Adhesion of *Pseudomonas aeruginosa* to silicone rubber in a parallel plate flow chamber in the absence and presence of nutrient broth

Marc B. Habash,¹ Henny C. van der Mei,² Gregor Reid^{1,3}
and Henk J. Busscher^{1,2}

Author for correspondence: Henk J. Busscher. Tel: +31 363 3140. Fax: +31 363 3159.
e-mail: H.J.Busscher@med.rug.nl.

¹ Department of Microbiology and Immunology, University of Western Ontario Health Sciences Centre, London, Ontario, Canada N6A 5C1

² Materia Technica, University of Groningen, Bloemsingel 10, 9712 KZ Groningen, The Netherlands

³ Lawson Research Institute, London, Ontario Canada N6A 4V2

The physico-chemical cell-surface properties of *Pseudomonas aeruginosa* AK1 and its adhesion to silicone rubber under flow were compared for cells suspended in phosphate-buffered saline (PBS) or PBS supplemented with 2% nutrient broth. Addition of 2% nutrient broth to cells suspended in PBS yielded minimal growth and did not significantly change the mean zeta potential of the organisms, which was around -13 mV. However, a comparatively larger proportion of the organisms had more negative zeta potentials in the presence of nutrient broth. This change was concurrent with a slight decrease in cell-surface hydrophobicity, as measured by water contact angles, from 119° to around 112° . The initial deposition rate of *P. aeruginosa* AK1 to silicone rubber, as studied in a parallel plate flow chamber, increased from $344\text{ cm}^{-2}\text{ s}^{-1}$ in the absence of nutrient broth to $505\text{ cm}^{-2}\text{ s}^{-1}$ in its presence. No stationary level of adhesion was observed in the presence of nutrient broth, instead the number of adhering cells increased steadily at a rate of approximately $85\text{ cm}^{-2}\text{ s}^{-1}$. Fluorescent staining of adhering cells demonstrated that for adhesion from buffer only 2% of the adhering cells were metabolically active, whereas in case of deposition from PBS supplemented with nutrient broth, 67% of the adhering cells were metabolically active. It is concluded that the deposition rates measured in the parallel plate flow chamber with 2% nutrient broth added to the PBS suspension represent an interplay of adhesion and surface-associated growth.

Keywords: silicone rubber, *Pseudomonas aeruginosa*, adhesion, growth, parallel plate flow chamber

INTRODUCTION

A wide variety of biomedical implants ranging from mammary and larynx prostheses to urinary and intravenous catheters are comprised of silicone rubber (Gristina *et al.*, 1988). However, common to nearly all biomedical implants is the adhesion of infectious microorganisms that may ultimately lead to removal of the implant. Silicone rubber is especially prone to becoming colonized with *Candida*, staphylococci, streptococci or *Pseudomonas* species depending on the site of implantation (Gristina *et al.*, 1988; Salzman & Rubin, 1995). The development of an infectious biofilm is initiated by the adhesion of bacteria to the implant

surface. The initial colonizing organisms are of importance as they play a pivotal role in the linking of the final biofilm to the implant surface (Busscher *et al.*, 1995).

Initial microbial adhesion to surfaces is generally considered to be an interplay of various physico-chemical properties of the interacting surfaces (i.e. the implant and the microbial cell surfaces), most notably their hydrophobicity and charge (Busscher & Weerkamp, 1987; Van Loosdrecht *et al.*, 1987a, b). Physico-chemical studies on microbial adhesion to surfaces are typically carried out with organisms suspended in buffers to avoid microbial growth or other associated complications (Van Loosdrecht *et al.*, 1990). *In vitro* studies have identified changes in antimicrobial efficacy (Evans *et al.*, 1990, 1991), protein expression (Harakeh & Matin,

Abbreviation: CTC, 5-cyano-2,3-ditolyl tetrazolium chloride.

1989), cell-surface hydrophobicity (Ljungh & Wadström, 1995) and adhesion (Fletcher, 1976; Zeng *et al.*, 1994), depending upon growth conditions. Alternatively, adhesion studies sometimes involve optimal growth conditions. Recently, Barton *et al.* (1996a) compared adhesion of *Staphylococcus epidermidis*, *Pseudomonas aeruginosa* and *Escherichia coli* to orthopaedic implant polymers for organisms suspended in phosphate-buffered saline (PBS), tryptic soy broth and hyaluronic acid. They found that adhesion in whole broth was decreased compared to adhesion in buffer, while the presence of hyaluronic acid increased bacterial adhesion.

