This article was downloaded by: *[Montana State University]*
On: *16 October 2009*
Access details: *Access Details: [subscription number 790568837]*
Publisher *Taylor & Francis*
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK

Biofouling
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454511

Effect of Polymer Surface Properties on the Reversibility of Attachment of Pseudomonas aeruginosa in the Early Stages of Biofilm Development

Mark Pasmore; Paul Todd [a]; Blaine Pfiefer [a]; Michael Rhodes [a]; Christopher N. Bowman [a]
[a] Department of Chemical Engineering, University of Colorado, Campus Box 424, Boulder, CO 80309-0424, USA.

First Published on: 01 January 2002

To cite this Article Pasmore, Mark, Todd, Paul, Pfiefer, Blaine, Rhodes, Michael and Bowman, Christopher N.(2002)'Effect of Polymer Surface Properties on the Reversibility of Attachment of Pseudomonas aeruginosa in the Early Stages of Biofilm Development',Biofouling,18:1,65 — 71
To link to this Article: DOI: 10.1080/08927010290017743
URL: http://dx.doi.org/10.1080/08927010290017743

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.informaworld.com/terms-and-conditions-of-access.pdf

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

Biofouling, 2002 Vol 18 (1), pp 65–71

Taylor & Francis
Taylor & Francis Group

Effect of Polymer Surface Properties on the Reversibility of Attachment of *Pseudomonas aeruginosa* in the Early Stages of Biofilm Development

MARK PASMORE[a,b,*], PAUL TODD[b], BLAINE PFIEFER[b], MICHAEL RHODES[b] and CHRISTOPHER N BOWMAN[b]

[a]*Center for Biofilm Engineering, Montana State University, Campus Box 173980, Bozeman, MT 59717-3980, USA;* [b]*Department of Chemical Engineering, University of Colorado, Campus Box 424, Boulder, CO 80309-0424, USA*

(Received 12 September 2001; in final form 26 October 2001)

Downloaded By: [Montana State University] At: 18:28 16 October 2009

Biofilm fouling is a common problem in industrial water and medical systems. Prevention of biofilm formation is often difficult because of the numerous potential attachment and adhesion mechanisms of bacteria, and therefore removal strategies are often necessary. Three surface properties, surface roughness, hydrophobicity (contact angle), and surface charge (zeta potential) were measured for several polymeric surfaces and related to specific biofilm characteristics. Biofilms of *Pseudomonas aeruginosa* were allowed to form on these surfaces for a period of 1–3 d, and the fraction of bacterial cells removed from each surface by exposure of biofilms to a standard shear stress was determined. Cells were most readily removed from the smoothest, most hydrophilic, neutral surfaces, with removal becoming more difficult at longer attachment times. This finding correlates directly with the finding that surfaces with these characteristics are most resistant to biofilm initiation. Therefore, it is demonstrated that by optimizing surface properties, it is possible to produce a surface from which bacteria can be more readily removed.

Keywords: biofilm; fouling; bacterial attachment; shear forces; membranes

INTRODUCTION

Biofilm fouling is a significant problem affecting a number of industrial and medical applications. Ships' hulls, pipes, heat exchangers, membranes, dental water lines and almost any other surfaces in regular contact with water are likely to be fouled by biofilms. Bacteria have evolved to fill almost every niche available, including colonization of solid surfaces (*i.e.* biofilms), where they are usually able to find nutrients and a protective environment (Characklis & Marshall, 1990; Costerton *et al.*, 1995). In order to attach and grow on surfaces, bacteria have developed a number of attachment mechanisms including flagellar attachment, type IV pili, adhesive membrane proteins and extracellular polysaccharides (Marshall *et al.*, 1989; Melo & Bott, 1992; Lau & Liu, 1993; Franklin & Ohman, 1996; O'toole & Kolter, 1998). As yet, there have been no surfaces found that are completely resistant to biofilm fouling (Costerton *et al.*, 2000). Thus, biofilm prevention is nearly impossible; however, surfaces with minimum roughness, charge and hydrophobicity do exhibit reduced susceptibility to biofouling (Pasmore *et al.*, 2001). Therefore surfaces with the weakest attraction for bacteria will be likely to require the least amount of effort to remove adsorbed organisms, and the strength of the attraction between the bacteria and the surface is expected to play an important role in the ability to remove biofilm from a surface. A surface that resists strong bacterial attachments would most likely foul slowly and be easier to clean. This hypothesis was tested in the current study by enumerating the bacteria removed with mechanical shear and detergent cleaning, from biofilms grown on materials of known surface properties.

