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Biocides incorporated into plasticized polyvinylchloride reduce adhesion of *Pseudomonas fluorescens* BL146 and substratum hydrophobicity

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C.R. JONES, P.S. HANDLEY, G.D. ROBSON, I.M. EASTWOOD AND M. GREENHALGH. 1996. A quantitative adhesion assay was developed to monitor attachment of *Pseudomonas fluorescens* BL146 to discs of plasticized polyvinylchloride (pPVC) with and without incorporated biocides. Adherent cells were quantified by radiolabelling with DL-[4,5-^{3}H]leucine. Adhesion reached a maximum after 6 h incubation at an initial cell concentration of 5×10^7 cells ml^{-1}. The adhesion assay was used to compare bacterial attachment to pPVC containing the biocides 10,10-oxybisphenoxyarsine (OBPA), 2-*n*-octyl-4-isothiazolin-3-one (OIT), 2,3,5,6-tetrachloro-4-(methylsulphonyl)pyridine (TCMP) and *N*-trichloromethylthiophthalimide (NCMP) at 0, 250, 750 and 2250 ppm. All four biocides reduced adhesion with increasing concentration, with statistically significant reductions in adhesion ($\geqslant 53\%$) occurring with OBPA, OIT and TCMP at 2250 ppm. Significant reductions in adhesion to pPVC containing OBPA were found whether adhering cells were viable or non-viable. The hydrophobicity of the pPVC surfaces was quantified by the measurement of water contact angles using the Wilhelmy plate technique. A trend of reduced hydrophobicity was observed with increasing biocide concentration. Incorporation of all four biocides at 2250 ppm caused statistically significant reductions in contact angle from 104·7° to a minimum of 93·5°. Incorporation of biocides into pPVC therefore concurrently reduces both bacterial adhesion and surface hydrophobicity.

INTRODUCTION

Biofouling of plasticized polyvinylchloride (pPVC) swimming pool liners results in the deterioration of both the appearance and the mechanical integrity of the pPVC (Lorenz 1990). In addition the associated microbial fouling of pool water may pose health problems, for example, levels of greater than 10^3 colony-forming units ml^{-1} of *Pseudomonas aeruginosa* in water have been found to constitute a significant threat of folliculitis to bathers (Price and Ahearn 1988). The routine application of biocides to pool water is usually effective in destroying those microbes freely suspended in the bulk water (Wyatt 1993). However, microbes adhering to the pPVC pool liner may be less susceptible to such treatments. The increased resistance of microbes adhering to surfaces over their planktonic counterparts is well recognized (Anwar *et al.* 1990; LeChevalier 1991). Incorporation of biocides into the pPVC liner attempts to address this problem by attacking microbes on, or in close proximity, to the interface. These agents may be directly effective through their biocidal activity, but they may also be active by causing changes in the physicochemical properties of the surface, discouraging microbial adhesion. Reductions in adhesion of *Ps. aeruginosa* have been shown following the incorporation of increasing concentrations of a biocide composed of polysubstituted quaternary amine and trialkylphosphate esters (Intersept), into the polymer, ethyl vinyl acetate (Price *et al.* 1991). In conjunction with reduced bacterial adhesion, Price *et al.* (1991) also suggested that the hydrophobicity of the polymer surfaces was reduced with increasing biocide concentration, although this was not described in detail.

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Reduced substratum hydrophobicity has been associated with reduced bacterial adhesion in a number of studies (Fletcher and Loeb 1979; Pringle and Fletcher 1983; Van Pelt *et al.* 1985; Bidle *et al.* 1993) although this is not always the case. For example, Busscher *et al.* (1990) found increased adhesion of *Streptococcus sanguis* to surfaces of reduced hydrophobicity whilst Denyer *et al.* (1993) found optimal adhesion of *Staphylococcus epidermidis* to polystyrene surfaces over a specific hydrophobicity range. Such conflicting evidence suggests that there is probably no clear relationship between the hydrophobicity of substrata and bacterial adhesion, so that the effects of changes in substratum hydrophobicity on adhesion must be considered separately for each substratum and bacterial species. The effect of the incorporation of biocides into pPVC on surface hydrophobicity and subsequent bacterial adhesion has not been previously examined.

In this study, we investigated the effect of incorporating biocides currently used in pPVC on the adhesion of viable and non-viable cells of *Pseudomonas fluorescens* BL146, and on the hydrophobicity of the pPVC surface.