Although there is a consensus that the metabolic activity of bacteria adhering to surfaces in natural environments under nutrient availability is influenced by the presence of a surface, experimental observations are not consistent. In a review, Van Loosdrecht *et al.* (1990) summarized studies reporting increased as well as decreased growth rates of bacteria on surfaces as compared to planktonic bacteria, while increased adhesion of metabolically active cells was also found. The mechanisms of surface-associated changes in bacterial behaviour are probably related to nutrient accumulation at the surface and thus are related to the physico-chemical properties of the substratum surface, including charge and hydrophobicity, and the physico-chemical nature of the nutrients themselves. The nutrient conditions around a biomaterials implant are extremely complex and possibly vary over time. Thus it is doubtful whether carrying out adhesion experiments with bacteria suspended either in buffers or under optimal laboratory growth conditions as in whole broth, represent clinically relevant conditions.

We have compared, in a parallel plate flow chamber, the adhesion to silicone rubber of a *P. aeruginosa* strain suspended in PBS or PBS supplemented with a minimal amount of nutrient broth. The metabolic activity of the adhering bacteria was determined under both nutrient conditions by utilizing a viable fluorescent stain (5-cyano-2,3-ditolyl tetrazolium chloride; CTC). Furthermore, the effects of supplementing the PBS with a minimal amount of nutrient broth upon the microbial zeta potentials and intrinsic cell surface hydrophobicities (by water contact angles) were measured.

METHODS

Strain and growth conditions. *Pseudomonas aeruginosa* AK1, a uropathogenic isolate, was used in this study. The strain was streaked and grown overnight at 37 °C from a frozen stock on a nutrient agar plate (Oxoid). The plate was then kept at 4 °C for not more than one week, at which time a new plate was streaked. Several colonies were used to inoculate a 'preculture' that was incubated at 37 °C in ambient air for 24 h. The 'preculture' was utilized to inoculate a second culture that was grown for 16 h. The bacteria from the second culture were harvested by centrifugation at 5000 g for 5 min at 10 °C and washed twice with Millipore-Q water. Subsequently, the bacteria were sonicated on ice three times for 10 s and then resuspended at a concentration of 3×10^8 cells ml⁻¹ in either

sterile PBS (10 mM potassium phosphate and 150 mM sodium chloride, pH 7.0) or PBS supplemented with nutrient broth.

To determine the concentration of nutrient broth constituting minimal growth conditions, growth curves were made over a 24 h time interval for cells suspended in PBS (control), in PBS supplemented with various concentrations of nutrient broth (varying from 0.5% to 10%) and in whole nutrient broth (optimal growth condition). Optical densities of the suspensions were measured with a Spectronic 20 (Bausch & Lomb) at 600 nm.

Silicone rubber. A silastic medical grade silicone rubber kit (Q7-4750, Nusil) was purchased and plates were produced following the procedures suggested by the manufacturer. Briefly, equal proportions of part A and part B were thoroughly blended together and injected into a mould at room temperature through a 3 mm diam. opening with a force of 3 tons. The silicone rubber was immediately cured at 200 °C for 50 min. Finally, samples were cleaned in a 2% RBS detergent solution under simultaneous sonication and thoroughly rinsed in demineralized water, methanol, again with demineralized water and lastly with Millipore-Q water.

Microbial cell-surface characterization. The *Pseudomonas* cells used in this study were characterized by their zeta potentials and surface hydrophobicity, as possible determinants for their adhesive cell-surface properties.

For zeta potential measurements (Hiemenz, 1977), three independently grown cultures were harvested and washed as described above. The collected cells were resuspended (3×10^7 cells ml⁻¹) in either sterile PBS (pH 7.0) or sterile PBS supplemented with 2% nutrient broth and kept at room temperature for up to 4 h, a time interval comparable with the duration of the adhesion experiments in the parallel plate flow chamber. Zeta potentials were measured either directly after resuspending the cells or after 4 h exposure of the bacteria to PBS or minimal growth conditions, i.e. PBS supplemented with 2% nutrient broth. The electrophoretic mobility of each sample was measured at 150 V using a Lazer Zee Meter 501 (PenKem) and converted into zeta potentials assuming that the Helmholtz-Smoluchowski equation holds. An automated version of the Lazer Zee Meter was employed (Noordmans *et al.*, 1993) allowing the determination of the zeta potential distribution of individual cells.

