Microbial detachment from biofilms

Gillian F. Moore,[1] Braden C. Dunsmore,[1] Steven M. Jones,[1] Christopher W. Smejkal,[1] Jana Jass,[2] Paul Stoodley[3] and Hilary M. Lappin-Scott[1]

[1] Environmental Microbiology Research Group, Exeter University, Exeter, UK
[2] Department of Microbiology, Umeå University, Umeå, Sweden
[3] Center for Biofilm Engineering, Montana State University, Bozeman, MT, USA

INTRODUCTION

This chapter reviews the broad area of biofilm detachment, the mechanisms of detachment and the methods used to study this important process. Two case studies are included: the first of these focuses on the control of clinical biofilms; the second case study examines detachment in the water industry.

Biofilms are dynamic structures found in a wide variety of both natural and man-made environments. Their formation has been well studied; for example, Characklis (1990) described eight different stages of biofilm accumulation (Table 1 and Fig. 1). There has been much research into the initial attachment of micro-organisms to surfaces, including the effect of electrostatic interactions and electrochemical forces (Bos *et al.*, 1999). The physiological changes that attaching cells undergo have also been examined; for example, the production of surface appendages such as fimbriae (Austin *et al.*, 1998). In contrast to the work undertaken on attachment, detachment has received little attention although many researchers regard it as a crucial stage of biofilm development (Stewart, 1993; Allison *et al.*, 1999).

Bryers (1988) classified the detachment process into four separate groups: abrasion, grazing, erosion and sloughing. Detachment from the biofilm can be directly caused by the collision or rubbing together of surfaces on which the biofilm has developed, leading to abrasive detachment. Larger organisms feeding on the biofilm can indirectly cause detachment through grazing. Erosion and sloughing refer to physical or chemical processes, which indirectly affect the biofilm structure, leading to detachment. Erosion

SGM symposium 59: Community structure and co-operation in biofilms. Editors D. Allison, P. Gilbert, H. Lappin-Scott, M. Wilson. Cambridge University Press: ISBN 0 521 79302 5 ©SGM 2000.

Table 1. Biofilm formation processes (Characklis, 1990)

Stage	Process
1	Organic preconditioning of the substratum
2	Transport of microbial cells to the substratum
3	Reversible adsorption of the cells to the substratum
4	Desorption of cells from substratum to bulk liquid
5	Irreversible adsorption of cells on the substratum
6	Growth of cells adsorbed to the substratum and production of extracellular polymeric substances
7	Attachment of other cells and particles from the bulk liquid to the biofilm
8	Detachment of portions of the biofilm

refers to the continual removal of cells or small groups of cells from the biofilm, whereas sloughing is the loss of discrete amounts of biofilm.

The detachment of pathogenic micro-organisms from a biofilm can have serious implications, such as the contamination of a water supply. Alternatively, increasing the rate of detachment by various types of intervention to control biofilm growth is one way of preventing harmful biofilm accumulation. To achieve this control, more must be understood about the detachment process within biofilms.

MECHANISMS OF BIOFILM DETACHMENT

The environment around and within the biofilm plays a key role in any detachment process. The physical and chemical properties of the environment can influence the whole biofilm or change the biological properties of the cells within the biofilm and this may lead to detachment. Abrasion, erosion and sloughing are all types of detachment processes. They may be regarded as distinct from grazing in that, once understood, they may be manipulated to alter detachment rates and control biofilms.

Detachment processes

Unlike erosion and sloughing, abrasion is caused by direct physical contact with the biofilm structure, for example, scraping a biofilm from a surface with cleaning utensils. The effect of abrasive detachment is particularly important in the water industry as it can seriously affect the performance of the fluidized-bed reactors that utilize biofilms to treat wastewater. Within these reactors, biofilms are developed on particles of an inert material and, in the treatment process, these can collide and rub together causing detachment of the biofilm (Chang *et al.*, 1991).

