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Journal of Membrane Science 138 (1998) 29–42

journal of MEMBRANE SCIENCE

Adhesion of waste water bacteria to reverse osmosis membranes

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Received 1 May 1997; received in revised form 31 July 1997; accepted 11 August 1997

Abstract

A stirred cell was used to study initial adhesion of three sewage bacteria belonging to the genus *Pseudomonas* to the three reverse osmosis (RO) membranes BW30, PVD and CAB2, and the nanofiltration membrane NF45. Membranes were immersed in suspensions containing 10^8 bacteria/ml for 10 min. All three strains were capable of rapidly colonising the four membranes, but to different extents. It was found that bacteria would sometimes aggregate upon adhering to particular RO membranes. The effects of solution ionic strength and pH, and conditioning of membranes (by prior exposure to filtrates of treated and untreated sewage) on the number of adherent bacteria were investigated. Minimal bacterial attachment occurred in a very low ionic strength milieu (deionised water). Salt concentrations corresponding to waste water and to twice that concentration resulted in significantly higher but statistically similar numbers of attached microbes. Adhesion of the three isolates was not affected by pH in the range of 4–8. The number of bacteria attaching to the membranes could be increased or reduced by conditioning films of sewage origin, conditioning films could also trigger or inhibit aggregation of adherent cells. Some surface properties of the membranes (roughness, hydrophobicity) and bacterial cells (electrophoretic mobility, functional groups by affinity chromatography) were also investigated. © 1998 Published by Elsevier Science B.V.

Keywords: Fouling; Microfiltration; Reverse osmosis; Bacterial adhesion; Conditioning films

1. Introduction

Microbiological fouling of reverse osmosis (RO) membranes is considered to be the main factor for flux decline and loss of salt rejection [1,2]. In general, bacterial fouling of a surface can be divided into three phases: transport of the organisms to the surface, attachment to the substratum, and growth at the surface. Attachment of bacteria to membranes initiates the formation of biological fouling layers, the biofilms [3]. Understanding the mechanisms of bacterial attachment may, therefore, assist in the development of novel antifouling technologies for membrane systems.

The transport of cells to surfaces in membrane modules is controlled by hydrodynamic forces. A bacterium must cross the hydrodynamic layer to reach the surface of the membrane. The thickness of this layer depends on the crossflow velocity, the permeate flux, and the fluid viscosity as well as the roughness of the membrane surface. It could extend to a range of 10–50 μm which is significantly more than the dimension of a bacterial cell [4]. Brownian motion, cell motility and/or diffusion are the mechanisms which assist organisms in traversing the boundary layer [4].

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PII S0376-7388(97)00196-8

In RO systems the permeate flux with a vertical vector to the membrane surface is an additional strong force assisting the cell to penetrate the viscous sub layer [5]. Crossflow, however, can promote lateral migration and/or shear enhanced diffusion away from the membrane [6,7], thereby reducing the convection of bacteria to the vicinity of the membrane.

The DLVO theory predicts the existence of two energy minima where stable adhesion of cells to surfaces is possible [8]. The secondary minimum is located at a greater distance from the surface than the primary minimum and represents a region where repulsive long range electrostatic forces are superseded by attractive long range van der Waals' forces. Bacteria attached in this secondary minimum are believed to be reversibly attached organisms which can be removed from the surface by relatively small shear forces [8]. Irreversible microbial attachment occurs in the primary minimum and involves short-range forces such as electrostatic, polar, non-polar, hydrophobic and hydrogen bonding type of interactions. The membrane surface becomes covered with a conditioning film containing both organic and inorganic compounds immediately after contact with the liquid phase [9]. Conditioning films have been shown to modify the surface charge of the substratum [10] as well as the Lifshitz–van der Waals and acid–base components of surface free energy [11]. The conditioned membrane/liquid interface therefore has physicochemical properties which may differ significantly from those of the clean membrane/liquid interface. Conditioning films can therefore alter the balance of DLVO long-range forces near the substratum surface. This may for example lead to enhanced removal of cells adhered in the secondary minimum from membrane surfaces by crossflow. No information is available on how conditioning films affect the adhesion of microbes to membrane surfaces.

Numerous factors have been reported to influence initial bacterial attachment to surfaces, including the type of micro-organism [12,13]; the concentration of cells in the suspension [14–16]; the stage in the growth cycle of the bacterium [12,17,18]; the amount and types of nutrients provided to the cells [9], particularly nutrient starvation [18]; cell surface charge [19] and hydrophobicity [3,19,20]; the presence of a glycocalyx (a coating of excreted polymers, the so-called extracellular polymeric substances, or EPS) [21,22]; pH [17]; temperature [23]; electrolyte concentration [8]; and presence of dissolved organic substances [24]. There are only very few systematic studies on microbial adhesion to membrane surfaces. Ridgway and coworkers [2,15,25,26] have postulated that hydrophobic interactions are the primary mechanism of adhesion of mycobacteria to cellulose acetate (CA) membranes. Flemming, however, reported no correlation between cell surface hydrophobicity and bacterial attachment to reverse osmosis membranes [27].

RO membranes have been used in waste water treatment for more than three decades and are now being commercialised as components of combined microfiltration–RO (MF–RO) systems. There are no systematic investigations on how MF treatment of secondary effluent would affect adhesion of bacteria to RO membranes. Such studies are important for evaluation of the biofouling propensity of RO membranes in such environments. The three bacteria used in this study were isolated from the early stages of biofilm development on RO membranes which had been used for final polishing of secondary effluent pretreated with MF. The effects of ionic strength, pH, RO membrane materials and conditioning films obtained from sewage related media on adhesion of these organisms under conditions typical for MF-treated secondary effluent were systematically investigated.

2. Experimental

2.1. Materials

All chemicals used were analytical grade reagents obtained from BDH. Deionised water (18 MΩ cm^{-1}) was used for preparation of all solutions. RO and nanofiltration (NF) membranes used in bacterial adhesion assays are listed in Table 1.