The contact angle measurements on *P. aeruginosa* AK1 lawns, of three individual cultures, were done as previously described using the sessile drop technique (van der Mei *et al.*, 1987) as a measure of the intrinsic cell surface hydrophobicity. Briefly, *P. aeruginosa* AK1 was kept either in water (as for standard contact angle measurements on microbial lawns), sterile PBS or PBS supplemented with 2% nutrient broth for up to 4 h at room temperature. Subsequently, bacteria were harvested, washed, resuspended in Millipore-Q water and deposited on 0.45 µm pore-size cellulose acetate filters to produce a lawn of approximately 50 stacked layers. The *P. aeruginosa* AK1 lawns were air-dried until so-called 'plateau contact angles' (Van Oss & Gillman, 1972) could be measured with water, followed by contact angle measurements with formamide, methylene iodide and α -bromonaphthalene.

The parallel flow plate chamber, image analysis and adhesion assay. The flow chamber (dimensions, $1 \times w \times h = 76 \times 38 \times 0.6$ mm) and image analysis system have been described in detail by Busscher & van der Mei (1995). Images were taken from the bottom plate (58 × 38 mm) of the parallel plate flow chamber which consisted of a thin square (15 × 15 × 0.5 mm) of silicone rubber affixed centrally into the

groove ($58 \times 15 \times 0.5$ mm) of the thicker (2.0 mm) perspex plate. The top plate of the chamber was made of glass. Deposition was observed with a CCD-MXRi camera (High Technology) mounted on a combined fluorescent/phase contrast microscope (Olympus BH-2) equipped with a $40 \times$ ultra long working distance objective (Olympus ULWD-CD Plan 40 PL). The camera was coupled to an image analyser (TEA, Difa).

Prior to each experiment, all tubes and the flow chamber were filled with either PBS or PBS with 2% nutrient broth added, taking care to ensure that all air bubbles were removed from the system. Once the system was filled, and prior to the addition of the bacterial suspension, the fluid was allowed to flow through the system at a flow rate of 0.025 ml s^{-1} corresponding with a shear rate of 10 s^{-1} for 10 min and subsequently switched to the appropriate bacterial suspension at the same flow rate. The bacterial suspension was perfused through the system for 4 h without recirculation and images were obtained in the phase contrast mode of the microscope system at predetermined time points. The increase in the number of adhering bacteria over time, both initially as well as in a more advanced state of the adhesion process was expressed in so-called deposition rates, i.e. the increase in the number of adhering bacteria per unit area and time.

Following 4 h perfusion of the flow chamber with bacterial suspension, flow was switched to buffer without bacteria to remove unbound organisms from the tubes and the flow chamber under the same flow rate and 3 ml of the fluorescent viable cell stain CTC (5 mM) was added without introducing any air bubbles or additional shear. The flow chamber was then incubated in the dark at 37°C for 2 h. CTC, a tetrazolium stain, fluoresces when taken up and reduced by the electron transport chain of metabolically active bacterial cells (Rodriguez *et al.*, 1992). The stain can then be observed as red fluorescent crystals within the bacteria at an emission maximum of 602 nm. The number of adherent, metabolically active cells were counted at 12 separate locations on the silicone rubber surface utilizing the fluorescent mode of the microscope, while the phase contrast mode of the microscope was used to enumerate the total number of adhering bacteria at the same locations.

RESULTS

Fig. 1 shows the growth curves of *P. aeruginosa* AK1 cells suspended in PBS supplemented with various amounts of nutrient broth. As can be seen, growth is virtually absent when less than 2% broth is added, while for higher concentrations of broth significant growth occurs. Therefore, minimal growth conditions in the remainder of this paper will refer to PBS with 2% nutrient broth added.

