Journal of Industrial Microbiology (1995) 15, 305–310

Quantification of the ease of removal of bacteria from surfaces

PJ Eginton[1], H Gibson[2], J Holah[2], PS Handley[3] and P Gilbert[1]

[1]*Department of Pharmacy, University of Manchester, Manchester, M13 9PL;* [2]*Campden Food and Drink Research Association, Gloucestershire, GL55 6LD;* [3]*School of Biological Sciences, University of Manchester, Manchester, M13 9PL, UK*

This paper describes a technique which reproducibly quantifies the ease of removal of microorganisms from surfaces. Tiles (22 mm × 22 mm) of various materials were colonised with *Staphylococcus epidermidis* NCTC 11047, *Escherichia coli* K12 HB101 or *Pseudomonas aeruginosa* PaWH, by submersion, for various times (2 min–48 h), in inoculated Tryptone Soya broth (37° C). Colonised tiles were blotted onto a Tryptone Soya agar plate for 1 min and the process was repeated through a succession of agar plates. The final plate contained tetrazolium salts (0.05% w/v) and was incubated *in situ* with the tile. Tetrazolium plates indicated that very few organisms remained on the tiles after 15 successive blots. In all instances, the number of recovered colonies per plate decreased exponentially with plate succession number, according to the relationship, $CFU = A.10^{-kN}$, where CFU is the number of colonies transferred, *k* is the removal exponent, A is the intercept and N is the plate succession number. Removal exponents differed significantly between organisms ($P > 0.95$), depended on the nature of the test surface, and decreased as the inital attachment and colonisation time was increased from 2 min–48 h. Intercept values (A) but not the gradients were dependent upon the initial numbers of bacteria in suspension. These data indicate that the gradients derived from counting recoverable viable cells from successive blots of test tiles onto agar is a measure of the strength of attachment of the organisms to the surface.

Keywords: biofilms; surface-attachment; surface-detachment; hygiene; contamination

Introduction

Bacteria in the environment are often found attached to surfaces growing as biofilms [6,8,13]. Such a mode of growth is thought to convey many advantages upon the cells in terms of nutrient acquisition [10,11,25], localisation of extracellular products [16] and protection from phagocytes and phagocytic protozoa [8]. Biofilms are functional consortia of microorganisms, embedded within exopolymer matrices, associated with and attached to surfaces [6,8]. The formation of biofilms on solid substrata commences with non-specific attachment of single cells or clusters of cells. Such cells are relatively loosely bound to the surface through electrostatic interactions such as van der Waals forces [20,24]. Although the regulatory mechanisms are unclear, such attachment is thought to initiate a cascade of physiological changes [9,12] in the cells which leads, amongst other things, to the overproduction of exopolymers [15,23]. The production of these exopolymers further strengthens the attachment of the organisms to the surface and provides the biofilm matrix within which the cells divide. Whilst the rate and extent of colonisation of surfaces has been reported to vary according to the physical properties of the surface, such as surface free energy, roughness and charge [20,24], this has been related more to the primary adhesion events than to biofilm development.

In order to colonise new surfaces, individual cells must be able to disperse from mature biofilms and reattach elsewhere [1,2]. This might involve detachment, through enzymatic cleavage of the matrix polymers by individual cells [21], or it might involve sloughing of cells together with matrix polymers caused by buffeting of the biofilm by particles or by the fluid dynamic forces of a turbulent liquid environment [19,22]. The ability of cells to transfer from an adherent biofilm to other surfaces is not only imperative for the long term survival of the organisms, but is also central to the problem of contamination of various products through contact with contaminated surfaces in the production environment. This is particularly the case in the food industry [17]. The ease with which microorganisms are shed from surfaces contributes to their ability to spread and contaminate product items or persons within the vicinity. Biofilms are often thought of as *foci* from which contamination and biospoilage arise. Much work has concentrated on the selection of surfaces, which resist colonisation by bacteria and aid maintenance of hygiene, for use within hygienic industries (food, pharmaceuticals and medicine). Such surfaces should be readily cleansed and disinfected. Bacterial biofilms are, unfortunately, not only difficult to remove but also notoriously recalcitrant even to the harshest antimicrobial regimens [3–5,7,14,18].