2.2. Membrane characterisation

2.2.1. DIC microscopy

RO membrane surfaces were photographed with an OLYMPUS IC inspection microscope (Model BH2-UMA, Olympus, Tokyo, Japan), using top illumination and Nomarski (or Differential Interference Contrast, N-DIC) optics at 100× total magnification.

Table 1
RO membranes used in attachment assays

Membrane	Supplier	Membrane type	Membrane material	Contact angle
CAB2	Hydranautics [a]	RO, asymmetric	Cellulose acetate blend	72°±2°
PVD	Hydranautics [a]	RO, thin film composite	Polyvinyl alcohol derivative	20°±1°
NF45	Filmtec [b]	NF, thin film composite	Polypiperazine amide	52°±2°
BW30	Filmtec [b]	RO, thin film composite	Aromatic	62°±3°

[a] Hydranautics, 8444 Miralani Dr., San Diego CA 92126, USA.
[b] Filmtec Corp., 7200 Ohms Lane, Minneapolis MN 55435, USA.

2.2.2. Field emission scanning electron microscopy (FESEM)

Membrane samples were fixed in glutaraldehyde. Dehydration was carried out in a water:ethanol series, followed by 100% acetone, then liquid CO_2 using a critical point dryer (BioRad Microscience, Watford, UK). The dried membranes were cut to a suitable size and mounted on bronze stubs with a shallow layer of silver paint, then coated with 2 nm of chromium using a sputter coating device (Xenosput 2000: Dynavac, Australia). Membranes were then examined using a Hitachi S-900 FESEM, at 2 kV accelerating voltage.

2.2.3. Surface roughness

Surface profiles of RO membranes were obtained with a surface profilometer instrument (Dektak IIA, Sloan, USA). The radius of the stylus of this instrument is 5 μm, and it can measure vertical features ranging in height from 0.01 to 65 μm.

2.2.4. Contact angle determination

Relative hydrophobicities of membranes were determined using contact angle measurement. Drops of distilled water were deposited on the surface of the membranes, which were fixed on a Perspex support. Contact angles of ten different drops were measured for each membrane with a goniometer (Erma, Tokyo, Japan) immediately after deposition of the water droplet.

2.3. Microbiology

2.3.1. Bacteria and culture

The three sewage bacteria used in attachment assays were isolated from early stages of biofilm growth on reverse osmosis membranes immersed in microfiltered secondary effluent [28]. The isolates were tentatively identified as presumptive *Pseudomonas* (PP1), *Pseudomonas aeruginosa* (PA2) and fluorescent *Pseudomonas* (FP3), respectively [28]. Bacteria were cultured in a defined artificial waste water medium (AWWM) which contained (in mg/l): $CaCl_2 \cdot 2H_2O$ (75.5); $MgCl_2 \cdot 6H_2O$ (24); $NaHCO_3$ (277.2); Na_2SO_4 (35.5), KCl (38), $NaH_2PO_4 \cdot 2H_2O$ (156), NH_4Cl (500) and CH_3COONa (2000). Trace elements and vitamins were prepared as stock solutions according to Schneider and Marshall [9] and added to AWWM after filter sterilisation. Broths (100 ml) were inoculated with 1 ml of overnight AWWM broth culture and agitated for 14 h at 30°C, until the optical density (OD, at 546 nm) of the culture reached 0.55–0.6, which corresponded to late log phase growth. The culture was harvested by centrifugation at 7000×g for 10 min. The supernatant was decanted and the cells were washed twice with artificial waste water salts (AWWS: AWWM without sodium acetate, ammonium chloride, vitamins or trace elements, buffered with 1 mM $NaH_2PO_4 \cdot 2H_2O$ to pH 7), and finally resuspended in this medium. The pH of the final suspension was adjusted to 7.5.

2.3.2. Morphology

Bacteria were adsorbed onto Colloidon coated copper grids, then stained with sodium phosphotungstate (1.5% w/v) for 5 min. After drying the grids were examined with a Hitachi H-7000 scanning transmission electron microscope at 75 kV accelerating voltage.

2.3.3. Electrophoretic mobility of bacteria

The surface charges of the three isolates were measured using a Doppler Electrophoretic Light Scattering Analyser (DELSA Model 440, Coulter Instruments UK) where the movement of particles in an applied electric field (electrophoresis) is analysed by

determining the Doppler shifts of scattered laser light. Triplicate samples of each isolate were diluted to an OD_{546} of 0.1 and electrophoretic mobilities determined as micrometer-centimetres per volt-second (μm-cm/V s).

2.3.4. Column chromatography

Column chromatography with Sepharose exchange resins was used to assess the types of cell-surface interactions occurring for each bacterial strain. Pasteur pipettes plugged with glass wool were filled with 1 ml of either Sepharose CL-4B; phenyl–Sepharose CL-4B; octyl–Sepharose CL-4B; DEAE–Sepharose CL-6B; or CM–Sepharose CL-6B gels (Pharmacia, Sweden). Columns were prepared in triplicate, and equilibrated with AWWS solution. The void fraction for all gels was determined to be 0.1 using a Dextran blue marker solution. Bacterial suspensions of the three isolates were prepared in AWWS to OD_{546} of 1.0, approximately 10^8 bacteria/ml. 1.0 ml of each of these suspensions was applied to each column, and eluted with AWWS. Four 1.0 ml eluates were collected from each column, and their optical densities measured using a spectrophotometer (546 nm). Optical densities of the eluates represented cells not retained in the gel. Each test was performed in triplicate, and the mean OD calculated. The affinity of isolates with gels was expressed as the percentage of cells initially retained compared with the total number of cells applied to the column, as follows:

$$\text{Retention}\% = \frac{OD_{546}\,\text{Original} - OD_{546}\,\text{First Eluate}}{OD_{546}\,\text{Original}} \times 100$$

Desorption of isolates was expressed as percentage of cells eluted compared with the total number of cells initially retained in the column as follows:

$$\text{Desorption}\% = \frac{\sum OD_{546}\,\text{Eluates} - OD_{546}\,\text{First Eluate}}{OD_{546}\,\text{Original} - OD_{546}\,\text{First Eluate}} \times 100$$

2.4. Adhesion assays

2.4.1. Standard procedure

The membranes were cut into strips of approximately 1 cm^2, washed three times with deionised water and subsequently dried in closed Petri dishes for 2–3 h. Dried membranes were glued to microscopic slides, which were mounted vertically on end in the stirred cell, a cylindrical glass vessel (10 cm diam.) on a magnetic stirrer. The cell was filled with bacterial suspension, with slow stirring to allow gentle mixing. After 10 min of immersion and stirring at 400 rpm the bacterial suspension was replaced by a rinse (AWWS). After a further 5 min the rinse was replaced by AWWS containing 2 g/l of crystal violet, with continued stirring. After 10 min the liquid was drained and the membranes were allowed to dry. Bacterial cells were visualised by bright field microscopy using the 100× oil immersion objective (no coverslip), ten or more fields were counted on each membrane.