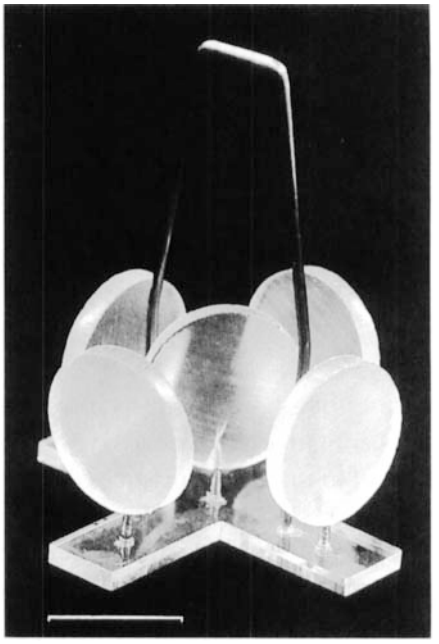

Fig. 1 Disc assembly. Bar represents 1 cm

MATERIALS AND METHODS

Bacterial strain and growth conditions

Pseudomonas fluorescens BL146 (kindly provided by Dr D. Greenway, University of Central Lancashire) was isolated from a biofilm on a pPVC swimming pool liner in the UK. *Pseudomonas fluorescens* BL146 was maintained on solid minimal medium (MM) containing (g l^{-1}): K_2HPO_4, 7·0; KH_2PO_4, 3·0; $Na_3C_6H_5O_7.2H_2O$, 0·5; $MgSO_4.7H_2O$, 0·1; $(NH_4)_2SO_4$, 1·0; glucose, 2·0; and Bacteriological Agar (Oxoid), 15, at 25°C. Batch cultures for use in the adhesion assay were grown in liquid MM and incubated at 30°C in a shaking waterbath (180 strokes min^{-1}) for 21 h.

Adhesion assay

The pPVC was formulated containing (parts per hundred resin): 571/102 Corvic (PVC resin), 100; dioctyl phthalate (plasticizer), 25; dioctyl adipate (plasticizer), 25; Lankromark LZ 935 (AKCROS) (calcium/zinc stabilizer), 2; Lankroflex Ed 6 (AKCROS) (Epoxidized oleate ester), 3; stearic acid, 0·2; and calcium stearate, 0·5. The biocides, 10,10-oxybisphenoxyarsine (OBPA), 2-*n*-octyl-4-isothiazolin-3-one (OIT), *N*-trichloromethylthiophthalimide (NCMP) and 2,3,5,6-tetrachloro-4-(methylsulphonyl)pyridine (TCMP) were incorporated separately into the pPVC formulation at concentrations of 0, 250, 750 and 2250 ppm. The biocides were added to the pPVC formulation together with the plasticizers. The pPVC was mixed on a two roll mill at 160°C for 1·5–2·0 min and laminated into 30 cm^2 sheets ≈2 mm thick on a 50 t hydraulic heated parallel press (Moore) at 160°C. These sheets were stored separately at room temperature in the dark to avoid u.v. degradation. When required, sheets were cut into discs using a 1·2 cm diameter stainless steel punch and die in conjunction with a fly press (Norburt 4). In order to avoid surface contamination the punch was swabbed with 70% ethanol prior to usage and discs handled by the edges only, using forceps. Five discs derived from the same sheet were secured vertically onto the stainless steel pins of a support, together composing one disc assembly (Fig. 1) which was then placed into a 25 ml beaker.

Pseudomonas fluorescens BL146 was grown in liquid MM containing 2 μCi ml^{-1} DL-[4,5-^{3}H]leucine (Amersham) for 21 h and harvested by centrifugation at 2800 *g* for 10 min. Cells were resuspended in an equal volume of phosphate-buffered saline (PBS) containing (g l^{-1}): NaCl, 8·00; KCl, 0·20; Na_2HPO_4, 1·15; KH_2PO_4, 0·20; $CaCl_2$, 0·10; and $MgCl_2$, 0·10, at pH 7·3 and centrifuged a second time. This washing procedure was carried out three times and the cells were then resuspended in PBS. Four 50 μl samples of this cell suspension were counted using a 2000 CA Tricarb liquid scintillation analyser (United Technologies, Packard) in 3·5 ml of scintillation fluid (Ecoscint A).

The above radiolabelled *Ps. fluorescens* BL146 cell suspension was aliquoted (13 ml) into 25 ml beakers, each containing one disc assembly. PVC discs were incubated in this cell suspension at 30°C in a shaking waterbath (180 strokes min^{-1}). Each assembly was dipped three times into 40 ml of distilled water to remove non-adherent cells. pPVC discs were counted in a liquid scintillation analyser in 3·5 ml of scintillation fluid. Percentage adhesion was calculated as follows:

% adhesion = (no. counts pPVC disc^{-1}/

no. counts ml^{-1} cell suspension) × 100

A cell concentration of $2{\cdot}5 \times 10^8$ cells ml^{-1} (O.D.$_{440\,nm}$ = 0·38) was used together with an incubation period of 1 h before adhesion assay conditions were optimized.

Reproducibility (intra-batch variation). Five biocide-free disc assemblies were prepared as described above and exposed to cells derived from one *Ps. fluorescens* BL146 batch culture. Five discs were sampled from each of five disc assemblies.