Fig. 2 shows the zeta potential distributions of *P. aeruginosa* AK1 under the various conditions relevant to this study. The zeta potential distribution of freshly cultured cells in buffer (Fig. 2a) is relatively narrow (SD 5 mV over 1015 cells) with a mean zeta potential of -13 mV . After prolonged exposure to buffer (Fig. 2b), however, zeta potentials tended to become more negative (-17 mV), with a higher SD of 11 mV over a population of 399 cells. Exposure of cells to minimal growth conditions (Fig. 2c) also yielded the appearance of greater numbers of more negatively charged cells,

Fig. 1. Growth of *Pseudomonas aeruginosa* AK1 suspensions with various concentrations of nutrient broth added: $\square$, 0%; $\triangle$, 0.5%; $\circ$, 1.0%; ∇ , 2.0%; $\blacksquare$, 2.5%; $\blacktriangle$, 5.0%; $\bullet$, 10.0%; $\blacktriangledown$, 100.0%.

though the mean zeta potential of the bacterial population as a whole remained around $-19 \pm 11 \text{ mV}$ (SD over 170 cells) prior to washing. Also after washing (Fig. 2d) a relatively wide zeta potential distribution of $-16 \pm 9 \text{ mV}$ (SD over 278 cells) was observed.

Contact angles, summarized in Table 1, indicate that *P. aeruginosa* AK1 is a hydrophobic organism with a water contact angle of around 106° , when measured routinely. Both exposure to PBS as well as exposure to PBS supplemented with 2% nutrient broth for 4 h yielded a slight increase in the intrinsic cell surface hydrophobicity as shown by water contact angles, a trend which was also reflected in the formamide contact angles. The contact angles measured with methylene iodide and α -bromonaphthalene, probing apolar cell surface properties, were not appreciably influenced. However, exposure of bacteria to PBS supplemented with 2% nutrient broth gave a slight decrease in water contact angles from 119° in PBS to 112° .

Fig. 3 shows the numbers of *P. aeruginosa* cells adhering to silicone rubber under both nutrient conditions. Interestingly, when the organisms were suspended in buffer a stationary level of adhesion was obtained within 60 min with an initial deposition rate of $344 \pm 83 \text{ cm}^{-2} \text{ s}^{-1}$ (SD over three separate experiments), while fluorescent microscopy indicated that only 2% of the adhering organisms were metabolically active. Under minimal growth conditions, the initial deposition rate

Fig. 2. Zeta potential (mV) distributions of *Pseudomonas aeruginosa* AK1 under various conditions. (a) Bacteria transferred to PBS (pH 7.0) immediately after harvesting ($n = 1015$ cells); (b) bacteria transferred to PBS (pH 7.0) immediately after harvesting and left for 4 h prior to measurement ($n = 399$ cells); (c) bacteria transferred to PBS (pH 7.0) supplemented with 2% nutrient broth and left for 4 h prior to measurement ($n = 170$ cells); (d) bacteria transferred to PBS (pH 7.0) supplemented with 2% nutrient broth, left for 4 h, washed and resuspended in PBS prior to measurement ($n = 278$ cells).

Table 1. Contact angles of water, formamide, methylene iodide and α -bromonaphthalene on *P. aeruginosa* AK1

The bacteria were kept in either PBS or PBS supplemented with 2% nutrient broth (PBS+) at 25 °C for up to 4 h, then washed in Millipore-Q water and collected on cellulose acetate filters (0.45 μ m). These results are based on contact angles measured for three separate cultures of *P. aeruginosa* AK1 $\pm$ SD.

Time (h)	Medium	Water	Formamide	Methylene iodide	α -Bromonaphthalene
0	Water*	106 $\pm$ 7	52 $\pm$ 3	52 $\pm$ 3	39 $\pm$ 5
4	PBS	119 $\pm$ 3	65 $\pm$ 2	52 $\pm$ 2	41 $\pm$ 1
	PBS+	112 $\pm$ 6	60 $\pm$ 4	50 $\pm$ 5	36 $\pm$ 4

*Standard contact angle measurement on microbial lawns according to van der Mei *et al.* (1987).