2.4.2. Statistical comparison of counts

The methods of Schneider and Marshall [9] were used. Briefly, mean and standard deviation of retained cells were determined for each membrane, and potential outliers were identified and excluded using a Grubb's test at P=90%. A new mean and standard deviation were calculated for the remaining data and the procedure repeated until all outliers were eliminated. In total 1.5% of the field counts could not pass the Grubb's test and were eliminated. The final means and standard deviations for each membrane used in parallel experiments, were then statistically compared (Student's t-test, P=99%). In no cases were replicates detected with statistically significant different numbers of bacteria. Finally the average numbers of cells and standard deviation were calculated for the experiment. Statistical comparisons between experiments were performed using Student's t-test at P=99%. The ten slide mounting positions in the stirred cell were found to be identical with respect to bacterial adhesion in a reliability test with isolate PA2 and the BW30 membrane. In addition, there was no significant difference in adhesion of three independent subcultures of isolate PA2 to BW30.

2.4.3. Salt and pH effects on bacterial adhesion

Ionic strength effects on bacterial adhesion were tested using three media: RO water (18 MΩ); AWWS and AWWS with double concentration of solutes. Effects of pH on bacterial attachment were investigated using AWWS with pH adjusted to 4, 6 and 8 using NaOH or HCl.

2.4.4. Conditioning of RO membranes

Solutions used to form conditioning films on membranes were obtained from a pilot water reclamation plant using a two stage membrane process (microfiltration and RO) installed at South Windsor sewage treatment works, near Sydney, Australia [29]. In this plant, raw sewage is screened through a discostrainer prior to microfiltration by a MEMCOR unit. The microfiltered sewage is then treated by reverse osmosis to obtain high quality reclaimed water. The conditioning fluids included raw sewage; secondary effluent; microfiltered primary effluent; RO permeate of the same; and AWWS (control). All of these solutions were filtered through a 0.1 μm polycarbonate membrane (Poretics) prior to use. The RO membranes were fixed on their supports and soaked in conditioning liquid for 1 h, after which the conditioned membranes were transferred to the stirred cells for adhesion assays.

Fig. 1. DIC microscopy of membrane surfaces. Contrast in b, c, and d has been enhanced by digital processing. Scale bar equals 100 μm. (a) CAB2; (b) PVD; (c) NF45; (d) BW30.

3. Results

3.1. Characterisation of membranes

3.1.1. DIC microscopy

All membranes had rough surfaces (Fig. 1). CAB2 showed three different features (Fig. 1(a)). The surface of the membrane was covered (2.9×10^5 cm^{-2}) with shallow pits (c. 15 μm diameter). Parallel scratches (5–10 μm width, 500–5000 μm length) were patchily distributed at a density of 8.7×10^5 cm^{-2}. A grid-like weave pattern was found to be imprinted on the membrane surface with an interval of approximately 500 μm.

PVD was covered with fine parallel wrinkles with a width of 0.5 μm or less and lengths of 100–1000 μm (Fig. 1(b)). On the top of this pattern larger irregular ridges and hummocks were observed. NF45 had a uniformly grooved surface (interval 0.5 μm) across the entire membrane sample (Fig. 1(c)). Some scratches (2.9×10^5 cm^{-2}) were observed, and there were some patches of irregular ridges or hummocks. The density, orientation and distribution of scratches on BW30 was similar to CAB2, but the scratches were narrower (1 μm) and varied in length between 50–1000 μm (Fig. 1(d)). In addition, the surface of this membrane was covered (not shown) by low relief raised surface inhomogeneities with dimensions of about 300 by 200 μm.

3.1.2. FESEM study

FESEM observation revealed that all membranes had small scale surface roughness (Fig. 2). CAB2 and BW30 had the most irregular surfaces, with polymeric branches standing vertically (Fig. 2(a, d)). Many spherical polymeric features (about 0.1 μm diam.) were observed on the surface of CAB2. The surface of PVD was composed of polymer blocks (about 70 by 30 nm), however, some strands up to 1 μm in length were observed (Fig. 2(b)). Features similar to pores (30–100 nm diam.) were observed on the NF45 membrane surface (Fig. 2(c)). It was assumed that these large features were not genuine pores, because the membrane had the operational characteristics of a typical nanofilter [28].

3.1.3. Surface roughness by profilometry

Due to curling, all membrane samples had curved profiles over horizontal distances in the order of a few hundreds of microns. They had surface roughness with amplitudes in the micron or submicron range. CAB2 showed two kinds of roughness on its surface. The first was a regular pattern which appeared in longitudinal profile as wave like, with interval of 400 μm and

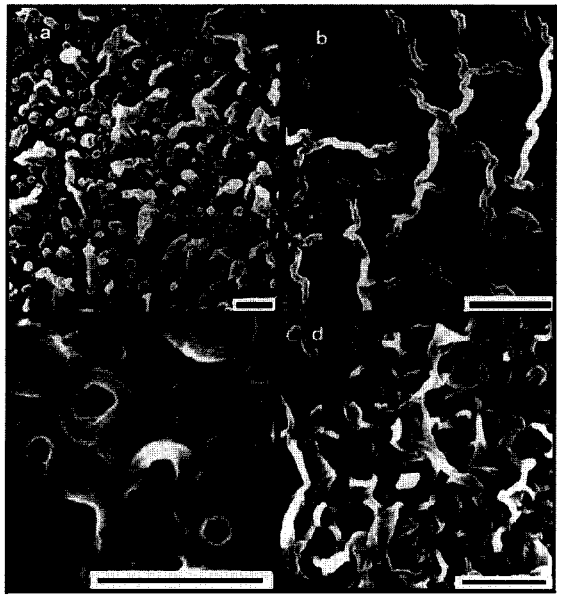

Fig. 2. FESEM of membrane surfaces prepared by critical point drying. Scale bars equal 500 nm. (a) CAB2; (b) PVD; (c) NF45; (d) BW30.