Reproducibility (inter-batch variation). Six biocide-free disc assemblies were exposed to cells derived from three *Ps. fluorescens* BL146 batch cultures. Ten discs were sampled from each batch.

Optimizing the time for maximum percentage adhesion. Six biocide-free disc assemblies were prepared as described above and five discs sampled at 2 h intervals from 0 to 12 h.

Optimizing the cell concentration for maximum percentage adhesion. Eleven biocide-free disc assemblies were exposed to *Ps. fluorescens* BL146 cell concentrations of O.D.$_{440\,nm}$ from 0 to 1·00. Five discs were sampled at each cell concentration after the time for maximum adhesion (6 h).

The effect of incorporated biocides in pPVC on percentage adhesion. Percentage adhesion was measured for pPVC containing the four different biocides OBPA, OIT, NCMP and TCMP at 0, 250, 750 and 2250 ppm using a cell concentration of 5×10^7 cells ml^{-1} and a 6 h incubation time. Five discs were sampled for each biocide concentration.

Adhesion of viable and non-viable cells to pPVC with incorporated biocide. Percentage adhesion was measured for pPVC containing the biocide OBPA at 0, 250, 750 and 2250 ppm using ultraviolet (u.v.)-irradiated cells and untreated cells at 5×10^7 cells ml^{-1} and a 6 h incubation time. Cells were u.v.-irradiated using a Model R-52G lamp 200–250 V, 50–60 Hz (UVP Inc., San Gabriel, USA) for 1 min, causing a reduction in viability of >99·999%. To determine percentage adhesion 10 discs were sampled at each biocide concentration for non-viable and viable cells.

Scanning electron microscopy

Plasticized PVC discs without incorporated biocide were incubated with 5×10^7 cells ml^{-1} *Ps. fluorescens* BL146 for 6 h (optimized assay conditions) and freeze-dried (Edwards, model B5 A) overnight. Discs were attached to stubs using Electrodag 915 (Acheson), dried overnight and sputter coated (S150, Edwards) with gold. Specimens were examined on a Stereoscan 360 Scanning electron microscope (SEM) (Cambridge Instruments).

Contact angle measurements

Contact angle measurements on pPVC surfaces were determined using a Dynamic Contact Angle analyser (DCA) (Cahn Instruments, CA) (Domingue 1990). DCA measurements employed the Wilhelmy plate technique allowing a large surface area to be sampled (Lander *et al.* 1993). A surface area of 2 cm^2 of pPVC was sampled by immersing 2 × 3 cm pPVC rectangles to a depth of 1 cm in distilled water at a stage speed of 19·6 μm s^{-1}. Contact angles were calculated automatically by the DCA from the equation:

$$\cos\theta = F/\,p.y$$

where θ is the contact angle, F is the force (dynes) measured by the electrobalance, p is the perimeter of the sample and y is the surface tension (dynes cm^{-1}) of the wetting liquid. Advancing contact angles (θ_a) were measured on control (biocide-free) pPVC, and pPVC containing the biocides OBPA, OIT, NCMP and TCMP at 250, 750 and 2250 ppm. Contact angle measurements were made on five replicate samples of each type of pPVC.

RESULTS

Adhesion assay

Reproducibility. The reproducibility of the assay was analysed statistically using analysis of variance. There was no significant variation ($P > 0{\cdot}05$) between adhesion values derived from discs incubated in the same batch of *Ps. fluorescens* BL146 cells (intrabatch variation). This was calculated for mean adhesion values from five discs, each from five separate disc assemblies incubated in one batch of cells. However, there was significant variation between adhesion values incubated in different batches of *Ps. fluorescens* BL146 cells (interbatch variation). This was calculated for mean adhesion values from 10 discs, from two disc assemblies, incubated in three different batches of cells. Percentage adhesion for different batches varied from ≈4 to 20%. In order to eliminate this source of variation subsequent adhesion experiments were carried out using cells from the same batch of cells.

Optimizing conditions for the adhesion assay. To determine the time taken to attain maximum adhesion, percentage adhesion of *Ps. fluorescens* BL146 to control pPVC without

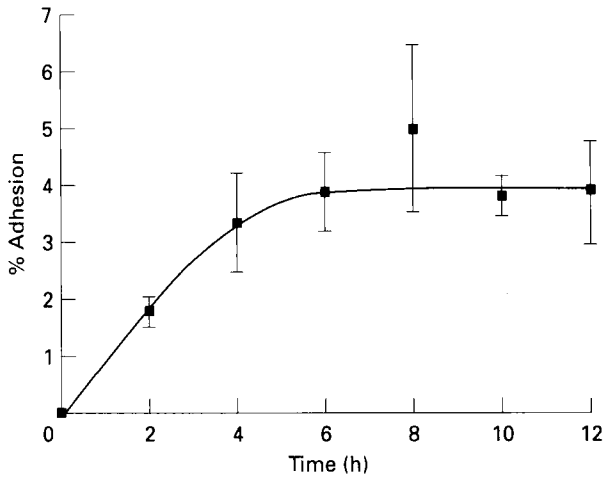

Fig. 2 Time course of adhesion of *Pseudomonas fluorescens* BL146 to control pPVC. Each data point represents the mean percentage adhesion from one pPVC assembly (five pPVC discs). The vertical bars show standard deviations