Absence of suitable methods for quantifying the strength of attachment of microorganisms to surfaces has severely restricted further developments in this field. Whilst vigorous cleansing programmes and the application of biocides and disinfectants are widely used in the food industry to remove or kill attached microorganisms, there have been few published attempts to examine the ease of transfer of microorganisms from surface to product. In addition, whilst there have been many studies which examine the ability of organisms to adhere to different surfaces, few have examined the ease with which the colonisers may be removed.

Correspondence: P Gilbert, Department of Pharmacy, University of Manchester, Manchester, M13 9PL, UK
Received 20 December 1994; accepted 30 March 1995

A major reason for the lack of such studies have been the absence of suitable methodologies to quantify bacterial detachment accurately. The majority of methods for quantifying the viable cells in biofilms rely on their removal, by swabbing, shaking or mild sonication, and enumeration in relation to the colonised surface area, methods which are notoriously inaccurate.

The present paper describes a novel method which quantifies the ease of removal of microorganisms from surfaces, and by inference gives a measure of the strength of attachment of cells to a surface.

Materials and methods

Organisms and culture maintenance

A stable, mucoid clinical isolate of *Pseudomonas aeruginosa* PaWH, *Staphylococcus epidermidis* NCTC 11047 and *Escherichia coli* K12 HB101, were used. Cultures were maintained on Tryptone Soya Agar (TSA, Oxoid CM131) slants, in the dark at 4° C, after overnight incubation at 37° C. Overnight cultures were prepared from the slopes by inoculating volumes of Tryptone Soya Broth (TSB, Oxoid CM129, 100 ml), contained in 250-ml Erlenmeyer flasks, and incubated at 37° C for 16 h in an orbital incubator (200 rpm).

Media and chemicals

Dehydrated culture media were obtained from Oxoid (Basingstoke, UK). 2,3,5-triphenyltetrazolium chloride (TT) was obtained from the Sigma Chemical Co (Poole, UK). All other reagents were of the purest available grade and were obtained from BDH Ltd (London, UK).

Preparation of test surfaces

Tiles (22 mm × 22 mm) were cut from sheet (1-mm thickness) Teflon, Formica, Polyethylene, polystyrene and stainless steel (Type 216, Grade 2B finish). Polyvinyl and glass coverslips (22 mm × 22 mm) were obtained from PGC Scientific (Bristol, UK) and BDH Ltd (London, UK) respectively.

Glass coverslips were washed in a neutral detergent, rinsed with distilled water and immersed in concentrated nitric acid (70% w/w) for 5 min. After five consecutive rinses in distilled water (50 ml), the slips were dried and sterilised by dry heat (150° C, 2 h). All other test surfaces were washed in a neutral detergent, rinsed five times with distilled water (50 ml) and sanitised by storing them in ethyl-alcohol (70% w/w).

Attachment of microorganisms and development of biofilms

Tiles made from the various test materials were clamped vertically within a cassette made out of Teflon. The cassette will take up to 16 tiles, held in a radial arrangement. When immersed in culture medium (200 ml) in a 500-ml beaker, the cassette allowed free circulation of liquid to all but two of the edges of the tiles. Beakers (500 ml) containing 200 ml TSB and loaded cassettes were sterilised by autoclaving them. Attachment of microorganisms was initiated, and biofilms allowed to form, by inoculation of the growth medium with an overnight culture, to give *ca* 10^4–10^5 cells ml^{-1}. The device was incubated in a shaking incubator (37° C, 200 rpm). Tiles were removed from the cassette 2 min, 8 h, 16 h and 48 h after inoculation.

Examination of colonised test surfaces (2-min colonisation)

Tiles were removed from the cassette after incubation for 2 min and rinsed separately in three successive 20-ml volumes of sterile saline. Viable counts performed on the rinsings established that less than 1 cfu ml^{-1} was carried passively into a fourth rinse. After the tiles were rinsed they were removed with forceps and placed gently, flat on the surface of a pre-dried (1 h at 55° C) TSA plate. After 1 min, the tile was removed to a second plate and the first plate spread with a glass spreader. The process was repeated through a succession of 15 TSA plates. Preliminary experiments had determined that the number of cfu transferred to the plates did not increase significantly if the contact time was extended beyond 1 min. The final plate contained TT (0.05% w/v) and was incubated (16 h, 37° C) with the tile *in situ*. Cfu were determined in all cases after overnight incubation at 37° C. In each case no less than eight replicate tiles were taken through the plate succession.