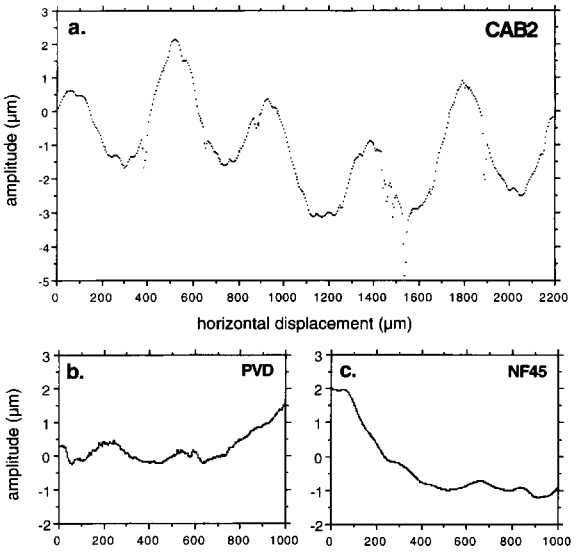

Fig. 3. Surface profiles of membranes. Vertical scale exaggerated 140-fold. (a) Transverse profile of CAB2, showing regular convolution of the surface with 400 μm wavelength, as well as narrow, deep features (scratches?), e.g. at 1530 μm. (b) PVD membrane, showing elevated patches of relatively intense small-scale roughness at 220 and 550 μm. (c) NF45 membrane, showing a relatively smooth surface.

amplitude of 3 μm, and in transverse profile as two peaks with regular intervals of 700 μm and 900 μm (Fig. 3(a)). This was presumably induced by the weave of the supporting cloth. Secondly, irregular rough sites with small features with amplitudes between 1–3 μm were observed (Fig. 3(a)). The 15 μm pits visible in DIC images were not resolved in the profiles.

The BW30 membrane (not shown) had an irregular surface with broad features of low amplitudes (0.1–3 μm). Elevated rough patches were observed on PVD membrane, where, in comparison to the other membranes, relatively high variations in gradient occurred over short transverse distances (Fig. 3(b)), but these features were of low amplitude (0.1–0.3 μm). NF45 had a relatively smooth surface (Fig. 3(c)) with occasional small scale irregularities of 0.3 μm amplitude or less.

3.1.4. Contact angles

Water contact angles of 20°±1°; 52°±2°; 62°±3°; and 72°±2° were observed for PVD (most hydrophilic), NF45, BW30 and CAB2 (most hydrophobic), respectively.

3.2. Characterisation of bacteria

3.2.1. Morphology

All three strains were small rod-shaped bacteria, approx. 1.0 by 0.5 μm. They were bipolarly flagellated, PA2 had a single flagellum at each pole whereas the other strains typically had 3–4.

3.2.2. Electrophoretic mobility

The electrophoretic mobilities of strains PP1, PA2 and FP3 were −0.77±0.03 μm-cm/V s; −0.29±0.03 μm-cm/V s; and −1.28±0.09 μm-cm/V s, respectively, corresponding to zeta potentials of −9.6±4 mV; −3.9±3 mV; and −16.5±6 mV.

3.2.3. Interaction chromatography

All strains adhered well to all matrix materials, but some adhered better to certain types than others. The results of interaction chromatography are given in Fig. 4. All strains showed 100% retention on DEAE–Sepharose (hydrophilic, positive charge), with no desorption. Isolate PP1 absorbed to both hydro-

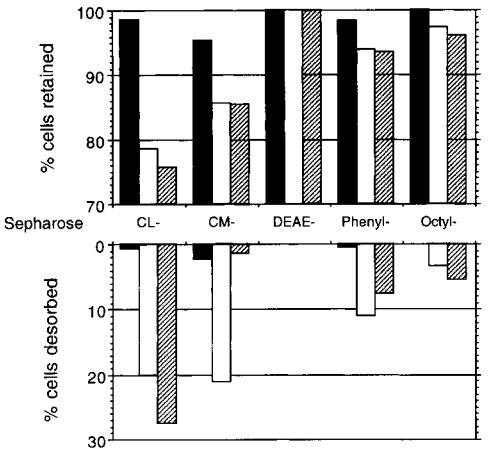

Fig. 4. Bacterial attachment mechanisms assessed by interaction chromatography with Sepharose gels. Retention (above) and desorption (below) of the three bacterial isolates from columns containing bead matrices with different surface properties: CL–Sepharose (neutral, hydrophilic); CM–Sepharose (negative, hydrophilic); DEAE–Sepharose (positive, hydrophilic); phenyl– and octyl–Sepharose (neutral, hydrophobic). ■ strain PP1; □ strain PA2; ▨ strain FP3. Sepharose is a gel prepared from the purified neutral fraction of agarose.