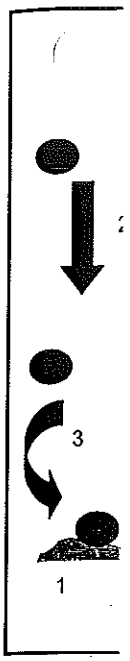

Fig. 1. Biof
eight stages
desorption;
7, attachme

Erosive d
interface.
those furt
the biofil
laminar st
the higher

It has beer
bulk liqui
(1994) de
denitrifyin

Factors
Detachme
bulk liqui
changes in

Physi
biofilm, ro

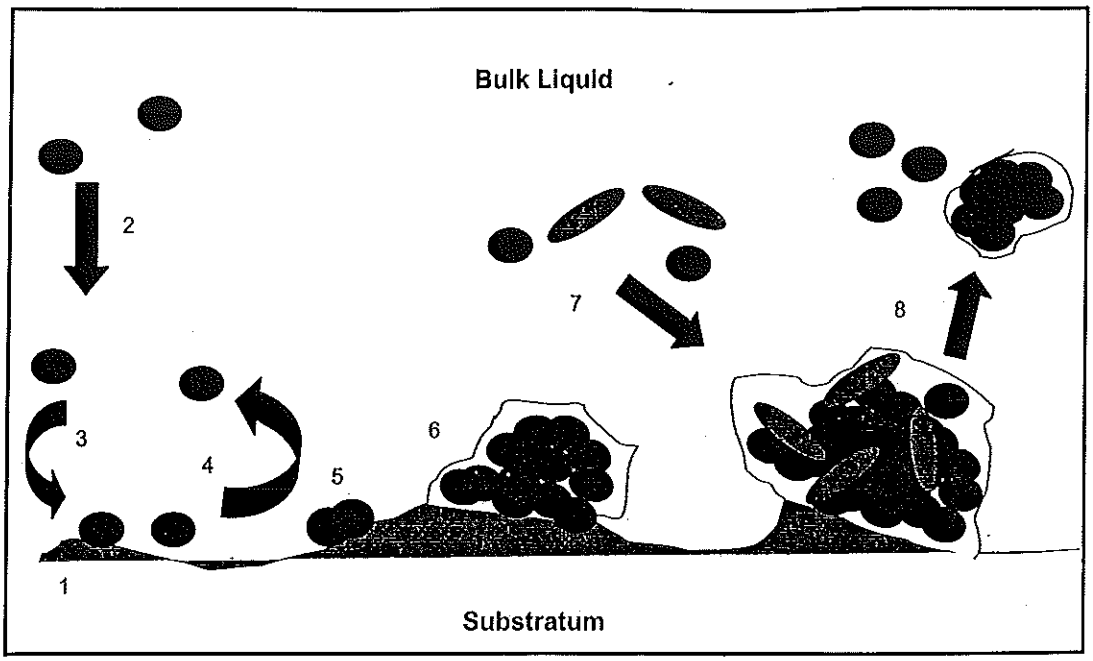

Fig. 1. Biofilm formation process (Characklis, 1990). Characklis (1990) described biofilm formation in eight stages: 1, preconditioning; 2, transport of cells to substratum; 3, reversible adsorption; 4, desorption; 5, irreversible adsorption; 6, growth and extracellular polymeric substances production; 7, attachment by other micro-organisms; 8, detachment.

Erosive detachment can be prompted by the fluid shear stress at the biofilm–fluid interface. Here there is a laminar sub-layer where shear forces are low compared to those further above the interface in the bulk liquid (Lappin-Scott *et al.*, 1994). When the biofilm grows and increases in thickness, its irregular surface grows above the laminar sub-layer and into more turbulent flows where cells are continually removed by the higher shear stresses (Characklis, 1990; Brading *et al.*, 1995).

It has been noted that chemical changes within the biofilm structure or in the surrounding bulk liquid can often cause sloughing of the biofilm. For example, Ohashi & Harada (1994) demonstrated that gas vacuoles of nitrogen formed in a mature biofilm of denitrifying bacteria. These were produced within the biofilm and weakened its structure.

Factors influencing detachment

Detachment processes can be linked to the environment within the biofilm or within the bulk liquid. These influences can be physical, chemical or biological and may lead to changes in the physiology of the cells in the biofilm and to eventual detachment.

Physical properties. Physical influences are mainly in the bulk liquid surrounding the biofilm, for example the liquid velocity. Chang *et al.* (1991) examined detachment rates

in a liquid fluidized-bed reactor and found that increases in liquid velocity and particle concentration lead to faster detachment rates.

The shear stress at the biofilm–liquid interface influences the erosion of a biofilm (Characklis, 1990). However, Stoodley *et al.* (1999a) suggested that the frequency of changes in shear, as well as the magnitude of shear, can lead to detachment. Between infrequent changes in stress, the biofilm may have an opportunity to repair itself, whereas in more frequent fluctuations, the biofilm reduces in thickness and detachment occurs.

There are a number of reports on the effect of physical forces on biofilm structure (Chang *et al.*, 1991; Nicolella *et al.*, 1997; Kwok *et al.*, 1998; Stoodley *et al.*, 1999b). Tijhuis *et al.* (1996) reported that with high detachment forces, the biofilm became smooth and strong. This was in contrast to the rough and weaker biofilms formed under low-force conditions.

Chemical properties. Changes in the chemical properties within the biofilm or the bulk liquid can cause detachment. Biofilm detachment can be influenced by changes in the availability of nutrients (Stoodley *et al.*, 1999b). Sawyer & Hermanowicz (1998) found that nutrient limitation at the biofilm–liquid interface of *Aeromonas hydrophila* increased the rate of detachment. However, other studies have shown that an increase in nutrient availability can lead to an increase in the detachment rate (Characklis *et al.*, 1990).