Examination of colonised test surfaces (8–48 h colonisation)

When the procedures described above were repeated with tiles that had been colonised for times greater than 4 h, confluent growth was obtained on the majority of the TSA plates. In order to render such plates countable, the bulk of the attached cells which formed the biofilms were removed by vigorous shaking in saline. This procedure was intended to leave those cells which were firmly attached to the test surface but to remove the majority of cells which were attached only to other cells. Tiles were removed from the cassette, after incubation for 8–48 h, transferred to individual Universal bottles containing 10 ml sterile saline and shaken vigorously in a flask shaker (Griffin and George Scientific, London, UK) for 15 min. Preliminary experiments, which followed the dispersal of bacteria from the tiles to the saline during shaking, established that no further removal of cells occurred after 10 min. Tiles were removed and further rinsed in three successive 20-ml volumes of sterile saline. Viable counts performed on the rinsings established that less than 1 cfu ml^{-1} was carried passively into a fourth rinse. After the tiles were rinsed they were transferred, using forceps, to a succession of TSA plates (as before). In each case no less than eight replicate tiles were taken through the plate succession.

Results and discussion

The initial intention of these experiments was to develop a cheap, reliable method by which the numbers of microorganisms that had colonised a surface could be readily quantified. Previous workers had made single impressions of colonised test surfaces onto agar, and had related the numbers of transferred organisms to the degree of surface attachment [15]. Such approaches had been used to relate the adhesiveness of various microorganisms with their surface characteristics ie cell surface hydrophobicity, cell sur-

face charge, fimbriation and growth phase [15,20]. Clearly, whilst such methods gave quantitative differences between different organisms and different test surfaces they accounted only for those organisms which could be removed by a single blot onto agar. It is unclear therefore whether the numbers obtained relate to the total number of organisms which attach to a surface or to that fraction which can be easily removed.

Attachment of microorganisms to surfaces

In the present study, conditions were established such that loosely attached cells were rinsed from the tiles before transfer to the agar plates. Therefore all colonies arising on the TSA plates were derived from cells which had resisted removal either by rinsing or, for 8 to 48-h biofilms, vigorous shaking in saline. Unlike methods previously described [15], a succession of impressions was made from each coverslip. Various initial cell densities were investigated for each organism and numbers were chosen which gave countable colonies on the succession of plates. *S. epidermidis* and *P. aeruginosa* were employed at an initial cell density of 10^5 cells ml^{-1} whereas *E. coli* was used at an initial cell density of 10^6 cells ml^{-1}.

Results were expressed as the mean of the number of cfu transferred from eight replicate tiles to each succession of 15 TSA plates. Initial experiments conducted with glass coverslips showed that the mean cfu decreased as an exponential function of the plate succession number (Figure 1). Regression analysis was performed using an SPSS Statistics Package, and lines of best fit were determined (Table 1). Data were fitted to Equation 1, where CFU is the number of organisms transferred to any given set of TSA plates, A is a constant, N is the plate succession number and k is a reduction exponent.

$$CFU = A.\ 10^{-kN} \qquad (1)$$

The reduction exponents for the lines of best fit differed significantly between *P. aeruginosa* and the two other organisms. Since k and A might have been affected by the numbers of organisms colonising the test surface at the time of taking the impressions, the 2-min contact experiments were repeated using lower initial cell densities. Data are illustrated in Figure 2 for *Pseudomonas aeruginosa* and described numerically for all three organisms in Table 1. These data show that whilst the constant A was affected by the initial cell density, and hence colonisation density, k was unchanged for any given organism.

Colonies appearing on the TSA plates correspond only to those cells which are able to detach during the manipulations. They would not indicate if any cells remained firmly attached to the glass at the end of the plate succession. Accordingly, the test tile was incubated, face-down on the surface of the final plate in the succession (number 16). This plate contained tetrazolium dye (0.05% w/v) which would stain metabolically active cells and microcolonies. After incubation, these plates were examined under a low power, optical microscope, and the number of microcolonies counted. These counts corresponded in all cases to the cfu predicted by the regression equation. This suggests that the equations describe the ease of removal of all