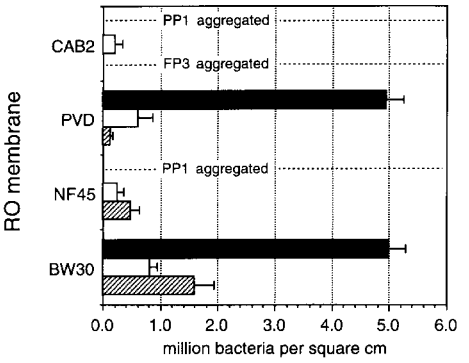

Fig. 5. Initial colonisation of RO membranes by three waste water bacteria. The membranes were immersed in AWWS, pH 7.5 containing 10^8 cells/ml for 10 min. In some cases (as noted) the bacteria attached as large aggregates, and could not be enumerated. ■ strain PP1; □ strain PA2; ▨ strain FP3.

phobic (phenyl–Sepharose, octyl–Sepharose) and hydrophilic (Sepharose–CL, CM–Sepharose and DEAE–Sepharose) gels similarly and strongly. Isolate PA2 adhered strongly to positively-charged (DEAE–Sepharose) and to hydrophobic matrices (octyl– and phenyl–Sepharose), but compared to PP1, it had low retention and was significantly desorbed from neutral (Sepharose–CL) and anionic (CM–Sepharose) hydrophilic matrices. Isolate FP3 interacted with most column materials in a manner similar to PA2, except it was not desorbed from CM–Sepharose.

3.3. Adhesion of sewage isolates to RO membranes

3.3.1. Standard adhesion assay (AWWS, pH 7.5)

Attachment of the three isolates to the four membranes is shown in Fig. 5. On BW30 and PVD isolate PP1 was retained in the largest numbers (approximately 5×10^6 cells/cm^2) while isolates PA2 and FP3 attached significantly less (0.2×10^6–1.5×10^6 cells/cm^2). A similar pattern of attachment was observed on NF45, except that isolate PP1 could not be accurately counted as it adhered in the form of large aggregates. Both strains PP1 and FP3 formed aggregates on the surface of CAB2, and isolate PA2 adhered in relatively small numbers (10^5 cells/cm^2).

Morphology of aggregates varied for each organism and membrane pairing. Small clumps of PP1 cells were distributed in an apparently regular fashion on NF45. On CAB2 the same organism was retained on the surface in large dense masses. These aggregates were distributed very randomly and most of the membrane surface was free of organisms. Isolate FP3 formed dendritic aggregates with bare spaces in between. The aggregates were distributed randomly and most of the surface was free of cells. In all cases some single cells were observed to be attached to the membrane in areas in between the aggregates.

3.3.2. Effects of changing ionic strength and pH

In all cases it was found that the number of organisms attached to membranes was lower in distilled water than in artificial salt media (Fig. 6), although in the case of adhesion of isolate PA2 to NF45, and possibly of FP3 to PVD membrane, the difference was not significant. Aggregation did not occur on any of the membranes when low ionic strength medium was used. Increased ionic strength did not promote or prevent aggregation compared to standard AWWS solution (Fig. 6).

In most cases there was no statistically significant effect of pH on attachment of the three sewage isolates to RO membranes (Fig. 7), save that strain FP3 had a possible adhesion minimum at pH 6 on BW30 and

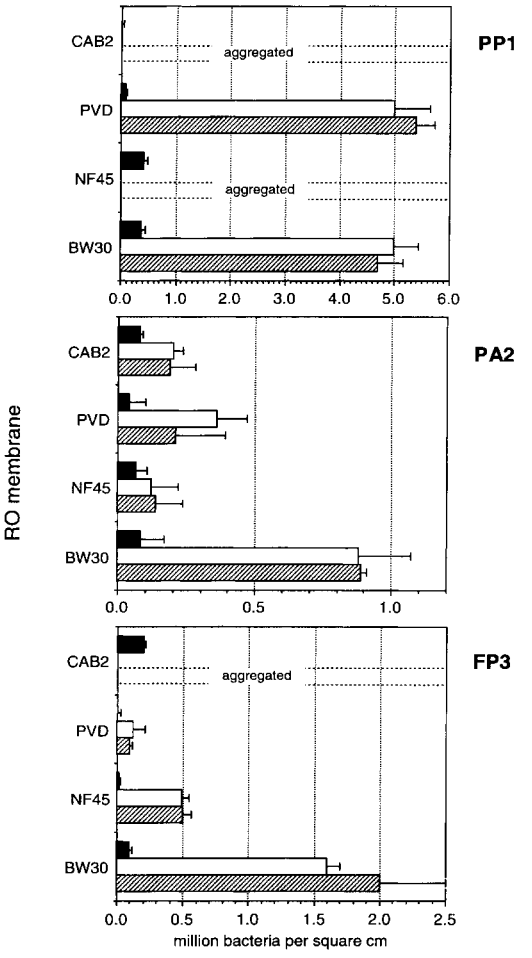

Fig. 6. Effect of ionic strength of the liquid milieu on the densities of initial bacterial colonisation of RO membranes. See also notes for Fig. 5. ■ deionised water; □ AWWS; ▨ double strength AWWS.

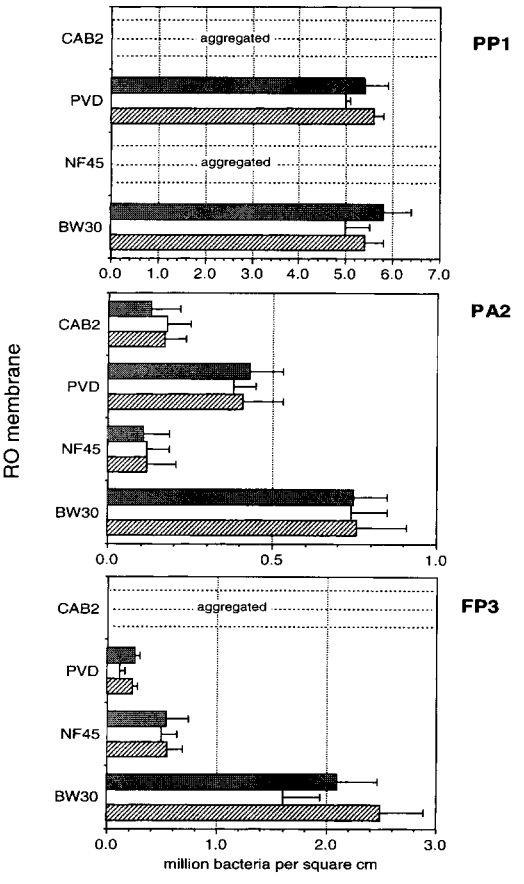

Fig. 7. Effect of pH of the liquid milieu on the densities of initial bacterial colonisation of RO membranes. See also notes for Fig. 5. ■ pH 4; □ pH 6; ▨ pH 8.