The electrochemical properties within the biofilm have been suggested to be a significant factor in detachment. Changes in these, associated with the requirement for protons needed for lactose transport into the cell, could cause the extracellular polysaccharide matrix to expand or contract with the change in potential and so lose its cohesive properties (Characklis *et al.*, 1990).

The effect of substrate limitation on detachment rates has also been investigated. Peyton & Characklis (1993) reported that substrate limitation decreased the rate of detachment in a *Pseudomonas aeruginosa* biofilm by limiting the growth rate of the cells within the biofilm. Other work has shown that the surface properties of bacterial cells change during different stages of growth and this may affect detachment rates (Gilbert *et al.*, 1991). Therefore, detachment may be mediated by the cell cycle (Allison *et al.*, 1990).

There are many different regulatory systems that control the expression of genes within bacteria. Some of these have been studied in the context of biofilm growth and offer

insights into detachment processes. The stationary phase sigma factor RpoS has been found to be involved in the regulation of the transcription of a variety of genes when the cell is subjected to stresses, such as nutrient limitation, that lead to the stationary phase of growth (Loewen *et al.*, 1998). The involvement of RpoS in the biofilm development of both *Escherichia coli* and *Salmonella enteritidis* PT4 has been reported (Adams & McLean, 1999; Moore *et al.*, 1999). The role of RpoS in biofilm formation may include the control of gene expression for fimbriae (Hammer *et al.*, 1995) and extracellular polymeric substances. However, the growth rate of the cells within the biofilm is increased after substrate loading, leading to the inactivation of RpoS and therefore the loss of activation of the proteins it regulates. This could result in a weakening of biofilm cohesion and detachment may occur.

Other investigators have also highlighted the relationship between growth phase and detachment. For example, *Clostridium thermocellum* has been shown to detach from the substratum during the stationary phase of growth (Lamed & Bayer, 1986). This bacterium adsorbs strongly to cellulose, which it then utilizes as a substrate. A cellulosome is produced and this enhances attachment to the substratum. When growth reaches the stationary phase, the cellulosome is released and the cell is free to attach elsewhere. It has been shown that, under certain conditions, bacteria within the biofilm can produce chemicals that destabilize the biofilm structure, leading to sloughing. Applegate & Bryers (1991) showed that in an oxygen-limited *Pseudomonas putida* biofilm, there was an increase in the calcium ion concentration and the production of extracellular polymeric substance. Calcium ions act as cross-linking agents within the biofilm and, along with the extracellular polymeric substance production, increase cohesiveness. Therefore, when sloughing occurred in oxygen-limited biofilms, it was on a large scale as the cells were firmly aggregated.

Biological properties. Some of the biological mechanisms involved in detachment from biofilms have been studied. *P. aeruginosa* biofilms produce alginate, a polysaccharide that enhances attachment of the cells to surfaces (Mai *et al.*, 1993). The bacterium also produces alginate lyase, which induces sloughing by degrading the polysaccharide that holds the biofilm together (Boyd & Chakrabarty, 1994). *Pseudomonas fluorescens* has also been reported to produce lyase, which degrades exopolysaccharides under starvation conditions and therefore increases detachment under these conditions (Allison *et al.*, 1998).

Enzymes produced by a range of organisms similarly bring about detachment. *Streptococcus mutans* produces surface protein releasing enzyme (SPRE) which releases proteins from the surface of the cell. The activity of SPRE increases the detachment of the bacterium from the biofilm (Lee *et al.*, 1996).

The importance of regulatory systems that control gene expression by bacteria has already been noted. The influence of other regulatory systems on biofilm formation and detachment has also been investigated. Davies *et al.* (1998) reported that mutants lacking the ability to synthesize specific signalling molecules formed undifferentiated biofilm, and these were sensitive to SDS. Other investigators have also demonstrated that homoserine lactones play a role in the formation, structure and detachment events by *P. aeruginosa* (Stoodley *et al.*, 1999c; Allison *et al.*, 1999). Homoserine lactones have also been reported to play a role in the biofilm detachment of *A. hydrophila* (Lynch *et al.*, 1999). Puckas *et al.* (1997) reported that *Rhodobacter sphaeroides* produces the homoserine lactone 7,8-*cis*-tetradecenoylhomoserine lactone. When cell aggregation by the bacterium was examined, it was noted that the wild-type grew as individual cells in suspension whereas a mutant deficient in the production of the homoserine lactone produced large aggregates.

The ability of cell–cell communication to influence biofilm structure suggests that it may regulate a number of physiological functions. Reports have implicated quorum sensing in the direct or indirect regulation of RpoS activity (Sperandio *et al.*, 1999). More research is needed in this new and complex area of regulatory systems in biofilms to clarify the specific roles they play. However, such systems could be the key to the development of novel agents to control biofilm growth.