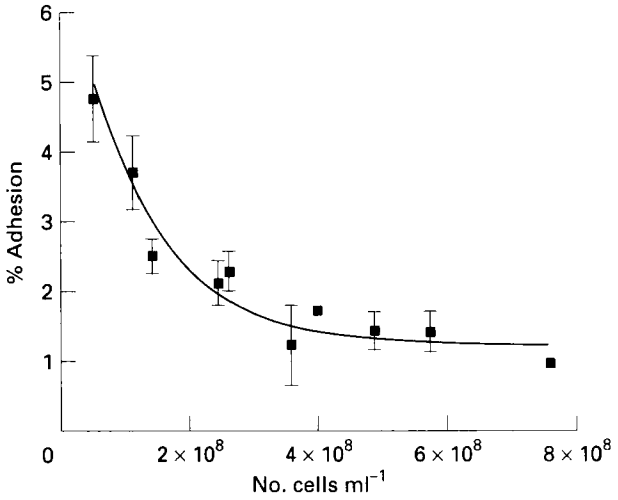

Fig. 3 The influence of cell density on adhesion of *Pseudomonas fluorescens* BL146 to control pPVC. Each data point represents the mean percentage adhesion from one pPVC assembly (five pPVC discs). The vertical bars show standard deviations

biocide was followed over a 12 h time period (Fig. 2). Adhesion increased steadily up to 4 h, slowing down between 4 and 6 h and reaching a plateau after 6 h at adhesion levels of $\approx 4\%$ for this batch of cells. Maximal adhesion occurred after 6 h, which was subsequently chosen as the incubation period for the adhesion assay. Observations of the *Ps. fluorescens* BL146 cell suspension by phase contrast microscopy showed $\approx 35\%$ of cells were motile after this 6 h incubation period.

To determine the optimum cell concentration for use in the adhesion assay, percentage adhesion was followed after 6 h at concentrations of *Ps. fluorescens* BL146 ranging from 5×10^7 cells ml^{-1} to $7{\cdot}5 \times 10^8$ cells ml^{-1}. There was a reduction in percentage adhesion with increasing cell concentrations up to $\approx 5 \times 10^8$ cells ml^{-1} (Fig. 3). At cell concentrations above 5×10^8 cells ml^{-1} percentage adhesion remained constant at $\approx 1\%$. A cell density of 5×10^7 cells ml^{-1} was chosen for use in future assays, since the high percentage adhesion at this cell concentration would be sensitive to reductions in adhesion due to the incorporated biocide.

SEM examination of the pPVC with adherent bacteria

SEM studies of adherent *Ps. fluorescens* BL146 cells under the optimized assay conditions of incubation in 5×10^7 cells ml^{-1} for 6 h, showed cells scattered singly over the PVC surface (Fig. 4). Cells were unevenly distributed over the pPVC, some fields of view showing no adherent cells at all. The pPVC surface was uneven with striations on the surface produced during the manufacturing process which were also visible to the naked eye. There was no difference in striations between batches of discs, as observed by SEM.