Fig. 3. The numbers of *P. aeruginosa* AK1 cells adhering to silicone rubber in a parallel plate flow chamber as a function of time when suspended in PBS (▲) or in PBS supplemented with 2% nutrient broth (■). Bars represent the SD over three experiments with separate bacterial cultures.

was slightly higher (505 ± 79 $\text{cm}^{-2} \text{s}^{-1}$) while no stationary level of adhesion was obtained. Instead, the number of adhering micro-organisms steadily increased, even after 200 min, at a rate of approximately

85 ± 31 $\text{cm}^{-2} \text{s}^{-1}$. However, the percentage of metabolically active cells under minimal growth conditions was much higher than in PBS and amounted to 67%.

DISCUSSION

In this work adhesion of *P. aeruginosa* AK1 to silicone rubber was studied in a parallel plate flow chamber, with both metabolically active and inactive bacteria. The conditions chosen to stimulate metabolic activity of the bacteria were such that minimal growth of bacteria in suspension occurred. The deposition kinetics observed in this study demonstrate that the number of bacteria adhering to the silicone rubber from PBS supplemented with 2% nutrient broth did not reach stationary numbers in contrast to bacteria suspended in PBS. Instead, even after 3 h, the number of bacteria on the silicone rubber increased at a rate of $85 \text{ cm}^{-2} \text{s}^{-1}$ in PBS supplemented with 2% nutrient broth. Considering that neither the zeta potentials nor the cell surface hydrophobicities of the majority of metabolically active bacteria change in a direction anticipated to be accompanied by increased adhesion (Van Loosdrecht, 1987a, b), we attribute this increase to surface-associated growth (Van Loosdrecht, 1990). We do not believe

that the presence of only 2% nutrient broth in suspension stimulates significant growth of planktonic bacteria, but accumulated nutrients may be available at the silicone rubber surface in sufficient concentration to yield significant growth of adhering bacteria (Pringle & Fletcher, 1986; Hawthron & Reid, 1990; Reid *et al.*, 1994; Marshall, 1985).

The deposition kinetics of metabolically inactive bacteria in a parallel plate flow chamber are governed by convective-diffusion and desorption (Sjollem *et al.*, 1989). Although bacterial desorption is measurable (Meinders *et al.*, 1994), desorption rates are usually small and in the order of 10^{-6} s^{-1} , which is negligible compared to the adsorption rate. The difference in initial deposition rates between bacteria suspended in PBS and in PBS supplemented with nutrient broth may be associated with a growth component (Bryers & Characklis, 1992). The difference in initial deposition rates under the two nutrient conditions amounts to $171 \text{ cm}^{-2} \text{ s}^{-1}$, of which 67% appeared as metabolically active bacteria by CTC staining. Consequently, it can be calculated that growth will contribute to the number of adhering bacteria at a rate of $114 \text{ cm}^{-2} \text{ s}^{-1}$, which corresponds well, within experimental reproducibility, with the increase in the number of adhering bacteria after 3 h, i.e. $85 \text{ cm}^{-2} \text{ s}^{-1}$ and thus constitutes a quantitative confirmation of the hypotheses on surface accumulation of bacteria by deposition and surface-associated growth (Van Loosdrecht *et al.*, 1990). Barton *et al.* (1996b) compared the growth rates of *S. epidermidis*, *P. aeruginosa* and *E. coli* in their planktonic state when suspended in tryptic soy broth and when adhering to various polymers. Generation times of planktonic bacteria were shorter than those of adhering bacteria. For adhering *P. aeruginosa*, generation times between 37 and 51 min were reported, depending on the substratum surface. From our present results, it can be estimated that under minimal growth conditions on silicone rubber, the generation time of *P. aeruginosa* AK1 amounts to 136 min, which is about threefold longer than the generation times reported by Barton *et al.* (1996b) for optimal growth conditions.