*Corresponding author; fax: (406) 994-6098; e-mail: mark_p@erc.montana.edu

ISSN 0892-7014 print/ISSN 1029-2454 online © 2002 Taylor & Francis Ltd
DOI: 10.1080/08927010290017743

MATERIALS AND METHODS

Bacterial Culture

A single bacterial species, *Pseudomonas aeruginosa*, was used. *P. aeruginosa* is involved in biofilm fouling of a number of industrial systems including membranes and pipes (Ridgeway *et al.*, 1984; Characklis & Marshall, 1990; Costerton *et al.*, 1995), and it is common in biofilms found in everyday life including the fouling of shower curtains, swimming pools and drinking water lines (Characklis & Marshall, 1990; Benkel *et al.*, 2000; Naumova *et al.*, 2000). It is also involved in infections where it has been shown to cause conjunctivitis, acute otitis media, endocarditits, and dermatitis (Brown & Baker, 1999; Costerton *et al.*, 1999; 2000). More commonly, the pathogenicity of *P. aeruginosa* is observed in infections of immune compromised patients such as those with hemodeficiencies, intravenous drug use, burn wounds or in the lung of cystic fibrosis patients, where it leads to a high mortality rate (Lam *et al.*, 1980; Potts *et al.*, 1995).

P. aeruginosa (ATCC# 27853) was obtained, cultured, and subdivided into a sufficient number of culture vials (approximately 300) to provide a fresh culture for each experiment. These vials were stored frozen at −70°C to maintain the genotype of the starter culture and the initial phenotype. The frozen cultures were incubated overnight in 25 ml of 30 g l^{-1} Trypic Soy Broth (TSB) (12–16 h) at 37°C with shaking at 150 RPM, to obtain a consistent late stationary phase culture of approximately 1×10^8 cells ml^{-1} as measured by optical density (OD). This 50 ml suspension, after adjustment of OD if necessary, was then used to inoculate the reactor.

TSB was used as the bacterial growth medium for this work. It was prepared by dissolving 30 g of TSB (T-8261, Sigma Chemical Company, St Louis, MO) per liter of deionized water and autoclaving for 45 min. The medium was placed into a sterile Mason Jar reactor (McFeters, 1992) and the impeller was rotated at 250 RPM (shear stress = 0.26 N m^{-2}), at 22–23°C. Biofilms were formed by allowing cells to attach and grow on coupons (triplicate samples of 1.5 cm^2) of the various sample materials on the wall of the reactor for up to 3 d after bacterial inoculation. The coupons were all exposed to the same nutrient and shear conditions.

Surface Materials

High-density polyethylene (PE) solid surface (Colorado Plastic Products, Boulder, CO) was used as a baseline reference surface in each experiment to facilitate comparison of various surfaces in the multiple experiments. To test the effects of hydrophobicity on bacterial removal, varying amounts of poly(ethylene glycol) were grafted to the surface of the polyethylene membranes (3M, St Paul, MN) using the acrylic photochemical technique described by Ma *et al.* (2000). The polyethylene membranes were of varying pore size and production procedure causing varying roughness of the surface. Nylon membranes (Pall-Gelman, East Hills, NY) were also used in this work to examine the effects of varying surface charge. Pall-Gelman membrane can be purchased with varying charges; these charges are produced by varying the ratio of acid (–COOH) and

TABLE I Values measured for the surface materials examined against the removal of bacterial biofilms