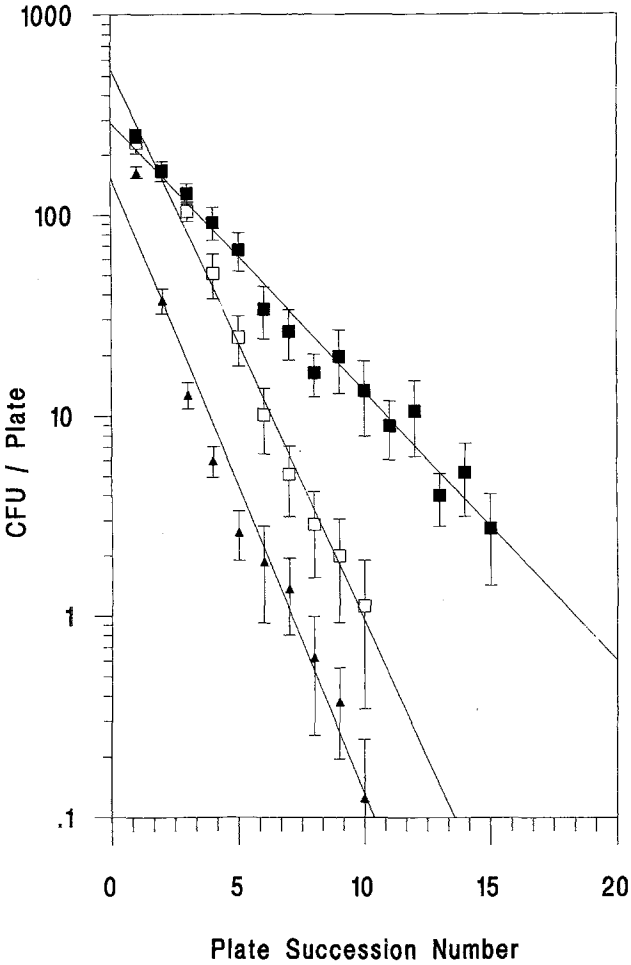

Figure 1 Removal of *Escherichia coli* (▲), *Pseudomonas aeruginosa* (■) and *Staphylococcus epidermidis* (□) from glass coverslips colonised for 2 min by immersion in inoculated medium, upon successive transfer and blotting on TSA plates. Error bars correspond to the standard error of the mean of eight replicates

Table 1 Regression analysis of cfu removed from glass coverslips colonised for 2 min by immersion in inoculated medium, upon successive transfer and blotting on TSA plates. CFU = $A.10^{kN}$, where CFU is the number of colonies transferred, k is the removal exponent, A the intercept and N the plate succession number

Organism	Initial cell density	A	k	r^2
Pseudomonas aeruginosa	10^5 cells ml^{-1}	290	−0.13	0.97
	10^4 cells ml^{-1}	38	−0.15	0.93
Escherichia coli	10^7 cells ml^{-1}	820	−0.28	0.93
	10^6 cells ml^{-1}	151	−0.31	0.97
Staphylococcus epidermidis	10^5 cells ml^{-1}	546	−0.28	0.99
	10^4 cells ml^{-1}	39	−0.29	0.87

of the microorganisms colonising the test pieces rather than a sub-population of cells of loosely attached cells.

Student t-tests, conducted on the k value of each organism generated for different initial cell densities, showed the lines to be parallel ($P > 0.95$). This indicated that the slope from such a relationship could be used to quantify the ease of removal of an attached organism from a surface. Such a value could be used indirectly to infer the strength of