METHODS FOR THE STUDY OF BIOFILM DETACHMENT

Many experimental systems are available to study biofilm growth (Ladd & Costerton, 1990). In this section, methods used for the study of the relatively new area of detachment from biofilms will be described. These methods can be broken down using a framework to assist the process of investigation (Fig. 2).

'Three choice' experimenting

When designing an experiment to study biofilm detachment, three choices must be made, that is, a culture method, a sample method and a method (or methods) of analysis (Fig. 2). The culture method refers to the situation in which the biofilm is established. The biofilm may already be established within its natural environment (a 'real' biofilm) or within a laboratory model system. Biofilms formed in the laboratory can be cultured in either a batch or flowing system to mimic their natural environment. If a flowing system is chosen, the rate of fluid flow is important to the experimental design as hydrodynamics have a profound effect on biofilms (Stoodley *et al.*, 1999d, e). Flow rate can affect the structure of the biofilm and lead to markedly different detachment events. Turbulent flow biofilms have been shown to be denser than, or of a different shape from, those grown under laminar flow (Chang *et al.*, 1991; Stoodley *et*

Culture Method	Sample Method	Method of Analysis
'Real' Environmental samples	Destructive	Macro (e.g. Plate counts, turbidity)
Batch system →	→	
Flowing system (Laminar/turbulent)	Non-Destructive	Micro (e.g. Light, epifluorescent and scanning electron microscopy)

Fig. 2. 'Three choice' experimental design: the necessary choices for design of experiments to study biofilm detachment.

al., 1999b). Finally, the flow rate should be chosen with reference to the environment that is being modelled.

Once a biofilm is established, the detachment events must be monitored. These methods can be either destructive or non-destructive. Non-destructive techniques allow *in situ*, real-time analysis, whereas destructive sampling involves the removal of the biofilm from its environment. Analysis of the sample is performed using either a macro-method, such as plate counting or turbidity measurement, or a micro-method involving the use of microscopy.

Critical evaluation of methods used for detachment studies

Macroscopic. A method which 'reproducibly quantifies the ease of removal of micro-organisms from surfaces' (Eginton *et al.*, 1995) gives a useful indication of cell detachment from a biofilm by measuring the strength of attachment. Here, tiles of different materials were suspended in media and biofilms developed. Tiles were then sequentially placed onto several agar plates and the resulting c.f.u. were counted. This methodology uses a batch system of growth followed by destructive sampling to allow detached cells to be measured macroscopically. The merits of this method are its simplicity and reproducibility.

Lee *et al.* (1996) investigated the role of surface proteins in detachment. In this study, hydroxyapatite rods were suspended in a chemostat containing *S. mutans*. The addition of SPRE increased the detachment from the biofilm. Detachment was measured by removal of the rods into buffer; these were then treated by gentle rocking or sonication. Viable cell counts were made from each treatment with detachment being measured as the difference between cells detached 'naturally' and the total of natural and sonicated counts. In the study, a batch culture was coupled to a destructive sampling technique and analysed macroscopically: it is one of the simplest, yet effective methods of studying detachment.

Nicolella *et al.* (1996, 1997) identified several of the parameters significant to detachment of bacteria from a fluidized bed. A laboratory-scale reactor, consisting of a glass column packed with sand, was used to establish a biofilm. The fluidized bed was designed to mimic the real system with fluid flow through the reactor. Suspended solids were drawn off and filtered to assess detachment. This method was non-destructive and the analysis was macroscopic.

Microscopic. Stoodley *et al.* (1999a) devised a real time, *in situ* flow cell system for non-destructive studies of biofilm processes, including detachment. Time-lapse image analysis was used to investigate mixed-species biofilms *in situ* within a glass flow cell (Fig. 3). Surface area measurements allowed both attachment and detachment to be monitored (Stoodley *et al.*, 1999c). The effect of specific conditions, such as changes in nutrient concentration or fluid flow, on detachment was captured and image subtraction was used to calculate detachment rates from the biofilm.

Sawyer & Hermanowicz (1998) used digital image analysis with time-lapse photography to demonstrate increased detachment rates of *A. hydrophila* under limiting nutrient conditions in a glass flow cell. Continuous video capture was also employed (Ohashi & Harada, 1994) to perform *in situ* analysis of a denitrifying bacterial biofilm formed on polyvinylchloride plates within a rectangular open channel reactor. Different types of detachment were described during the progression of biofilm formation. By combining a flowing culture system with non-destructive image analysis by microscopy, an effective tool was created for detachment studies. Images taken *in situ* may be used to describe properties of the biofilm such as morphology, surface area covered, as well as the mechanisms of detachment.