Material	Roughness (μm)	SD	Contact angle (°)	SD	Zeta Potential (mV) pH 7	SD
Polyethylene of varying roughness						
PE solid	0.1	0.0	76	3		
PE membrane #1	0.4	0.3	98	3		
PE membrane #2	1.4	0.2	99	4		
PE membrane #3	1.4	0.1	102	6	−4.75	0.38
PE membrane #4	1.5	0.3	99	2		
PE membrane #5	2.7	1.6	106	2	−6.3	
PE membrane #6	2.8	1.4	99	4		
PE membrane #7	3.4	1.7	106	2		
Nylon of varying zeta potential						
Nylon Membranes +	1.1	0.1	< detection		−20.3	0.76
Nylon Membranes A	1.7	0.3	< detection		−12.55	1.7
Nylon Membranes B	1.7	0.8	< detection		−4.135	4.3
Nylon Membranes C	2.5	1.0	< detection		+3.395	1.5
Grafted material of varying contact angle Polyethyleneglycol(200) on PE						
PE 18 (weight gain = 5.7%)	1.8	0.4	73.2	3.4	−11.41	4.41
PE 25 (wg = 4.13%)	1.5	0.2	82.1	5.2		
PE 30 (wg = 2.60%)	2.2	0.4	98.7	4.0		
PE 27 (wg = 1.81%)	1.5	0.3	103.0	4.4	−7.17	7.07

SD = the standard deviation of the measured values; < detection = samples that were below the detection limit of the contact angle measurement technique (< approximately 3°, also termed "zero" in the text)

Downloaded By: [Montana State University] At: 18:28 16 October 2009

Downloaded By: [Montana State University] At: 18:28 16 October 2009

base ($-NH_2$) groups within Nylon 6,6. All of these materials and various properties are presented in Table I.

Surface Property Analyses

The surface properties of interest in this study are surface roughness, hydrophobicity, and surface charge. These properties were measured by profilometry, contact angle and streaming potential, respectively. The details of these measurements are described briefly below and in greater detail by Pasmore *et al.* (2001). The measured values are shown for the range of materials used in biofilm testing in Table I.

Determination of hydrophobicity by contact angle measurement

Static contact angles were measured by goniometry. The sample material was placed on a level horizontal platform. A 10-μl droplet of deionized water was then placed on the test material, and an image was taken using a CCD camera (Sony XC-75, Sony Electronics Incorporated, Park Ridge, NJ). The image was analyzed using NIH Image (National Institutes of Health, Bethesda, USA) image analysis software. The droplet image was magnified 50x and analyzed to determine the internal angle (contact angle) between the water and the surface (Yasuda & Okuno, 1994). This measurement was repeated three times for each material tested, and the contact angle was measured on both sides of the drop to be sure that the drop was uniform. This technique could not be used for surfaces that absorb water and wet or swell; therefore, the contact angle for these types of materials is reported as zero degrees. Contact angles on both sides of the drop were averaged for each material.

Determination of roughness by profilometry

Surface roughness was determined using a Dektac (Sloan Technology Corporation, Santa Barbara, CA) profilometer. For consistency the same samples were used in both the contact angle and profilometer experiments. The samples were analyzed by scanning with a stylus having a 25 μm diameter diamond tip. The profilometer was set to scan 5 mm lines on the surface with a 5 μg force. This experiment was performed three times, and the root-mean-squared roughness was calculated and recorded. These results are shown in Table I. Additionally, to confirm the reproducibility of the measured values some of the surface materials were examined 18 times from random locations on the membrane sample, including certain solid PE samples, PE membrane 3 and polyethyleneglycol grafted PE 25.

Streaming potential for the determination of charge

In preparation for streaming potential measurements the surface materials were each cut into two 55 mm × 105 mm sheets. The samples were clamped into a flow cell with a Teflon spacer separating them to provide a channel with a height of 0.8 mm, a width of 10 mm and a length of 80 mm.

An acetic acid buffer solution, the pH of which was systematically varied from 4 to 10, was made by dissolving acetic acid and potassium acetate in deionized water. The ratio of acid to base was changed to vary the pH. The conductivity was maintained at approximately 1.0 msiemen by adding a small amount of potassium chloride. For example, a solution at pH 5.0 was made by adding 0.32 ml of acetic acid and 1 g of potassium acetate to 1 l of DI water followed by the addition of 0.04 g of potassium chloride to obtain the desired conductivity. pH was monitored throughout the streaming potential experiments and did not change significantly during measurements.

