zone of reduced adhesion downstream of an adhering bacterium (Bos et al., 1999).

Finally, it has been shown that standard deviations over triplicate experiments in the PP flow chamber are never better than 20–30% (Wit et al., 1997), while in the SP flow chamber standard deviations of 10% have been observed. Theoretically, accumulated errors in temperature and diffusion coefficients, concentration of bacterial suspension, flow chamber dimensions and flow rates could account for 10–12% of the variations observed regardless of the type of flow chamber involved (Yang et al., 1999), leaving a discrepancy between theoretically accountable and experimentally observed reproducibility in the PP flow chamber. Hypothetically, we attribute the lower reproducibility in the PP flow chamber to the fact that mass transport in this system depends much more on chance processes like diffusion and collisions between flowing and adhering bacteria than in the SP flow chamber, in which mass transport is mostly convection controlled.

It is difficult to give a preference for either of the two flow chamber devices evaluated in this paper, particularly as the adhesion mechanisms seem to differ under different modes of mass transport while both modes of mass transport are ecologically relevant. Although the SP flow chamber might appear more attractive due to its faster kinetics and the higher reproducibility of the results, the PP flow chamber poses less specific requirements for transparency of the substratum, and yields better image quality. Moreover, flow is homogeneous in the PP flow chamber over large areas, which allows adhesion to be studied over different microscopic fields of view. Also from the present results, it can be seen that larger differences are observed in the SP flow chamber than in the PP flow chamber for different combinations of strains and substrata, indicating that adhesion in the SP flow chamber is strongly interaction controlled. Considering the impact of the methodology on adhesion mechanisms, however, it is concluded that the choice for a specific flow chamber should a priori be made on the basis of relevance for the problem under investigation.

**ACKNOWLEDGEMENTS**

This work was supported by IOP milieutechnologie/Zware Metalen, Senter, The Netherlands.
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Received 25 July 2001; revised 5 October 2001; accepted 10 October 2001.