PVD. When aggregation occurred, it was observed across the entire range of pH tested.

3.3.3. *Conditioning films*

The data are presented in Fig. 8, and the effects are summarised in Fig. 9. The interactions of isolate PA2 and FP3 with membranes were influenced by conditioning films. Isolate PP1 was not affected, except that aggregation on NF45 was suppressed by a coating from MF sewage. In contrast some conditioning films appeared to induce aggregation, as did most of the treatments for strain PA2 on CAB, and RO permeate for FP3 on NF45.

The interaction of isolate PA2 with membranes could be strongly influenced by conditioning films. The effect of each type of conditioning film was not consistent, for example coatings from raw sewage doubled the numbers of PA2 cells retained on the BW30 membrane, but strongly reduced its attachment to the other three membranes. Nor was there a uniform conditioning effect for each bacterium/membrane combination. For example, a conditioning film of secondary effluent tripled attachment of PA2 to NF45, but the other three conditioning films strongly diminished the attachment of this isolate to this membrane (Fig. 8).

Although adhesion of strain FP3 was also affected by conditioning the surface, the pattern of effects was strikingly different to those on PA2 (Fig. 9). Many

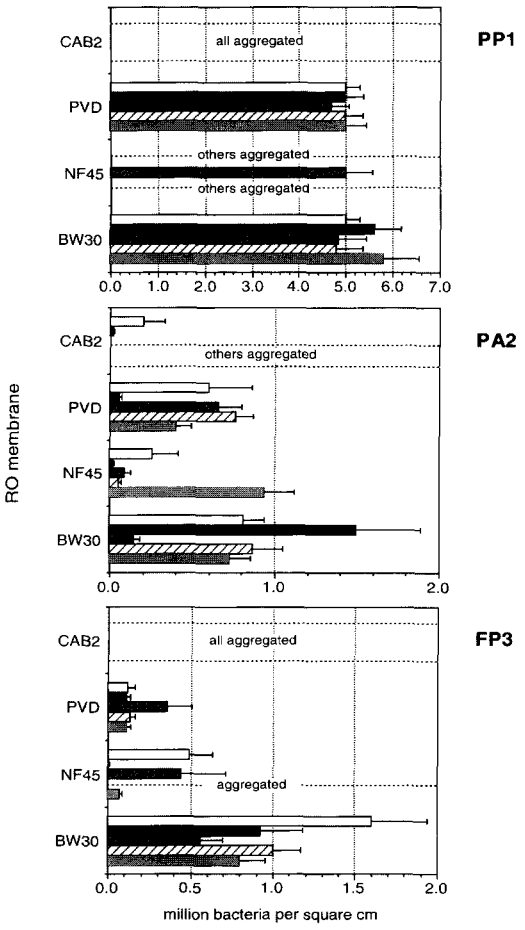

Fig. 8. Effect of conditioning films on the densities of initial bacterial colonisation of RO membranes. The membranes were soaked in filtrates of sewage-based conditioning agents for 1 h prior to the adhesion assay. See also notes for Fig. 5. Conditioning agents were: ☐ AWWS (control); ■ primary effluent; ■ microfiltered primary effluent; ▨ RO permeate from MF primary effluent; ■ secondary effluent.

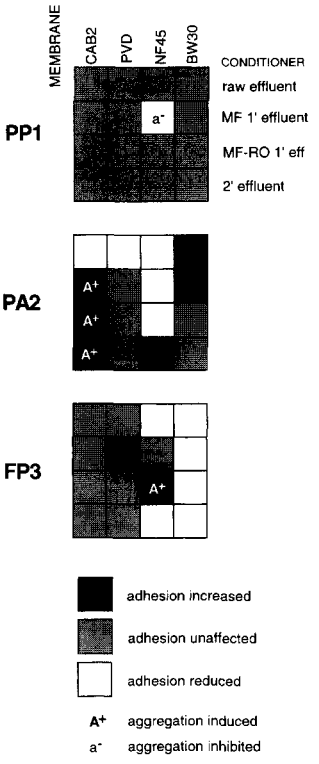

Fig. 9. Diagrammatic representation of the effects of conditioning films on initial attachment of the three bacteria (strains PP1, PA2, and FP3) to membranes. Only statistically significant effects are indicated. ■ adhesion increased; ■ adhesion unaffected; ☐ adhesion reduced; A^+ aggregation induced; a^- aggregation inhibited. Due to the large (but unknown) numbers of cells in aggregates, the induction of aggregates is considered to indicate a significant increase in bacterial colonisation, and their inhibition the reverse.

conditioning films decreased retention of isolate FP3, in fact a conditioning film from raw sewage prevented attachment to NF45.

4. Discussion

Bacteria attached to RO membranes either as individual cells or as aggregates. Adhesion of the three sewage isolates was affected by membrane material, the bacterial species and ionic strength of the medium, however pH had little effect on adhesion of the organisms. Conditioning films could promote, decrease or inhibit bacterial adhesion. They could even cause or prevent aggregation of bacteria on the surface of the membrane.

The retention value of microbes measured in column chromatography combines both reversible and irreversible adhesion. The desorption numbers are indicative of the proportion of bacteria which adhered reversibly to the respective columns. As might be expected given their negative charge, all bacteria adhered strongly and irreversibly to an anion exchange matrix. They also favoured hydrophobic matrices, clearly demonstrating that these organisms were

capable of forming strong linkages with a diversity of surface chemistries. Column chromatography results also indicated that the surface of each organism contained hydrophilic sites capable of interacting with the substratum via hydrogen bonds in addition to charged groups. The high desorption of PA2 and FP3 from a neutral polar matrix (Sepharose–CL) showed that their hydrogen bonds were not as firm as those of PP1. Similarly, the strong desorption of isolate PA2 from CM–Sepharose would indicate that this organism is not capable of forming the same strong linkages with negatively charged surfaces as the other two bacteria. This weaker bonding was not solely determined by electrostatic repulsion since PA2 had the lowest electrophoretic mobility of the three strains. Clearly, these bacteria would have been capable of interacting with the membranes by a diversity of mechanisms, all of which could have been employed simultaneously.