The materials were soaked in the buffer solution for 24 h prior to analysis. The material and solutions were then placed in the AP Paar, Electro Kinetic Analyzer (EKA, Brookhaven Instruments Corporation, Holtvill, NY), and the analyzer system was flushed with buffer (Kim *et al.*, 1996). The temperature was allowed to equilibrate at 25°C, and a series of pressure ramp runs going from 0 to 50, 100 or 150 mbar was performed. The streaming potential was then recorded while monitoring pH, conductivity, temperature, and pressure. During each run the pressure ramp was performed twice to 50 mbar, four times to 100 mbar, and twice to 150 mbar. The experiments were repeated three times for each material. From the streaming potential data zeta potential was calculated automatically by the EKA system software, and the results are reported in Table I.

Determination of Reversibility of Bacterial Attachment

At specified times, samples were removed from the reactor and soaked in PBS to remove any suspended cells. The surface samples covered with the biofilm were placed individually into the reaction flask of the Rototorque reactor (Characklis & Marshall, 1990) with 100 ml of PBS and then exposed to shear forces for 10 min. For all experiments the Mason Jar reactor the biofilms were grown at 250 RPM (shear stress = $0.26\,N\,m^{-2}$). Initially, the effects of shear on 1, 2 and 3-d old biofilms were tested at 0, 200, 250 and 300 RPM (shear stress = 0, 0.22, 0.28 and $0.34\,N\,m^{-2}$, respectively). Once the analysis of the effects of shear stress on removal had been performed, all subsequent experiments were conducted at 300 RPM in

the Rototorque reactor (shear stress = $0.34\,N\,m^{-2}$) to provide a standard detachment force.

The PBS solution was removed from the reactor in which the biofilms were sheared. The suspended cells removed from the surface were treated by adding Tween 20 to the PBS removal solution to final concentration 0.1% (v/v), approximately the critical micelle concentration (CMC), to break up cellular aggregates. Three separate 300 μl samples were analyzed with a Coulter Counter (Coulter Electronics, Hialeah, FL), which was filled with the same PBS solution as the cell suspension.

The remaining biofilm was also treated with Tween 20 at 0.1% (v/v), to aid in detachment, and these detached cells were counted using the same Coulter Counter technique as used for the suspended cells. This second removal step was often necessary due to the fact that the remaining biofilm was in some cases still too thick to be properly enumerated. After the second removal technique only single cells and small colonies remained on the surface, and these could be easily enumerated by direct microscopy. Therefore the surfaces were stained with acridine orange by placing the sample coupons into an aqueous solution of $1\,g\,l^{-1}$ acridine orange and 1.5% glutaraldehyde. The sample was stained for 24 h, after which time it was removed and rinsed three times in 5 ml of PBS. Immersion oil was placed on the sample, and the sample was analyzed by epifluorescence microscopy (Pasmore *et al.*, 2001).

An epifluorescence microscope was used to directly count the bacteria remaining on the surface at 600× magnification. The area of the field of view was determined using a calibrated ocular micrometer and was approximately $0.02\,mm^2$. Ten fields were counted on each sample, and each sample was analyzed in triplicate for each run for a total of 30 unique areas analyzed per run.

The total number of cells removed was calculated by multiplying the suspended cell concentration by 100 ml, and the number of cells remaining was determined using the total area of the coupon. By summing the total removed and total remaining cells, the total number of cells was determined, and the percent removed was calculated.

RESULTS AND DISCUSSION

P. aeruginosa biofilms appeared to form stronger attachments on some surfaces than on others, and the attachment to all of the surfaces grew stronger with time. The data presented here indicated that time, roughness, hydrophobicity and surface charge are all important in determining the strength of a specific attachment and therefore the likelihood of removing the bacteria.

Figure 1 demonstrates that increasing shear stress applied to attached biofilms increased removal of bacteria from fouled surfaces. The samples were grown on PE at the same shear stress as the 250-RPM removal treatment; therefore, very little removal at shear stress of equal or lower values than that of the 250-RPM treatment would be expected. After 1 day of biofilm growth the PE samples had an average of approximately 5×10^8 cells, which corresponds to a near complete coverage of approximately 75% or the coupon surface (multiple cells thick). On days 2 and 3 the average number of cells increased to 1.3×10^9 and 3.2×10^9, respectively. Of greatest interest in Figure 1 is that although the percentage of bacteria removed by 300 RPM is nearly constant on each of the 3 days of analysis the percentage removed by 200 and 250 RPM shears dropped substantially on day 2