Physico-chemical cell-surface characteristics of more metabolically active bacteria were on average identical to those of inactive bacteria, although the water contact angles indicate a minor decrease in intrinsic cell surface hydrophobicity (see Table 1). Unfortunately, water contact angles do not give information on individual bacteria. Particulate microelectrophoresis is capable of providing zeta potentials of individual bacteria and showed that a population of metabolically more active bacteria contains a larger proportion of more negatively charged bacteria than a suspension of metabolically inactive bacteria. These observations suggest that metabolically active cells change their cell surface properties during their division cycle. Whereas the cell surface hydrophobicity changed only slightly, newly formed cells had significantly more negative zeta potentials. Earlier, Gilbert *et al.* (1991) also found that the growth phase of *E. coli* and *S. epidermidis* affected the electro-

phoretic mobility of these bacteria. *E. coli* was shown to be less negatively charged in its lag phase than in its early exponential growth phase (Allison *et al.*, 1990b), *S. epidermidis* showed a decrease in negative surface charge towards neutrality with progression of the growth phase into mid-exponential phase and restoration of the negative charge after entry into the stationary phase. Our present results on the intrinsic hydrophobicity of *P. aeruginosa* under growth confirm the conclusion by Allison *et al.* (1990a) that newly formed daughter cells of this organism are more hydrophilic.

In summary, this paper demonstrates that quantitative data on adhesion and surface-associated growth of *P. aeruginosa* can be interpreted to yield the separate contributions of deposition by convective-diffusion and of growth. Analysis of bacterial deposition data obtained in the parallel plate flow chamber under minimal growth conditions is more complicated than in the absence of nutrients in suspension, but is more relevant to the events occurring in natural environments without impeding a physico-chemical approach.

REFERENCES

- Allison, D. G., Brown, M. R., Evans, D. J. & Gilbert, P. (1990a). Surface hydrophobicity and dispersal of *Pseudomonas aeruginosa* from biofilms. *FEMS Microbiol Lett* **59**, 101–104.
- Allison, D. G., Evans, D. J., Brown, M. R. W. & Gilbert, P. (1990b). Possible involvement of the division cycle in dispersal of *Escherichia coli* from biofilms. *J Bacteriol* **172**, 1667–1669.
- Barton, A. J., Sagers, R. D. & Pitt, W. G. (1996a). Bacterial adhesion to orthopedic implant polymers. *J Biomed Mater Res* **30**, 403–410.
- Barton, A. J., Sagers, R. D. & Pitt, W. G. (1996b). Measurement of bacterial growth rates on polymers. *J Biomed Mater Res* **32**, 271–278.
- Bryers, J. D. & Characklis, W. G. (1992). Biofilm accumulation and activity: a process analysis approach. *NATO ASI Ser Ser E Appl Sci* **223**, 221–238.
- Busscher, H. J. & van der Mei, H. C. (1995). Use of flow chamber devices and image analysis methods to study microbial adhesion. *Methods Enzymol* **253**, 455–477.
- Busscher, H. J. & Weerkamp, A. H. (1987). Specific and non-specific interactions in bacterial adhesion to solid substrata. *FEMS Microbiol Rev* **46**, 146–173.
- Busscher, H. J., Bos, R. & van der Mei, H. C. (1995). Initial microbial adhesion is a determinant for the strength of biofilm adhesion. *FEMS Microbiol Lett* **128**, 229–234.
- Evans, D. J., Allison, D. G., Brown, M. R. W. & Gilbert, P. (1990). Effect of growth-rate on resistance of Gram-negative biofilms to ceftrimide. *J Antimicrob Chemother* **26**, 473–478.
- Evans, D. J., Allison, D. G., Brown, M. R. W. & Gilbert, P. (1991). Susceptibility of *Pseudomonas aeruginosa* and *Escherichia coli* biofilms towards ciprofloxacin: effect of specific growth rate. *J Antimicrob Chemother* **27**, 177–184.
- Fletcher, M. (1976). The effects of culture and age, time, and temperature on bacterial attachment to polystyrene. *Can J Microbiol* **23**, 1–6.
- Gilbert, P., Evans, D. J., Duguid, I. G., Evans, E. & Brown, M. R. W. (1991). Cell surface properties of *Escherichia coli* and *Staphylococcus epidermidis*. In *Microbial Cell Surface Analysis – Struc-*

tural and Physico-Chemical Methods, pp. 339–356. Edited by N. Mozes, P. S. Handley, H. J. Busscher & P. G. Rouxhet. New York: VCH Publishers.

Gristina, A. G., Naylor, P. & Myrvik, Q. (1988). Infections from biomaterials and implants: a race for the surface. *Med Prog Technol* **14**, 205–224.