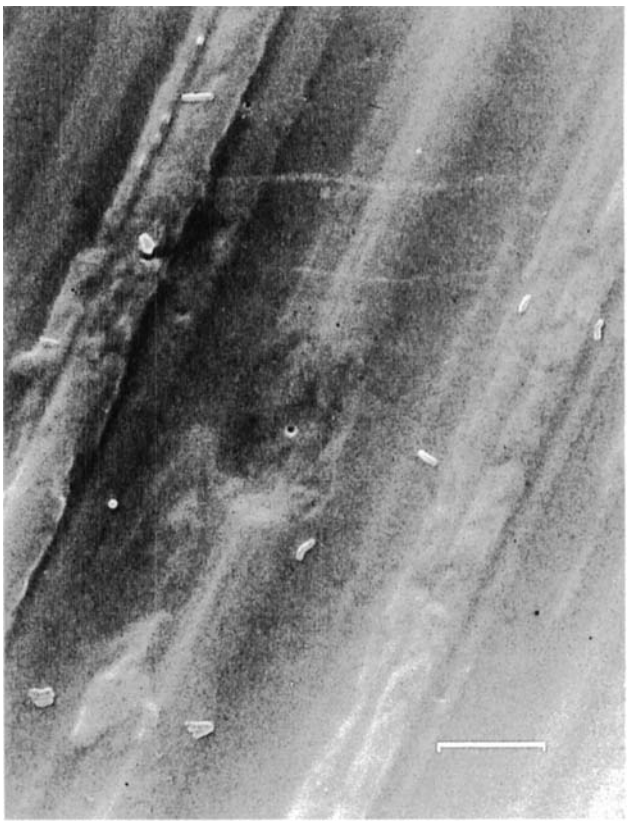

Fig. 4 SEM of the pPVC surface following incubation in 5×10^7 *Pseudomonas fluorescens* BL146 cells ml^{-1} for 6 h and subsequent washing in distilled water to remove non-adherent cells. Single cells were distributed sparsely over the pPVC surface. Some areas of the pPVC were void of any attached cells. Bar represents 10 μm

Adhesion and water contact angles of pPVC with incorporated biocides

The effect of biocide incorporation into pPVC on the percentage adhesion of *Ps. fluorescens* BL146 and the water contact angle of the surface are shown in Fig. 5. For each of the four biocides, both percentage adhesion and water contact angle decreased as the concentration of the incorporated biocide increased. Unpaired *t*-tests were used to compare the different concentrations of the four biocides with respect to percentage adhesion and water contact angles. Following the incorporation of 250 ppm OBPA into pPVC (Fig. 5a), adhesion was significantly reduced by 34% compared with the control ($P < 0{\cdot}02$). Subsequent increases in levels of OBPA in pPVC to 750 ppm caused a further reduction in adhesion by 62% ($P < 0{\cdot}001$). However, only an additional 10% decrease was achieved by increasing OBPA levels to 2250 ppm. The contact angle declined significantly ($P < 0{\cdot}02$) from 104·7° for control pPVC with no biocide to 97·9° at 2250 ppm OBPA. The reduction in contact angle was gradual, with no significant difference ($P > 0{\cdot}05$) between 250 and 750 ppm, and between 750 and 2250 ppm, respectively.

Incorporation of OIT at 250 ppm in pPVC (Fig. 5b) had no significant effect on *Ps. fluorescens* BL146 adhesion ($P > 0{\cdot}05$); however, further increases in the concentration of this biocide significantly reduced adhesion by 35% at 750 ppm ($P < 0{\cdot}02$), and 55% at 2250 ppm compared with the control ($P < 0{\cdot}002$). The contact angle was reduced significantly by 9° ($P < 0{\cdot}002$) following the incorporation of OIT at 2250 ppm compared with the control. However, there was no significant difference between the contact angles on pPVC with incorporated OIT at 250, 750 and 2250 ppm.

Incorporation of TCMP (Fig. 5c) at 250 and 750 ppm did not significantly reduce adhesion relative to pPVC without biocide ($P > 0{\cdot}05$). TCMP at a concentration of 2250 ppm caused a significant reduction (53%) in percentage adhesion ($P < 0{\cdot}002$). The contact angle of water on the pPVC surface