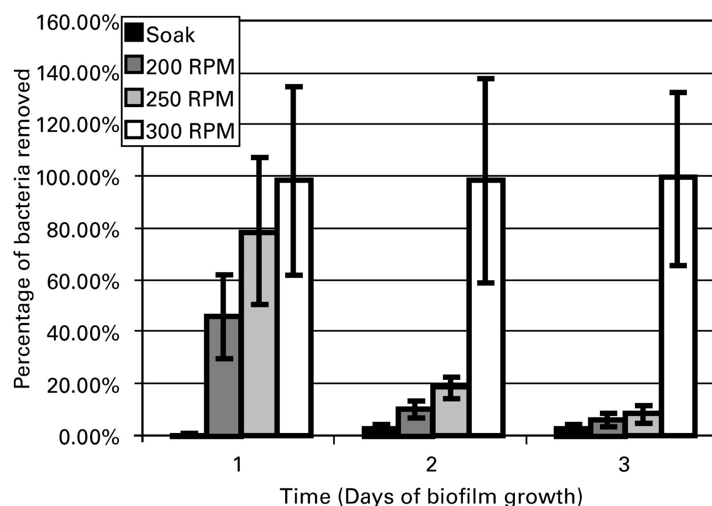

FIGURE 1 Plot of the percentage of bacteria removed from a $1.5\,cm^2$ polyethylene surface under varying amounts of shear at different times. Soak = ~0 shear; 200 RPM = $0.22\,N\,m^{-2}$; 250 RPM = $0.28\,N\,m^{-2}$; 300 RPM = $0.34\,N\,m^{-2}$ shear stress exerted on the surface during removal. The samples were grown in the growth reactor at 250 RPM or $0.26\,N\,m^{-2}$ shear stress.

Downloaded By: [Montana State University] At: 18:28 16 October 2009

Downloaded By: [Montana State University] At: 18:28 16 October 2009

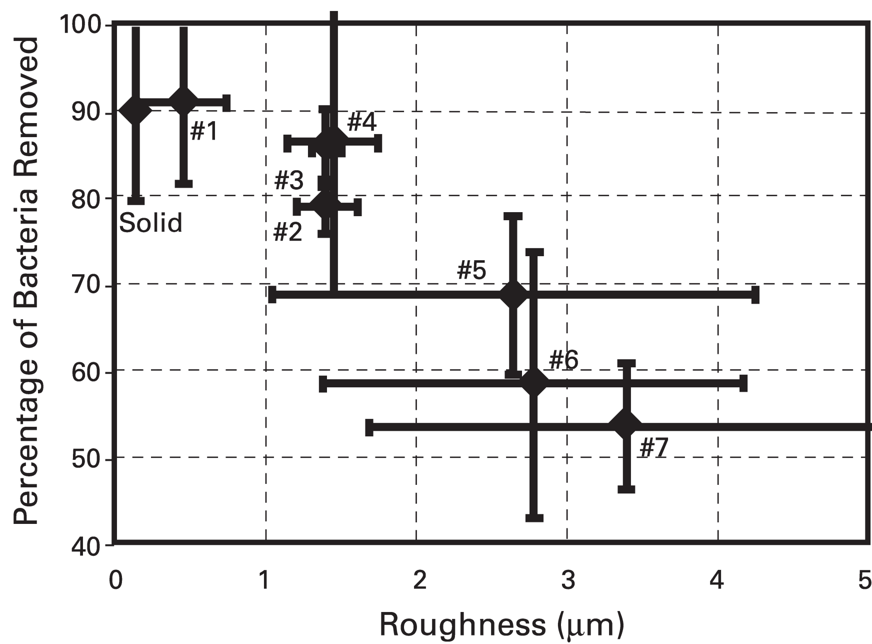

FIGURE 2 Plot of percentage of bacteria removed by shear at 300 RPM from polyethylene *vs* the surface roughness, tested after 72 h biofilm growth. Numbers on points correspond to surfaces listed in Table I.

and to a lesser extent on day 3. There is thus a change in cell adhesion over the three days of this experiment. The number of cells removed at 200 and 250 RPM remained relatively constant over the 3 days of experimentation, but the drop in percentage removed indicates an increase in the overall attachment strength of the bacteria.