Combined macroscopic and microscopic analysis. Biofilms formed on basalt particles in airlift reactors were studied by Kwok *et al.* (1998). Biofilm was established under reproducible conditions, then the effect of changing the substrate loading and fluid force on detachment was investigated. Detachment was assessed by sonicating th

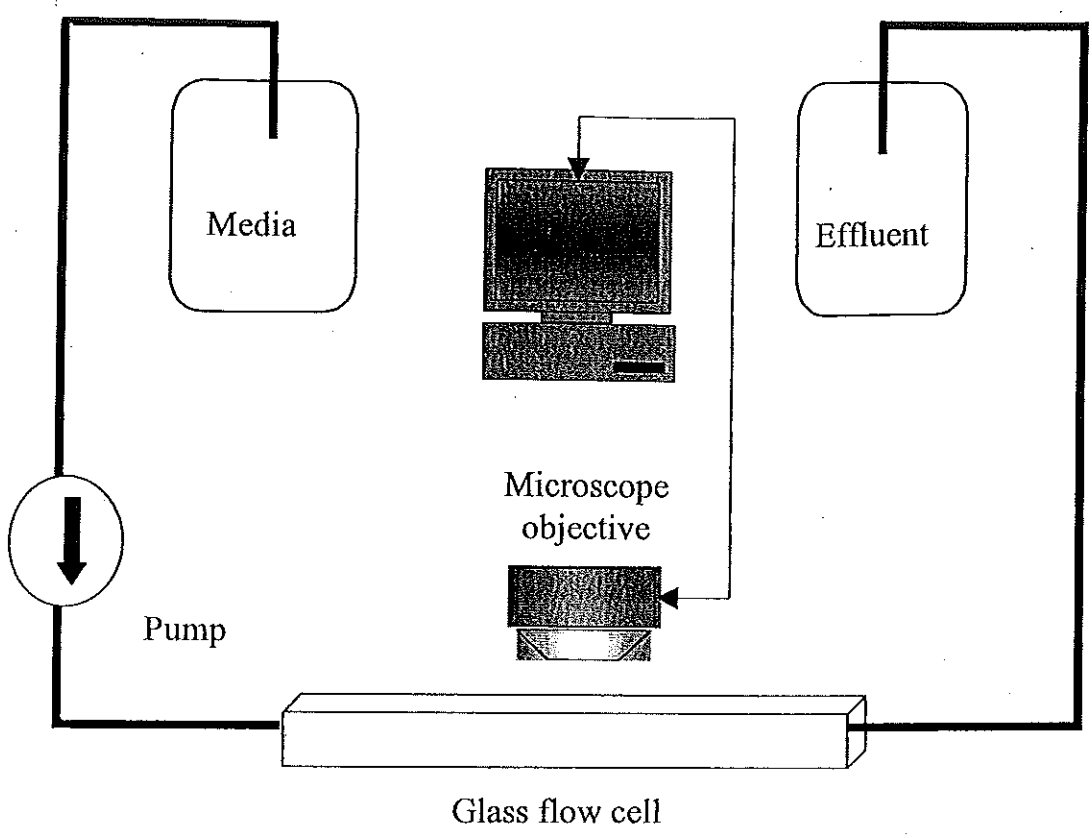

Fig. 3. Schematic of a simple image analysis system to study biofilm detachment. Systems can be more sophisticated to include: recirculating flow (useful for high flow rates), non-pulsed flow (useful for slow flow rates) and multiple flow cells.

particles to remove the biofilm, then the organic carbon was determined by dry weight. Biofilm morphology was also monitored using an image analysis system.

In summary, few methods are available for the quantification of detachment in biofilms. However, there are examples of experimental design that enable the researcher to measure detachment. The culture method chosen to develop the biofilm will largely depend on the system to be modelled.

Destructive sampling can provide insight into detachment and may be currently the most fruitful way of studying real biofilms. The danger of using such sample methods for measuring detachment is that the destructive nature of the sampling may alter the process of detachment and, for this reason, non-destructive sampling is preferable. The strengths of macroscopic sampling lie with the ability to take large samples. This can be especially useful when sampling industrial systems. Macro-analysis is highly reproducible when compared to microscopic analysis. The latter does, however, have the advantage of providing greater insight into the mechanisms behind detachment.

CASE STUDY: THE CONTROL OF CLINICAL BIOFILMS

In clinical situations, biofilms can colonize human tissues and man-made surfaces introduced into the human body, such as medical prostheses. These biofilms can prove potentially fatal to the patient. It is important that control measures are formulated specifically to detach and eradicate these biofilms. The following section discusses commonly applied antimicrobial agents used to control biofilms by inducing detachment.