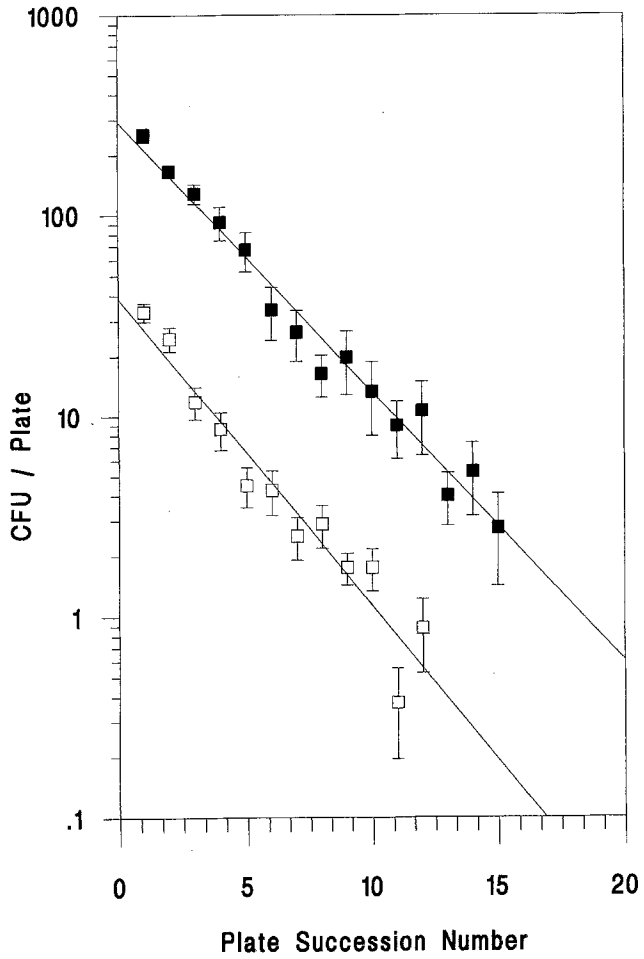

Figure 2 Removal of *Pseudomonas aeruginosa* from glass slides, colonised for 2 min by immersion in inoculated medium, upon successive transfer and blotting on TSA plates. Initial cell densities in the inoculated medium were 10^5 cells ml^{-1} (solid symbols) and 10^4 cells ml^{-1} (open symbols). Error bars correspond to the standard error of the mean of eight replicates

attachment. In this respect it is interesting to note that whilst *S. epidermidis* is the organism with the most hydrophobic surface, and from the colonisation densities and previous published work [15,24] would be predicted to attach most avidly to the surface, this is not reflected in its ease of removal from glass, or indeed from any of the other test surfaces (Table 2). Accordingly, of the three test strains this organism attaches to glass to the greatest extent yet is most easily removed. Such observations have not previously been reported.

Effect of test surface on the ease of removal

Further experiments were conducted with *P. aeruginosa* and *S. epidermidis* to determine the effect that different substrata might have on the ease of removal. Tiles, of the same dimension as coverslips, were made from materials which differ in terms of their surface chemistries, surface free energies and roughnesses. Surface properties such as these are widely reported to affect the attachment of bacteria [15,20,24] but were not measured for the test materials since their use was intended to demonstrate whether or not the removal exponent could distinguish between different

Table 2 Regression analysis of cfu removed from various test surfaces, colonised for 2 min by immersion in TSB inoculated either with *Pseudomonas aeruginosa* (10^5 cells ml^{-1}) or *Staphylococcus epidermidis* (10^5 cells ml^{-1}), upon successive transfer and blotting on TSA plates. $CFU = A.10^{kN}$, where CFU is the number of colonies transferred, k is the removal exponent, A is the intercept and N is the plate succession number

Test surface	A	k	r^2
(a) Pseudomonas aeruginosa			
Stainless steel	308	−0.09	0.99
Formica	297	−0.07	0.97
Teflon	296	−0.15	0.99
Polyvinylchloride	221	−0.10	0.96
Polystyrene	281	−0.14	0.97
Polyethylene	346	−0.10	0.99
Glass	290	−0.13	0.97
(b) Staphylococcus epidermidis			
Stainless steel	440	−0.23	0.94
Formica	477	−0.23	0.95
Teflon	233	−0.25	0.98
Polyvinylchloride	799	−0.54	0.92
Polystyrene	283	−0.37	0.98
Polyethylene	470	−0.24	0.99
Glass	546	−0.28	0.99

substrata rather than form a separate study of adhesion phenomena.

For both organisms, incubation of the blotted test pieces on TT agar confirmed, as for the glass slides, that there were very few residual attached cells. Optically opaque materials were overlayed, after blotting, with TT agar in order to facilitate viewing.

Figure 3 illustrates the results for *P. aeruginosa* whilst results for both organisms are summarised in Table 2. These data indicate different strengths of attachment for each organism onto each surface type, with high values of k indicating a weaker attachment and *vice versa*. Exponents were generally higher for *S. epidermidis* than *P. aeruginosa*, indicating a looser attachment of the *S. epidermidis* strain. Exponents for the various test surfaces, varied from −0.07 to −0.15 for *P. aeruginosa* and from −0.23 to −0.54 for *S. epidermidis*. Such results clearly demonstrate the potential of this approach to distinguish between different materials in terms of their colonisation and shedding potential.