It has been repeatedly demonstrated that bacterial attachment can be affected by substratum nature [2,20,26,30]. The RO membranes used in this work were made of organic polymers of different compositions. BW30 and NF45 are polyamides – BW30 is cross-linked aromatic polyamide and NF45 is a polypiperazine amide. CAB2 is a cellulose acetate blend, and PVD is a polyvinyl alcohol derivative. These distinctive material properties were reflected in different water contact angles. Adhesion of PP1 was independent of contact angle, and adhesion of PA2 did not correlate with surface wettability (Fig. 10), indicating that attachment depends on the particular nature of the surface chemistry of the substratum rather than average physicochemical interface parameters, such as hydrophobicity when measured by contact angle. However, attachment of FP3 increased with contact angle, which suggested that this organism might have adhered to the membranes primarily via hydrophobic interactions (Fig. 10). Retention of FP3 on phenyl– and octyl–Sepharose columns also indicated that hydrophobic interaction is one of the effective mechanisms of adhesion of this sewage isolate (Fig. 4).

Surfaces of RO and NF membranes may have an intrinsic negative charge [31,32], or would soon acquire one upon immersion [33]. The bacteria had negative surface charges, and one would therefore expect that cells would have experienced a repulsive force caused by the overlap of two electric double

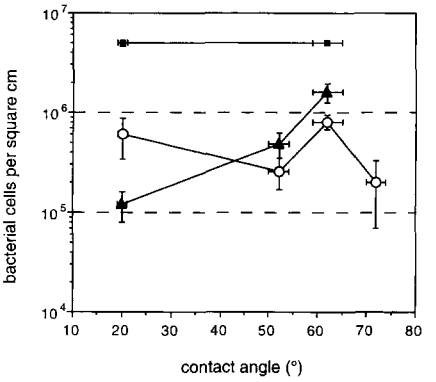

Fig. 10. Relationships of membrane hydrophobicity, measured as contact angles, to densities of initial colonisation by the three bacterial isolates. From left (most hydrophilic): BW30, NF45, PVD, CAB2 (most hydrophobic). Due to cell aggregation some colonisation values could not be measured, but they could be considered as higher than 5×10^6 cells cm^{-2}, the maximum measured colonisation density. —■— strain PP1; —○— strain PA2; —▲— strain FP3.

layers when approaching membrane surfaces. If it can be assumed that the more hydrophilic membranes have more polar surface groups imparting surface charge, then isolate FP3, which had the greatest negative charge, probably experienced a stronger repulsion as the hydrophilicity of the membranes increased. The better adhesion of this organism to the more hydrophobic membranes is in accord with this hypothesis (Fig. 10). No such relationship was, however, observed in the other two organisms. Strain PA2 colonised all membranes, but in low numbers, and in column chromatography it exhibited high desorption values from most matrices. These results may indicate a reversible adhesion mechanism, which is characteristically non-discriminating as it does not depend on specific polymer interactions, but is weak, as the cell is held at the secondary minimum by non-covalent forces. Thus the bulk of attached cells may have been removed in the rinsing and staining stages.

In general column chromatography results were in accordance with the membrane adhesion study. For example, strain PP1 adhered strongly and indiscriminately to all column matrices, and colonised all membrane surfaces heavily regardless of preconditioning. Inconsistencies should be expected. From the retention values obtained it is obvious that most or all of the total cell population adhered to Sepharose surfaces in

column chromatography but, due to hydrodynamic considerations, it is likely that only a small proportion of the cells originally present in the liquid adhered to membranes in the stirred cell. Other researchers have shown that batch-grown cells are diversified in their adhesive ability [34]. Therefore, results of the stirred cell experiment could have been determined by the relative proportion of adhesion variants whose cell properties favoured recruitment by a particular surface, rather than being a measure of general cell-to-surface adhesiveness. The experimental setup did not allow us to evaluate the 'sticking efficiency' of organisms, i.e. the proportion of cells contacting the surface that actually adhered [35,36].

All RO membranes used in this work had rough surfaces at microscopic scales. Regular surface features such as the grooves of NF45, the fibrillar structures of PVD and the gridlike pattern of CAB2 appeared to be properties imparted on the membranes by the support material. However, the irregular and sometimes deep scratches which occurred on every membrane surface were probably introduced during manufacturing or handling of the finished products. If these scratches penetrate the semipermeable layer, they might compromise the 'absolute barrier' function of RO in removing microbial pathogens (especially viruses) in waste water treatment applications [37].

Roughness is believed to increase microbial adhesion [38]. The degree of surface roughness was not quantified, but it appeared that NF45 was the smoothest membrane, and CAB2 the roughest. In general, NF45 was the membrane least susceptible to colonisation, while CAB2 was heavily colonised by individual cells or aggregates. The effects of roughness on adhesion to membranes will be difficult to assess, unless a means of altering it without changing other surface properties can be found. Mechanisms for the effect of roughness would depend on the dimensions of the irregularities. Structures of multimicron size may cause uneven flow distribution or channelling of flow over the surface of the membrane. Also, they may act as physical barriers and entrap bacteria or other particles. Irregularities in the micron range, as revealed by FESEM on the surface of all membranes, could influence the structure of the static boundary layer by increasing its thickness at some locations, possibly shielding attached cells from shear forces. Roughness in the nanometre range (not measured in this work) would impact on the structure of the electric double layer.