Man-made surfaces

The introduction of foreign materials into the human body, such as contact lenses or catheters, can provide new surfaces for biofilm formation. *P. aeruginosa* (Stapleton & Dart, 1995), *Staphylococcus epidermidis* (Gabriel *et al.*, 1996), *Bacillus* sp. (Gopinathan *et al.*, 1997) and *Acanthamoeba* (Marciano-Cabral *et al.*, 2000) are commonly associated with biofilm formation on contact lenses. If these biofilms are not removed, and the user continues to wear the lenses, conditions such as keratitis and acute conjunctivitis can occur (Stamler, 1998). Biofilms on contact lenses are removed by subjecting the lens to a rigorous cleaning regime. Research by Landa *et al.* (1998) examined the efficacy of ophthalmic solutions, such as all-in-one solutions and a detergent mixture. The latter consisted of 0.25% (w/v) sodium lauryl sulphate (SLS) and 0.2% (w/v) sodium methyl cocoyl taurate (Tauranol). These solutions were tested against attached *P. aeruginosa* biofilms on gas permeable and soft contact lenses. The results showed that the all-in-one solutions were successful at inducing minor bacterial detachment of *P. aeruginosa* biofilms and the SLS/Tauranol mixture was shown to detach 95% of the *P. aeruginosa* biofilm. A study by Gavin *et al.* (2000) demonstrated that even after 30 min exposure to 3% (w/v) hydrogen peroxide, 11–13% of the *P. aeruginosa* biofilm cells were actively respiring and recoverable by standard culture methods.

In addition to the bacterial presence, protozoa can also exist on contact lens surfaces. The most significant of these protozoa is *Acanthamoeba polyphaga*, which grazes upon the biofilm. Gorlin *et al.* (1996) reported that, in the absence of a biofilm, the detaching fluids or saline rinses essentially removed all amoebae from the lens surface. However, the cleaning solutions were less effective at controlling the *Acanthamoeba* in the presence of a biofilm of *P. aeruginosa*.

Methicillin-resistant *Staphylococcus aureus* (MRSA) can colonize catheters (Steinberg *et al.*, 1996). The effects of antimicrobial agents on MRSA biofilms grown on silastic are being studied in our laboratory (S. M. Jones, T. J. Humphrey & H. M. Lappin-Scott, unpublished observations). Detachment of a 48 h MSRA biofilm established on silastic rubber was induced after exposing the biofilm to 100 μg vancomycin ml^{-1}. The biofilm detached, the surface area coverage being reduced by 4.76% min^{-1} after

administering the antibiotic (Figs 4 and 5), thus demonstrating that high concentrations of vancomycin are effective at increasing detachment of MRSA biofilms.

Human tissues

Biofilms can form on natural surfaces within the human body and lead to serious health problems. For example, endocarditis is a life-threatening infection of the heart valves. Once established, the mortality rate may be as high as 70%. The bacteria commonly associated with endocarditis are viridans streptococci (Douglas *et al.*, 1993), *S. aureus* (Kelly & Barnass, 1999), *Enterococcus faecium* (Houry & Crisman, 1999) and *S. epidermidis* (Costa *et al.*, 1999). Research by Entenza *et al.* (1999) examined the efficacy of trovafloxacin, vancomycin and ciprofloxacin in the treatment of experimental staphylococcal and streptococcal endocarditis. Results showed that trovafloxacin and ciprofloxacin were successful in inducing detachment of staphylococcal biofilms from heart valves and were comparable to vancomycin in terms of effectiveness. However, the use of ciprofloxacin resulted in the selection of resistant strains of staphylococci (Entenza *et al.*, 1999). These results showed that the antibiotics tested have varying degrees of success for treating endocarditis.

The recent discovery of MRSA strains that have a reduced susceptibility to vancomycin (VISA) have also reduced its effectiveness in treating heart valve colonization. Several studies have demonstrated that the administration of vancomycin against a VISA biofilm situated on a rabbit heart valve was ineffective, with no evidence of detachment (Patron *et al.*, 1999; Backo *et al.*, 1999). Probiotics, such as *Lactobacillus* spp., have been suggested as a novel treatment strategy. Such biofilms are encouraged to develop on natural surfaces and reduce their availability for pathogenic organisms. In addition, these harmless species may produce substances that prevent adhesion of pathogens (Hawthorn & Reid, 1990; Reid *et al.*, 1990).

Antimicrobial agents have been shown to have some effect on biofilm detachment. However, it is well documented that biofilms have a greater resistance to antimicrobial agents than their planktonic counterparts (Costerton *et al.*, 1995). An improved understanding of biofilm detachment will inform the search for new agents that are more effective against biofilms.