Since the physical characteristics of the test surfaces have not been fully characterised, it is not possible to discuss their relative merits for use as hygienic surfaces. Materials which are used routinely as food production surfaces, such as stainless steel and Formica, were however notable not only in terms of their high colonisation potential (A) but also in that they retain both species of bacteria the most strongly. It is also interesting that, with the exception of Formica and stainless steel, the rank order of adhesiveness of the test surfaces differed between organisms. *In situ* this would lead to a selective concentration, with time, of one organism relative to another and lead to an enrichment on the surface of particular organisms. If such an organism were a potential pathogen such as *Listeria* spp then the implications to food manufacturing areas are of concern.

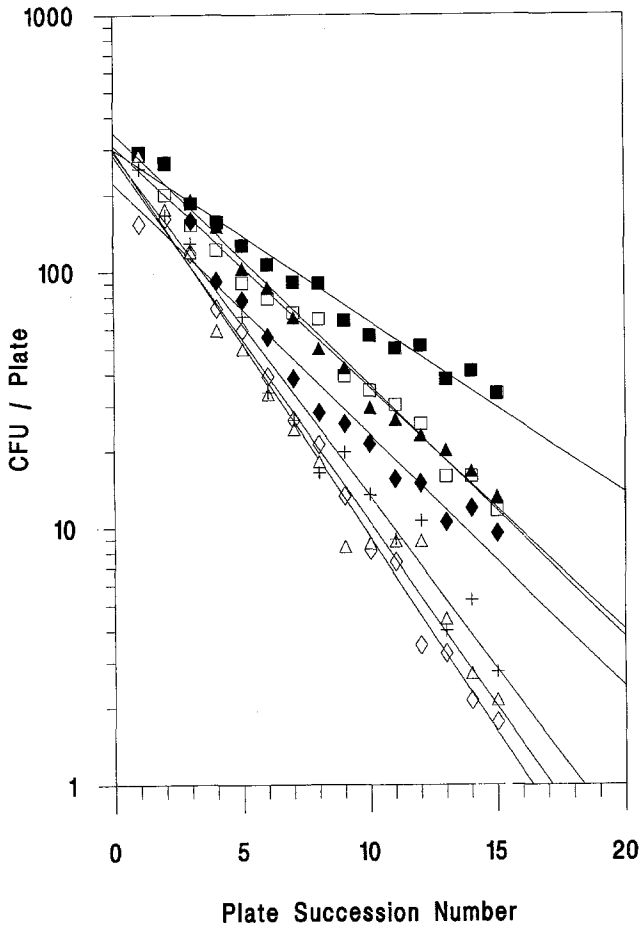

Figure 3 Removal of *Pseudomonas aeruginosa* from tiles of various materials colonised for 2 min by immersion in inoculated medium, upon successive transfer and blotting on TSA plates. Tiles were made of steel (□); Formica (■); Teflon (◇); PVC (◆); Polystyrene (△); polyethylene (▲) and glass (+)

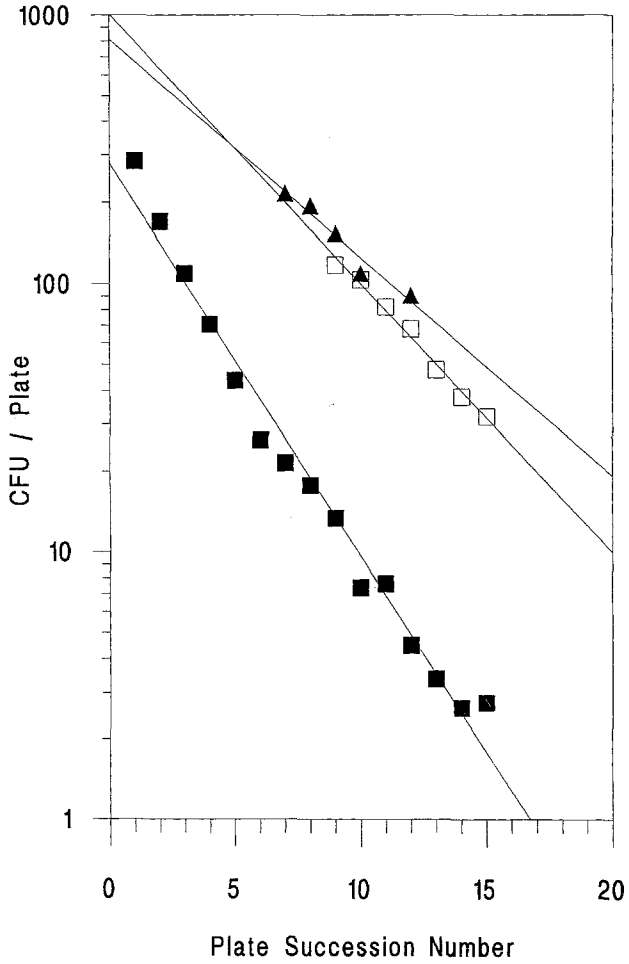

Figure 4 Removal of *Pseudomonas aeruginosa* from glass slides, colonised for various times by immersion in inoculated medium, upon successive transfer and blotting onto TSA plates. Colonisation times were (■) 8 h; (□) 16 h and (▲) 48 h