The effects of the three surface properties on the reversibility of cellular attachment are presented in Figures 2–4. The rough surfaces provide both increased surface area for attachment and low-shear areas where shear-induced detachment is likely to be decreased. For these reasons the results shown in Figure 2 are expected. The plot shows that as the roughness of the surface increased, the percentage of cells that can be removed became reduced.

The hydrophobicity and charge of the surface can affect the attractive and repulsive forces between the

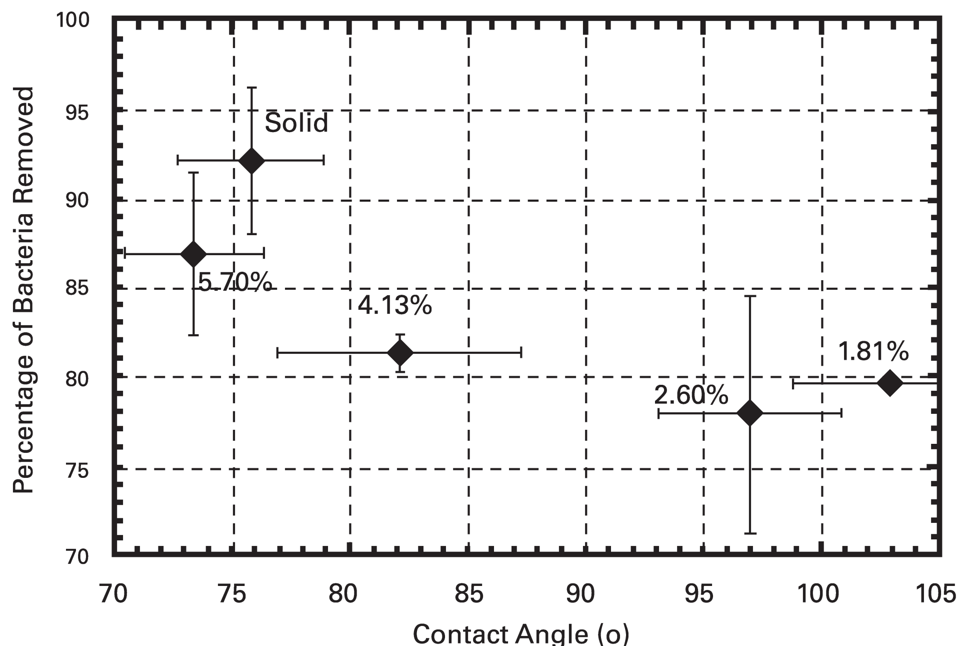

FIGURE 3 Plot of percentage of bacteria removed by shear at 300 RPM from the surface *vs* the contact angle or hydrophobicity of varying consistencies of polyethylene/poly(ethylene glycol) membranes, tested after 72 h biofilm growth. The numbers at each point indicate % weight gain due to grafted poly(ethylene glycol), and the untreated solid polyethylene sample.