In RO or NF processes, solute concentrations in the boundary layer are unlikely to exceed twice the amounts of the compounds in the bulk [7]. In this study doubling of ion concentration had no significant effect on attachment of the sewage isolates. The reduced retention of cells we have observed in a low ionic strength medium is predicted by the DLVO theory. At low ionic strength, the electrical double layer surrounding each bacterium is expanded and occupies a larger volume around the surfaces. Under such circumstances, repulsive electrostatic interactions between two approaching bodies occur at relatively large distances. The compaction of the electric double layer in high ionic strength media allows closer approximation of bacteria and substrata, facilitating the contact of bacterial cell surface polymers with the substratum [2]. In addition, divalent ions such as Ca^{2+} and Mg^{2+} may stabilise adhesion by ion-bridging between anionic functional groups on the substratum surface and in the cell glycocalyx [13]. There have been conflicting reports about the influence of the electrolyte concentration on initial adhesion. In some cases, adhesion has increased with increasing electrolyte concentration [17], but in other reports no significant correlation between initial adhesion and electrolyte concentration was found [2,24,39].

Varying the pH had little effect on adhesion of the three bacteria in this work. Ridgway et al. [2] also found that pH had little influence on adhesion of mycobacteria to cellulose acetate membranes. Changing of pH may result in the modification of the localised charges on cell and membrane surfaces, thereby influencing microbial adhesion by modification of double layer structure by alteration of the total amount of charge on the interface, and (by charge neutralisation) creating opportunities for chemical interaction by non-ionic mechanisms. In addition, the conformation of macromolecules is pH-dependant, therefore, changes could alter the viscosity [40] and thickness of the bacterial glycocalyx. However, our results showed that none of these effects were strong enough to influence adhesion over a modest pH range.

The three bacterial isolates had flagella at their poles. The principal components of flagella are proteins; for example, the flagellum of *Escherchia coli* is

a long helical structure composed of a single protein subunit [41]. Flagella may assist in attachment in two ways. Firstly, flagella may be used by a bacterium to swim and cross the boundary layer [4]. By increasing the kinetic energy of cells they assist them to overcome the electric repulsive forces which exist between the bacterium and the substratum [42]. Secondly, flagella or other fine bacterial appendages such as fimbriae, pili and EPS could physically bridge the free energy barrier, as cell-surface contact is energetically favoured by decreased radius of curvature of the approaching regions [43]. These appendages may also be responsible for cooperative effects between cells in colonisation of a surface [44].

Although conditioning films were capable of increasing or decreasing adhesion, the different effects for the three isolates and the three membranes mean that for a mixed culture the initial adhesion may be rather selective. This may influence the species composition and rate of development of the biofilm. The diverse effects of a single conditioning solution when applied to different membranes may indicate that membrane surface properties are still 'showing through' a thin conditioning film, or may have been due to variations in film composition caused by selective adsorption of different molecular subsets from the liquid by membranes according to their individual surface chemistries. Raw sewage, secondary effluent, microfiltered primary effluent and RO permeate used in this work contained an unknown variety of organic and inorganic compounds which could have been incorporated into conditioning films. It was not possible to identify which chemicals in these extremely complex conditioning films influenced adhesion, though it can be assumed that the surface chemistry of a conditioned membrane will be different from that of the clean polymer surface. Such differences may include the type and quantity of hydrophobic or hydrophilic functional groups. Bacteria may therefore have adhered to conditioned interfaces by other mechanisms than those used for attachment to clean membranes. Adsorption of conditioning film macromolecules will also increase nanometre-scale surface roughness. This may introduce structures to the interface which cross the double layer, particularly in high ionic strength solutions where it is compact [9]. In such cases cells could be capable of physicochemically interacting with the substratum while located in the secondary minimum. These results show the importance of testing adhesion in the correct milieu, and the problem of transferring conclusions from one experimental system to another.

Bacterial aggregation occurred on the membrane surfaces, not in the suspension medium, where cells were observed to be present as individual entities. Why could aggregation of bacteria occur only on the surfaces of RO membranes? Localisation at a surface may favour the kinetics of aggregation by limiting the geometry of approach to two dimensions, and by lowering the velocities of interacting particles in the viscous boundary layer. The observed uneven distribution of aggregates could have been due to local variations in substratum properties such as surface charge, type of exposed functional groups, or hydrophobicity. The presence of attached bacteria could favour recruitment of suspended cells streaming close to the surface, perhaps by simple physical entrapment. Such a process would be self-reinforcing, and could rapidly produce large aggregates.

As could be predicted from the DLVO theory [45], aggregation was inhibited in a deionised environment. This result was consistent with previous studies [46,47] on microfiltration of bacteria, where bacterial cakes formed at high salt concentration were found to be much more stable than those formed at low ionic strength. An outcome of this is that an ion-depleted (RO permeate) water may be of use as a RO cleaning or storage solution. Aggregations of bacteria on membranes could facilitate biofilm formation by accelerating the growth of microcolonies and production of the polysaccharide matrix responsible for their stabilisation [9]. Membranes that appear to catalyse aggregation of attached bacteria may, therefore, be unsuitable for water and waste water industry applications. The simple procedures we have developed could be used to screen candidate membranes.

5. Conclusions

Adhesion of three waste water bacteria to RO membranes was investigated under varying conditions pertinent to secondary effluent treatment. Attachment was influenced by the ionic strength, but not pH of the suspending medium, and by the nature of the membrane surface. The effects of conditioning treatments

showed that a membrane's susceptibility to colonisation may be altered even by short term exposure to a complex menstruum.

Seeking a non-fouling surface is an attractive option because, ideally, the requirement for biofouling management should be minimal during the membrane's operating life. While carefully controlled laboratory trials are necessary to understand mechanisms of bacterial attachment, each particular membrane's propensity to biofouling should not be inferred from our results. The different colonisation strategies displayed by our three test organisms can only hint at the capabilities of populations containing hundreds of bacterial species, additionally bacteria have the ability to adapt to suit their environment, given time. However, the procedures we have developed can be used as guide. Thus, where significant aggregation or adhesion occurs the membrane is likely to biofoul, although a membrane that does not induce aggregation is not necessarily capable of fouling control.

Acknowledgements

This work was supported financially by the Ministry of Culture and Higher Education of Iran; the Commonwealth Special Research Centre and UNESCO Centre for Membrane Science and Technology; and the CRC for Waste Management and Pollution Control, an Australian Government initiative funded through the Co-operative Research Centres Programme.

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