Effect of colonisation time on the ease of removal of P. aeruginosa *from glass*

Biofilms of *P. aeruginosa* were formed on glass coverslips over 8-h, 16-h and 48-h incubation periods (Figure 4). The numbers of loosely attached biofilm cells were reduced by vigorous shaking in a flask shaker. This procedure was intended to leave those cells which were firmly attached to the test surface but removed the majority of cells which were attached only to other cells. The ease of removal of the remaining cells was determined by the plate succession technique. In most instances vigorous shaking was sufficient to reduce the numbers of attached organisms to levels which permitted colony counts to be performed on the blotted TSA impression plates, for the 16- and 48-h biofilms, however, the first few plates of the succession were overgrown and could not be counted. Summary data are presented in Table 3. Intercept values (A) were increased markedly by prolonged incubation of the biofilm beyond 8 h. Since growth of the planktonic cells had progressed into stationary phase by 8 h, this probably indicates an increase in the proportion of cells which were able to resist removal by vigorous shaking rather than continued growth of the biofilm. Coupled with this increase in the proportion of firmly attached cells, the strength of attachment, indicated by the k value, was substantially increased with biofilm aging. This strengthening of the attachment continued up to 48 h and might reflect continued matrix polymer deposition after cessation of cell division. Alternatively the data might indicate qualitative changes in exopolymers during biofilm maturation.

Table 3 Regression analysis of cfu removed from various test surfaces, colonised by immersion in TSB inoculated with *Pseudomonas aeruginosa* (10^5 cells ml^{-1}) and incubated at 37° C for 8–48 h, upon successive transfer and blotting on TSA plates. CFU = $A.10^{kN}$, where CFU is the number of colonies transferred, k is the removal exponent, A is the intercept and N is the plate succession number

Incubation time (h)	A	k	r^2
8	227	–0.15	0.98
16	1007	–0.10	0.99
48	813	–0.08	0.95

In the present study conformity to first order kinetics of the numbers of transferred colonies with plate succession number suggests a uniform strength of attachment of the organisms to the surfaces with fixed probabilities of

removal at each blotting step. The removal exponents derived from these relationships were independent of the numbers of organisms attached initially (Figure 2), but differed between organisms (Figure 1), between different substrata for the same organism (Figure 3) and changed with age of the developed biofilm (Figure 4, Table 3). Such changes will reflect not only the surface properties of the bacteria and their ability to produce exopolymers, but also the physical characteristics of the substrata, such as hydrophobicity, charge and roughness. It is our proposition that the removal exponent could be used as a simple, cheap and effective measure of the strength of attachment of organisms to a variety of surfaces. The method is able to distinguish between different surfaces and microorganisms and will prove useful in the design of treatment regimens for surface hygiene and in the selection of suitable materials for construction of the fabric of hygienic installations. Modification of the approach through the use of successive impression plates on surfaces *in situ* is currently being investigated as a means to field testing of surfaces and hygienic cleaning regimens.

Acknowledgements

PJE would like to acknowledge an EPSRC Total Technology Studentship awarded in conjunction with the Campden Food and Drink Research Association, which has made this work possible.