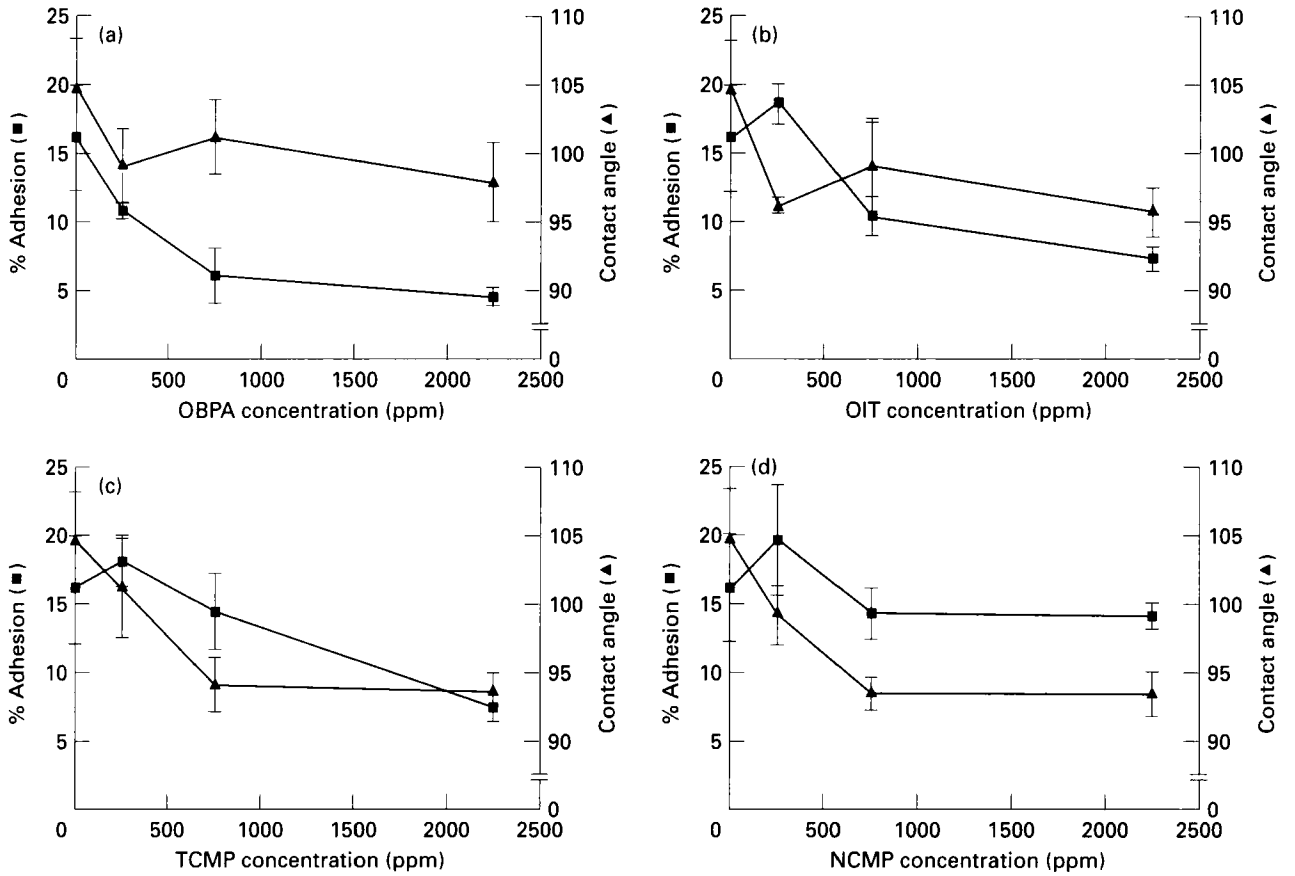

Fig. 5 The effect of the biocides (a) 10,10-oxybisphenoxyarsine (OBPA), (b) 2-*n*-octyl-4-isothiazolin-3-one (OIT), (c) 2,3,5,6-tetrachloro-4-(methylsulphonyl)pyridine (TCMP) and (d) *N*-trichloromethylthiophthalimide (NCMP), incorporated into pPVC on adhesion of *Pseudomonas fluorescens* BL146 to the pPVC surface and the water contact angle of the pPVC. Data points (■) represent the mean percentage adhesion derived from one pPVC assembly (five pPVC discs). Data points (△) represent the mean of five replicate contact angle measurements. The vertical bars show standard deviations

decreased with the incorporation of increasing concentrations of TCMP. At 250 ppm this reduction (3·4°) in contact angle was not significantly different from the control; however, significant reductions of 10·5° were found at 750 ppm ($P < 0{\cdot}001$) and 11° at 2250 ppm ($P < 0{\cdot}001$).

Incorporation of NCMP (Fig. 5d) in pPVC at the highest concentration examined (2250 ppm) caused only a 13% decrease in adherent *Ps. fluorescens* BL146. This was not significantly different from adhesion levels to the control ($P > 0{\cdot}05$) although there was an overall trend towards reduced adhesion. The contact angle of the pPVC was reduced significantly following incorporation of NCMP at 250 ppm by 5·6° ($P < 0{\cdot}02$), and at 750 ppm by 11·2° ($P < 0{\cdot}001$). Additional increases in levels of NCMP to 2250 ppm did not significantly reduce the contact angle any further.

Adhesion of viable and non-viable cells to pPVC with incorporated biocides

Treatment of the cell suspension with u.v. irradiation caused a net reduction in adhesion to pPVC of approximately one-third compared to untreated cells at all biocide concentrations (Fig. 6). There was a trend of reduced adhesion with increasing OBPA concentration for both viable and non-viable cells. Adhesion was significantly reduced relative to the control for both viable and non-viable cells at 750 ppm OBPA ($P < 0{\cdot}01$), and at 2250 ppm OBPA ($P < 0{\cdot}02$ and $P < 0{\cdot}001$, respectively).