CASE STUDY: BIOFILM DETACHMENT – THE POTENTIAL HAZARDS IN THE WATER INDUSTRY

Biofilms in water systems

Many processes in the water industry rely on biofilms for water treatment. In such cases, biofilms are beneficial (Costerton, 1999) as the micro-organisms degrade soluble

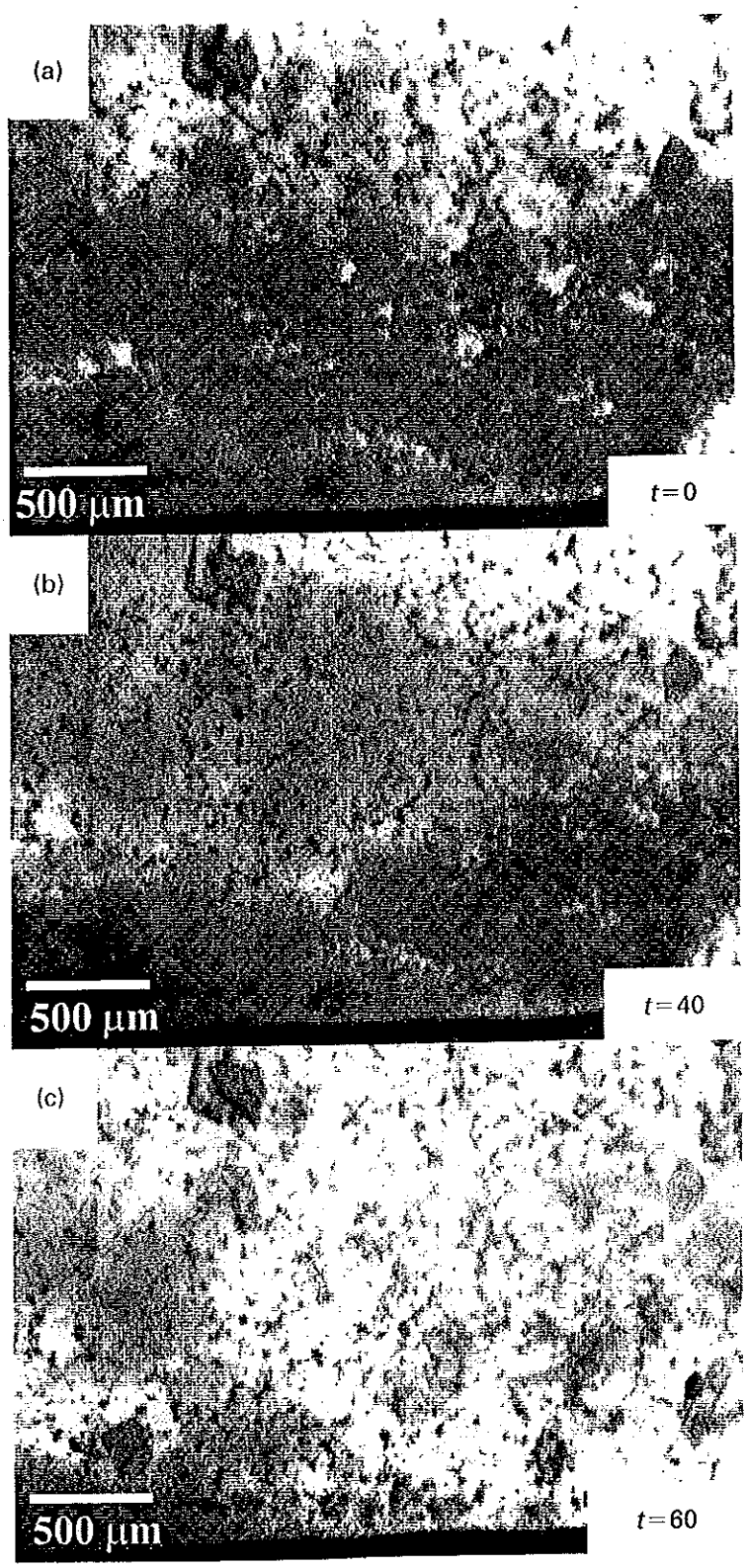

Fig. 4. Detachment of MRSA biofilm after administration of vancomycin. A 48 h MRSA biofilm formed on silastic rubber was exposed to 100 μg vancomycin ml^{-1}. (a) MRSA biofilm at $t=0$, before exposure to vancomycin; (b) biofilm after 40 min exposure; (c) biofilm after 60 min exposure (S. M. Jones, T. J. Humphrey & H. M. Lappin-Scott, unpublished results).