References

1 Allison DG, MRW Brown, DJ Evans and P Gilbert. 1990. Surface hydrophobicity and dispersal of *Pseudomonas aeruginosa* from biofilms. FEMS Microbiol Lett 71: 101–104.
2 Allison DG, DJ Evans, MRW Brown and P Gilbert. 1990. Possible involvement of the division cycle in dispersal of *Escherichia coli* from biofilms. J Bacteriol 172: 1667–1669.
3 Anwar H, MK Dasgupta, K Lam and JW Costerton. 1989. Tobramycin resistance of mucoid *Pseudomonas aeruginosa* biofilm grown under iron-limitation. J Antimicrob Chemother 24: 647–655.
4 Anwar H, JL Strap and JW Costerton. 1992. Establishment of aging biofilms: possible mechanism of bacterial resistance to antimicrobial chemotherapy. Antimicrob Agents Chemother 36: 1347–1351.
5 Brown MRW, DG Allison and P Gilbert. 1988. Resistance of bacterial biofilms to antibiotics: a growth rate related effect. J Antimicrob Chemother 22: 777–789.
6 Costerton JW, RT Irvin and KJ Cheng. 1981. The bacterial glycocalyx in nature and disease. Ann Rev Microbiol 35: 399–424.
7 Costerton JW and ES Lashen. 1984. Influence of biofilm on efficacy of biocides on corrosion-causing bacteria. Mat Perform 9: 13–17.
8 Costerton JW, KJ Cheng, GG Geesy, TI Ladd, JC Nickel, M Dasgupta and TJ Marrie. 1987. Bacterial biofilms in nature and disease. Ann Rev Microbiol 41: 435–464.
9 Dagostino L, AE Goodman and KC Marshall. 1991. Physiological responses induced in bacteria adhering to surfaces. Biofouling 4: 113–119.
10 Evans E, MRW Brown and P Gilbert. 1994. Iron chelator, exopolysaccharide and protease production on *Staphylococcus epidermidis*: a comparative study of the effects of specific growth rate in biofilm and planktonic culture. Microbiology 140: 153–157.
11 Fletcher M. 1984. Comparative physiology of attached and free-living bacteria. In: Microbial Adhesion and Aggregation (Marshall KC, ed), pp 223–232, Springer-Verlag, Berlin.
12 Gambello MJ, S Kaye and BH Iglewski. 1993. *LasR* of *Pseudomonas aeruginosa* is a transcriptional activator of the line protease gene (*apr*) and an enhancer of exotoxin A expression. Infect Immun 61: 1180–1184.
13 Gibbons RJ and J van Houte. 1975. Bacterial adherence in oral microbial ecology. Ann Rev Microbiol 29: 19–44.
14 Gilbert P, PJ Collier and MRW Brown. 1990. Influence of growth rate on susceptibility to antimicrobial agents: biofilms, cell cycle and dormancy. Antimicrob Agents Chemother 34: 1865–1868.
15 Gilbert P, DJ Evans, E Evans, IG Duguid and MRW Brown. 1991. Surface characteristics and adhesion of *Escherichia coli* and *Staphylococcus epidermidis* in batch culture. J Appl Bacteriol 71: 72–77.
16 Giwercman B, ET Jensen, N Hoiby, A Kharazmi and JW Costerton. 1991. Induction of β-lactamase production in *Pseudomonas aeruginosa* biofilms. Antimicrob Agents Chemother 35: 1008–1010.
17 Holah JT, SF Bloomfield, AJ Walker and H Spenceley. 1994. Control of biofilms in the food industry. In: Bacterial Biofilms and Their Control in Medicine and Industry (Wimpenny J, W Nichols, D Stickler and H Lappin-Scott, eds), pp 163–168, Bioline Press, Cardiff.
18 Hoyle BD, J Jass and JW Costerton. 1990. The biofilm glycocalyx as a resistance factor. J Antimicrob Chemother 26: 1–6.
19 Lappin-Scott HM and JW Costerton. 1989. Bacterial biofilms and surface fouling. Biofouling 1: 323–342.
20 Marshall KC, R Stout and R Mitchell. 1971. Mechanism of the initial events in the sorption of marine bacteria to surfaces. J Gen Microbiol 68: 337–348.
21 May TB and AM Chakrabarty. 1994. *Pseudomonas aeruginosa*: genes and enzymes of alginate synthesis. Trends Microbiol 2: 151–157.
22 McCoy WF, JD Bryers, J Robbins and JW Costerton. 1981. Observations in fouling biofilm formation. Can J Microbiol 27: 910–917.
23 Sutherland IW. 1977. Bacterial polysaccharides—their nature and production. In: Surface Carbohydrates of the Procaryotic Cell (Sutherland IW ed), pp 27–96, Academic Press, London.
24 van Loosdrecht MCM, J Lyklema, W Norde and AJB Zehnder. 1990. Influence of interfaces on microbial activity. Microbiol Rev 54: 75–87.
25 Zobell CE. 1943. The effect of solid surfaces upon bacterial activity. J Bacteriol 46: 39–56.