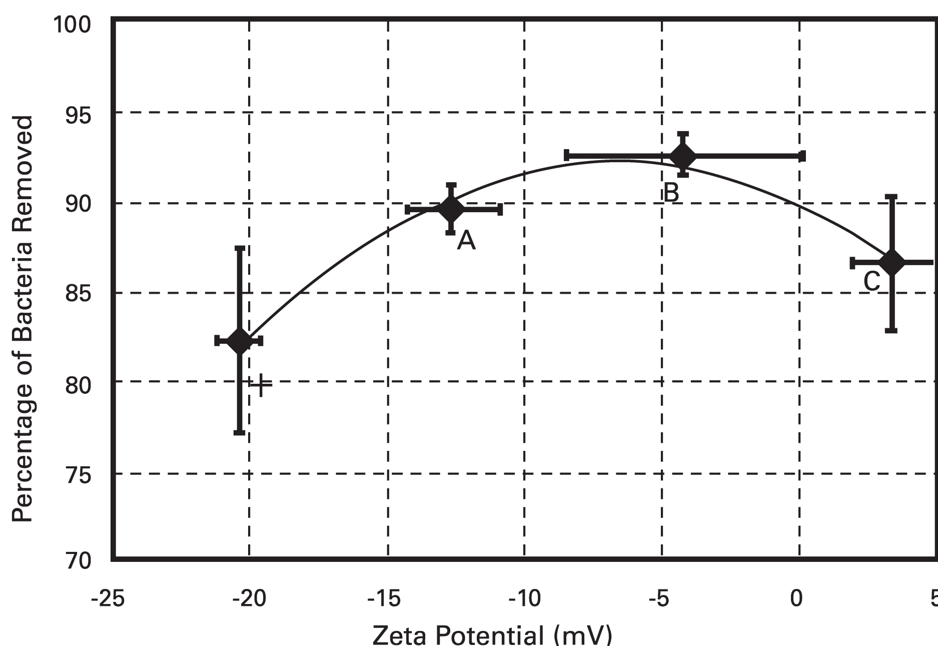

FIGURE 4 Plot of percentage of bacteria removed by shear at 300 RPM from the surface *vs* the zeta potential of nylon surfaces after 72 h biofilm growth. Letters on points correspond to surfaces listed in Table I.

Downloaded By: [Montana State University] At: 18:28 16 October 2009

bacterial cells and substrate (Pasmore *et al.*, 2001). Bacteria are usually moderately hydrophilic with negative surface charge (Madigan & Martinko, 1997). Figure 3 shows that a lower percentage of bacteria were removed from hydrophobic surfaces than from hydrophilic surfaces, while Figure 4 demonstrates that the maximum removal took place when bacteria attached to surfaces with neutral or small negative charges. Furthermore, as the surface charge increased, either with positive or negative charges, the cell removal decreased.

This work does not explore why the strength of biofilm attachment varies, but it does correlate well with the results of others, who have shown that biofilms are difficult to remove from surfaces (Characklis & Marshall, 1990; Davies *et al.*, 1993; Lau & Liu, 1993) One explanation is that the extracellular polysaccharides (EPS) produced by *P. aeruginosa* absorb/adhere more strongly to specific surfaces (Davies *et al.*, 1993). EPS is one or more the group of sticky carbohydrate-based polymer that many bacteria secrete and which has shown to be the main structural component of the biofilm matrix material (Characklis & Marshall, 1990).

CONCLUSIONS

The amount of force used is critical in the removal of bacteria from biofilms. Time (age of biofilm) is also an important factor; as a biofilm is allowed to develop the strength of attachment increases, and, after a specific time, the amount of biofilm removed by a given technique is greatly reduced.

Although the three surface properties studied (roughness, charge and hydrophobicity) for conventional materials may not prevent biofilm formation, these properties are significant in determining biofilm removability. Differences of between 15 and 50% are attributed to changes in a single surface property (roughness, charge and hydrophobicity), and each property optimized individually enabled 91–93% removal. If all three properties of the surface are optimized, it is conceivable that more significant increases in removal are possible. This study has shown that it is most facile to remove bacteria from young biofilms grown on smooth, hydrophilic, neutral surfaces. In this context it can be noted that the "Nylon B" sample used in the study had the lowest negative zeta potential, zero contact angle and minimal roughness (Table I), and this membrane permitted the greatest biofilm removal of all of the surfaces evaluated.

It was of interest that the relationship between surface properties and removal appears to reflect the corresponding relationship determined for biofilm formation, as shown previously (Pasmore *et al.*, 2001). That is to say that the materials that permitted the most biofilm growth initiation were the surfaces that allowed the least removal of bacteria. Therefore, although biofilms grow on almost all surfaces, the strength of attachment will determine both the extent of biofilm development and the ease of its removal.

Acknowledgments

We would like to thank Dawn Lowman Baker, Lee Halevi, Sara Smith, and Jill Fletcher for their help in

the laboratory, as well as William B Krantz, Bart Van Zeghbrock, and John J Pellegrino for use of their equipment. Funding for this project was provided by the University of Colorado Membrane Applied Science and Technology Center, National Science Foundation, and the Colorado Advanced Technology Institute.