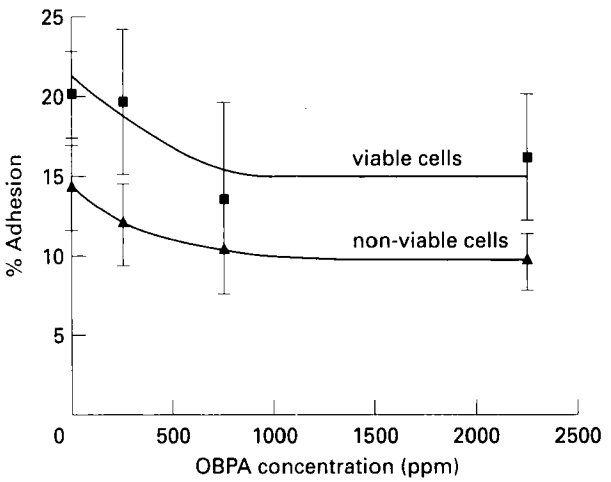

Fig. 6 The effect of the biocide 10,10-oxybisphenoxyarsine (OBPA) incorporated into pPVC on adhesion of viable (■) and non-viable (△) *Pseudomonas fluorescens* BL146 to the pPVC surface. Data points represent the mean percentage adhesion derived from two pPVC assemblies (10 pPVC discs). The vertical bars show standard deviations

DISCUSSION

The adhesion assay developed allowed accurate quantification of adherent *Ps. fluorescens* BL146 cell numbers. Adhesion levels were statistically reproducible within experiments using the same batch of cells. However, there were statistically significant differences between the adhesion levels for separate batches of cells, although the same overall trends in adhesion were always observed. Radiolabelling adhesion assays are widely used (Pringle and Fletcher 1983; Van Loosdrecht *et al.* 1989; Van der Mei *et al.* 1993), although few studies have investigated the reproducibility of the assay system. Significant differences in the percentage adhesion between different batches of cells have previously been reported for *Staphylococcus epidermidis* adhesion to polyurethane (John *et al.* 1995). Variation in adhesion levels between different batches of cells is therefore an important factor that should be considered in the design and interpretation of adhesion experiments. The reasons for intrabatch variation in adhesion levels are not understood. Cells harvested at different stages in the growth cycle in batch culture can have very different adhesive abilities (Gilbert *et al.* 1990), but this factor should not have influenced the results reported here, as cells of *Ps. fluorescens* were always harvested in stationary phase. In practice, since adhesion levels were consistent within batches, adhesion to pPVC surfaces with and without biocides could be validly compared.

Adhesion of *Ps. fluorescens* BL146 cells to pPVC reached a maximum after 6 h. Beyond 6 h there was no further net change in percentage adhesion. Lopez-Lopez *et al.* (1991) also found maximal adhesion of *Ps. aeruginosa* to PVC catheters after 6 h. However, other studies have reported maximum adhesion occurring after less than 5 min for a *Pseudomonas* strain adhering to hydrophobic polystyrene (Van Loosdrecht *et al.* 1989) compared to 70 min for *Ps. aeruginosa* on stainless steel (Vanhaecke *et al.* 1990). *Pseudomonas fluorescens* has also been shown to take five times longer to adhere to stainless steel and glass surfaces than *Ps. aeruginosa* in a fed flow system (Mueller *et al.* 1992). Such variations in the time taken to reach equilibrium are determined by the physicochemical properties of the bacteria, the substrata and the suspending media involved (Absolom *et al.* 1983). These factors will govern both the numbers of available binding sites and how rapidly these sites are filled.

Percentage adhesion levels of *Ps. fluorescens* BL146 to the pPVC surface were maximum at low cell concentrations. Increasing the concentration of cells in suspension caused a reduction in percentage adhesion. This reflects the high increases in numbers of unbound planktonic cells relative to comparatively low increases in numbers binding to the pPVC. Thus the pPVC may contain only a finite number of binding sites which become saturated at higher cell concentrations, in this case at concentrations above 5×10^8 cells ml^{-1}. Doyle

(1991) has observed that this is a general phenomenon in bacterial adhesion. SEM observations of *Ps. fluorescens* BL146 cells on pPVC indicate that these binding sites are sparsely distributed over the substratum. The uneven distribution of such binding sites suggests that the pPVC may be heterogeneous with respect to its surface physicochemistry, necessitating the large sample sizes used in this study.

An overall trend in reduced adhesion of *Ps. fluorescens* BL146 was observed following the incorporation of increasing concentrations of the four antimicrobials into the pPVC. At the highest concentration studied (2250 ppm), the greatest reduction was observed using the biocide OBPA (72%), whilst OIT and TCMP were less active (reducing adhesion by 55% and 53%, respectively), and NCMP, the least effective (giving a 13% reduction). Incorporation of the antimicrobial, Intersept, into the plastic ethyl vinyl acetate used in carpet tile backing systems has also been shown to reduce the adhesion of *Ps. aeruginosa* (Price *et al.* 1991) but the reasons for this are not clear. The incorporation of biocides into a surface may reduce bacterial adhesion either directly through their biocidal activity on cells, or indirectly through changes caused in the physicochemical properties of the surface.