Harakeh, S. & Matin, A. (1989). Influence of nutrient-limited growth on pathogenesis-associated outer membrane proteins of *Yersinia enterocolitica*. *J Appl Bacteriol* **67**, 209–212.

Hawthorn, L. & Reid, G. (1990). The effect of protein and urine on uropathogen adhesion to polymer substrata. *J Biomed Mater Res* **24**, 1325–1332.

Hiemenz, P. C. (1977). Electrophoresis and other electrokinetic phenomena. In *Principles of Colloid and Surface Chemistry*, pp. 452–487. Edited by J. J. Lagowski. New York & Basel: Marcel Dekker.

Ljungh, Å. & Wadström, T. (1995). Growth conditions influence expression of cell surface hydrophobicity of staphylococci and other wound infection pathogens. *Microbiol Immunol* **39**, 753–757.

Marshall, K. C. (1985). Mechanisms of bacterial adhesion at solid–water interfaces. In *Bacterial Adhesion: Mechanisms and Physiological Significance*, pp. 133–161. Edited by D. C. Savage & M. Fletcher. New York: Plenum Press.

van der Mei, H. C., Weerkamp, A. H. & Busscher, H. J. (1987). A comparison of methods to determine hydrophobic properties of streptococcal cell surfaces. *J Microbiol Methods* **6**, 277–287.

Meinders, J., van der Mei, H. C. & Busscher, H. J. (1994). Physico-chemical aspects of deposition of *Streptococcus thermophilus* B to hydrophobic and hydrophilic substrata in a parallel plate flow chamber. *J Colloid Interface Sci* **164**, 355–363.

Noordmans, J., Kempen, J. & Busscher, H. J. (1993). Automated image analysis to determine zeta potential distributions in particulate microelectrophoresis. *J Colloid Interface Sci* **156**, 394–399.

Pringle, J. H. & Fletcher, M. (1986). Influence of substratum hydration and adsorbed macromolecules on bacterial attachment to surfaces. *Appl Environ Microbiol* **51**, 1321–1325.

Reid, G., Khoury, A. E., Preston, C. A. K. & Costeron, J. W. (1994). Influence of dextrose dialysis solutions on adhesion of *Staphylococcus aureus* and *Pseudomonas aeruginosa* to three catheter surfaces. *Am J Nephrol* **14**, 37–40.

Rodriguezs, G. G., Phipps, D., Ishiguro, K. & Ridgway, H. F. (1992). Use of a fluorescent redox probe for direct visualization of actively respiring bacteria. *Appl Environ Microbiol* **58**, 1801–1808.

Salzman, M. B. & Rubin, L. G. (1995). Intravenous catheter-related infections. *Adv Pediatr Infect Dis* **10**, 337–368.

Sjollem, J., Busscher, H. J. & Weerkamp, A. H. (1989). Experimental approaches for studying adhesion of microorganisms to solid substrata: applications and mass transport. *J Microbiol Methods* **9**, 73–78.

Van Loosdrecht, M. C. M., Lyklema, J., Norde, W., Schraa, G. & Zehnder, A. J. B. (1987a). The role of bacterial cell wall hydrophobicity in adhesion. *Appl Environ Microbiol* **53**, 1893–1897.

Van Loosdrecht, M. C. M., Lyklema, J., Norde, W., Schraa, G. & Zehnder, A. J. B. (1987b). Electrophoretic mobility and hydrophobicity as a measure to predict the initial steps of bacterial adhesion. *Appl Environ Microbiol* **53**, 1898–1901.

Van Loosdrecht, M. C. M., Lyklema, J., Norde, W. & Zehnder, A. J. B. (1990). Influence of interfaces on microbial activity. *Microbiol Rev* **54**, 75–87.

Van Oss, C. J. & Gillman, C. F. (1972). Phagocytosis as a surface phenomenon. I. Contact angles and phagocytosis of non-opsonized bacteria. *J Reticuloendothel Soc* **12**, 283–292.

Zheng, D., Taylor, G. T. & Gyananath, G. (1994). Influence of laminar flow velocity and nutrient concentration on attachment of marine bacterioplankton. *Biofouling* **8**, 107–120.

Received 20 January 1997; revised 21 March 1997; accepted 3 April 1997.