References

Benkel D H, McClure E M, Woolard D, Rullan J V, Miller G B, Jenkins S R, Hershey J H, Benson R F, Pruckler J M, Brown E W, Kolczak M S, Hackler R L, Rouse B S, Breiman R F (2000) Outbreak of Legionnaires' disease associated with a display whirlpool spa. *Int J Epidemiol* **29**: 1092–1098

Brown M R W, Baker J (1999) Unexplored reservoirs of pathogenic bacteria: protozoa and biofilms. *Trends Microbiol* **7**: 46–50

Characklis W G, Marshall K C (1990) *Biofilms*. John Wiley & Sons, New York

Costerton J W, Stewart P S (2000) Biofilms and device-related infections. In: Nataro J P, Blaster M J, Cunningham-Rundles S (eds) *Persistent Bacterial Infections*. ASM Press, Washington DC, pp 423–439

Costerton J W, Stewart P S, Greenberg E P (1999) Bacterial biofilms: a common cause of persistent infections. *Science* **284**: 1318–1322

Costerton J W, Lewandowski Z, Caldwell D E, Korber D R, Lappin-Scott H M (1995) Microbial biofilms. *Annu Rev Microbiol* **49**: 711–745

Davies D G, Chakrabarty A M, Geesey G G (1993) Exopolysaccharide production in biofilms: substratum activation of alginate gene expression by *Pseudomonas aeruginosa*. *Appl Environ Microbiol* **59**: 1181–1186

Franklin M J, Ohman D (1996) Identification of algI and algJ in the *Pseudomonas aeruginosa* alginate biosynthetic gene cluster which are required for alginate O acetylation. *J Bacteriol* **178**: 2186–2195

Kim K J, Fane A G, Nystrom M, Pihlajamaki A, Bowen W R, Mukhtar H (1996) Evaluation of electroosmosis and streaming potential for measurement of electric charges of polymeric membranes. *J Membr Sci* **116**: 149–159

Lam J, Chan R, Lam K, Costerton J W (1980) Production of mucoid microcolonies by *Pseudomonas aeruginosa* within infected lungs in cystic fibrosis. *Infect Immunol* **28**: 546–556

Lau Y L, Liu D (1993) Effect of flow rate on biofilm accumulation in open channels. *Water Res* **3**: 355–360

McFeters G A (1992) Effects of culture conditions and biofilm formation on the iodine susceptibility of *Legionella pneumophila*. *Can J Microbiol* **38**: 423–429

Ma H, Davis R H, Bowman C N (2000) A novel sequential photoinduced living graft polymerization. *Macromolecules* **33**: 331–335

Madigan M, Martinko J M (1997) *Brock Biology of Microorganisms*. Prentice Hall, Upper Saddle River, NJ

Marshall P A, Loeb G I, Cowan M M, Fletcher M (1989) Response of microbial adhesives and biofilm matrix polymers to chemical treatments as determined by interference reflection microscopy and light section microscopy. *Appl Environ Microbiol* **55**: 2827–2831

Melo L F, Bott T R (1992) *Biofilms – Science and Technology*. Kluwer Academic Publishers, Boston, MA

Naumova E N, Chen J T, Griffiths J K, Matyas B T, Estes-Smargiassi S A, Morris R D (2000) Use of passive surveillance data to study temporal and spatial variation in the incidence of giardiasis and cryptosporidiosis. *Public Health Rep* **115**: 436–447

O'toole G A, Kolter R (1998) Flagellar and twitching motility are necessary for *Pseudomonas aeruginosa* biofilm development. *Mol Microbiol* **30**: 295–304

Pasmore M, Todd P, Smith S, Baker D, Silverstein J, Coons D, Bowman C N (2001) Effects of ultrafiltration membrane surface properties on *Pseudomonas aeruginosa* biofilm initiation for the purpose of reducing biofouling. *J Membr Sci* **194**: 15–32

Potts S B, Roggli V L, Spock A (1995) Immunohistologic quantification of *Pseudomonas aeruginosa* in the tracheobronchial tree from patients with cystic fibrosis. *Pediatr Pathol Lab Med* **15**: 707–721

Ridgeway H F, Justice C A, Whitaker C, Argo D G, Olson B H (1984) Biofilm fouling of RO membranes—its nature and effect on treatment of water for reuse. *J Am Water Works Assoc (JAWWA)* 94–102

Yasuda T, Okuno T (1994) Contact angle of water on polymer surfaces. *Langmuir* **10**: 2435–2439

Downloaded By: [Montana State University] At: 18:28 16 October 2009