Direct biocidal activity on the bacterial cells may reduce adhesion in three possible ways. Firstly, the biocide may leach from the pPVC surface, reducing the viability of cells in the suspension and hence reducing the numbers of motile cells. Reduced motility may subsequently reduce percentage adhesion since motile *Ps. fluorescens* cells adhere four times faster than non-motile strains (Korber *et al.* 1994). Secondly, the high biocide concentration at the pPVC surface may generate a negative chemotactic response in the bacterial cells, also reducing percentage adhesion. In both of these cases, the *Ps. fluorescens* BL146 cells used in the assay would have to be motile and thus viable to achieve the observed reduction in adhesion with increasing biocide concentration. However, reduced adhesion with increasing concentration of the biocide, OBPA, was evident for both viable and non-viable *Ps. fluorescens* BL146 cells. Clearly this biocide did not reduce adhesion by its biocidal activity. Thirdly, leached biocide may reduce adhesion by affecting cell surface properties. Changes in cell surface hydrophobicity, for example, have been reported by Gorman (1991) following biocide treatment of cells of *Escherichia coli, Staphylococcus saprophyticus, Staph. epidermidis* and *Candida albicans*. In this way adhesion could be altered for both viable and non-viable cells.

The u.v. irradiation of *Ps. fluorescens* BL146 cells caused a reduction in adhesion independent of the biocide concentration. This reduced adhesion may be attributed to a loss of cell motility, a known determinant of adhesion (Korber *et al.* 1994), since motility was observed in $\approx 36\%$ of *Ps. fluorescens* BL146 cells under normal assay conditions. Alternatively the reduction in adhesion could be attributed to an additional effect of u.v. on the physicochemical properties of the cell surface. Fletcher (1980) similarly found u.v. irradiation reduced adhesion of a marine pseudomonad.

Alternatively biocides incorporated into pPVC may affect microbial adhesion indirectly by changing the physicochemical properties of the pPVC surface, such as the hydrophobicity. Reduced adhesion was observed in conjunction with reduced hydrophobicity following the incorporation of all four biocides. The hydrophobicity properties of the pPVC surface without biocide are derived primarily from the highly non-polar plasticizers. These plasticizers tend to migrate to the surface of the PVC, causing plasticized PVC to be considerably more hydrophobic, with $\theta_a = 105°$ in this study, than unplasticized PVC, $\theta_a = 80°$ (Pringle and Fletcher 1983). Incorporation of biocide into the pPVC formulation may produce a chemically heterogeneous surface where both biocide and plasticizing agents are exposed. Since the biocides are less hydrophobic than the plasticizers, a net reduction in the hydrophobicity of the pPVC surface may result. The hydrophobicity of the biocides may be measured by the partition coefficient between octanol and water (log P_{ow}). Log P_{ow} values obtained for the biocides used in this study rank in the order OBPA > OIT > NCMP > TCMP, from hydrophobic to hydrophilic (I.M. Eastwood, personal communication). The more hydrophilic the biocide incorporated in the pPVC at 2250 ppm, the greater was the observed reduction in surface hydrophobicity.

Surface hydrophobicity can also be influenced by differences in surface rugosity (Hazlett 1993). In this study surface rugosity (R_a), the arithmetical average value of all departures of the profile from the mean line during the sample length, varied by only 0·3 μm between pPVC with and without biocide (data not presented). There was no trend in these differences in R_a of the pPVC with biocide incorporation, nor were any differences in rugosity visible under SEM. This suggests that surface rugosity of the pPVC is unaffected by biocide incorporation and is unlikely to be responsible for the observed reductions in hydrophobicity. Therefore any differences in contact angle between pPVC with and without biocide must be attributed to the effect of the biocide on surface hydrophobicity or charge properties. This reduction in surface hydrophobicity may subsequently cause the observed reduction in adhesion of *Ps. fluorescens* BL146.

Hydrophobicity has previously been linked directly to microbial adhesion. An early study of this phenomenon discovered a linear relationship between decreasing surface hydrophobicity and decreased adhesion of a marine *Pseudomonas* spp. (Fletcher and Loeb 1979). Subsequent investigations have also demonstrated decreased adhesion with decreased surface hydrophobicity (Van Pelt *et al.* 1985; Bidle *et al.* 1993). In this current study, the addition of biocides was found to reduce the hydrophobicity of the pPVC surface. However, the incorporation of biocide in the pPVC produced only a limited range of contact angles (94–105°), thus poten-

tially restricting the magnitude of the effect observed on the adhesion of *Ps. fluorescens* BL146.

In conclusion, the incorporation of antimicrobial agents OBPA, OIT, TCMP and NCMP into pPVC reduced adhesion of *Ps. fluorescens* BL146. These agents are incorporated into pool liners to prevent biodeterioration of the constituent pPVC by their effect on viability on microbes on or in close proximity to the surface. However, this study provides evidence to suggest that the incorporation of antimicrobial agents may also be successful by conferring changes in the hydrophobicity of the pPVC which discourage microbial adhesion.

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