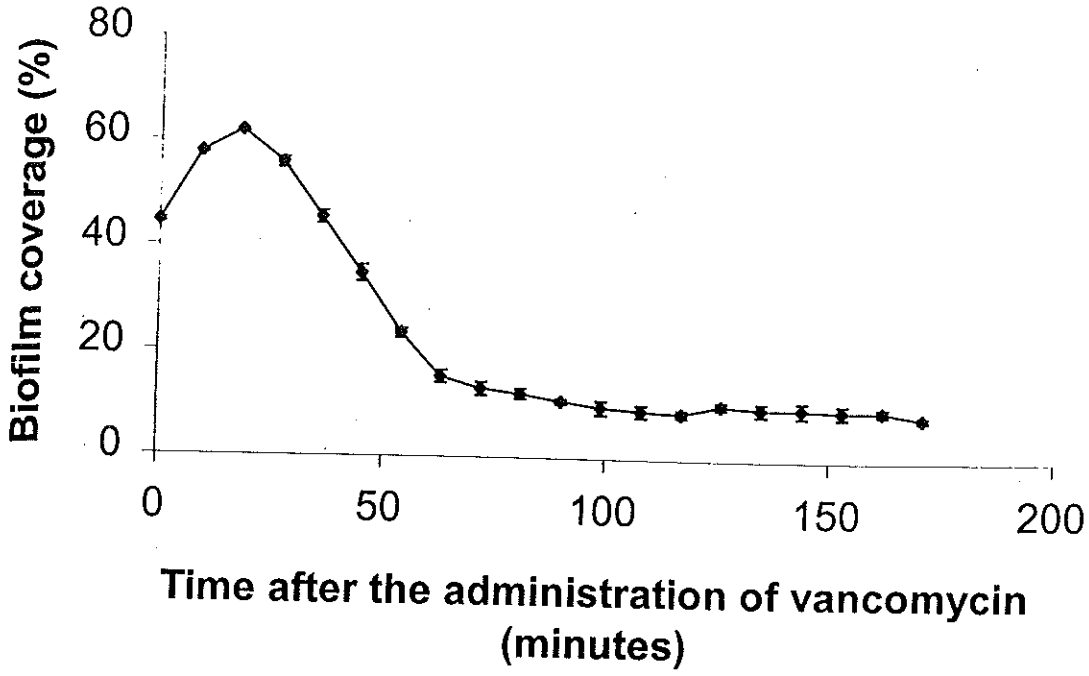

Fig. 5. Per cent surface biofilm coverage of an MRSA biofilm after exposure to vancomycin. A 48 h MRSA biofilm was established and exposed to 100 μg vancomycin ml^{-1}. Detachment was monitored by calculating biofilm coverage (%) (S. M. Jones, T. J. Humphrey & H. M. Lappin-Scott, unpublished results).

organic and nitrogenous waste materials and utilize their by-products as carbon sources. However, there are instances where biofilms have adverse effects such as in the biodeterioration, biofouling and contamination of water supplies. The harbouring of potential pathogens within biofilms has been described (Lappin-Scott & Costerton, 1989) and this can lead to detrimental effects. These pathogens can be lethal to humans and other animals because many release endotoxins which can be introduced into the water system during the sloughing of the biofilm (Rioufol *et al.*, 1999). Therefore, detachment of biofilms may involve the release of a whole array of micro-organisms, including potential pathogens, adsorbing onto microbial flocculents (flocs) and surfaces. The pathogens that are released may include *Entamoeba coli*, *Giardia lamblia*, *Shigella* spp., *Salmonella* spp. (Daly *et al.*, 1998) and *E. coli* (Mackerness *et al.*, 1993).

Biofilms in water treatment systems consist mainly of bacteria; however, it is important to consider constituent eukaryotic organisms that may be harmful or influence detachment. Microalgae form a major component in water exposed to light (Christensen & Characklis, 1990), and in hard-water reservoirs and river systems, the light and water chemistry enable the biofilm to become calcitic, leading to substantial corrosion of water pipes (Callow *et al.*, 1995). Filamentous fungi are also important in

h MRSA biofilm
biofilm at $t=0$, before
mi posure (S. M.

water treatment plants and there is now an improved understanding of their ability to form biofilms (Elvers *et al.*, 1998; Roberts *et al.*, 1999a, b). Phycomycetes are found in water and are primarily responsible for corrosion and deterioration in that environment. In addition, the unicellular protozoa are frequently found grazing on bacteria, algae and other particulate matter in trickling filters.

Methods of water treatment

The principal methods of water treatment include: the reverse osmosis system, the activated sludge method and the fluidized-bed system. The reverse osmosis system is designed to decontaminate water by membrane separation of organic solutes (Altman *et al.*, 1999; Koyuncu *et al.*, 2000), inorganic solutes (Buhrmann *et al.*, 1999; Jaouen *et al.*, 1999; Padilla & Tavani, 1999) and bacteriophages (Governal & Gerba, 1999). The effectiveness of this process is reduced by biofilm formation as the semi-permeable membrane surfaces are particularly susceptible to colonization (Bremere *et al.*, 1999).

The activated sludge method of water treatment uses a mixed microbial population in the form of flocs. Suspended and colloidal material in waste water adhere and adsorb to the flocs. The flocs are held together by extracellular polymeric substances produced by many micro-organisms such as *Zoogloea ramigera*. The waste materials are then digested and the sludge is separated from the water by sedimentation. Floc stability is determined by the ionic strength of the medium and can be explained by the Derjaugin, Landau, Verwey and Overbeek theory (Zita & Hermansson, 1994). When the ionic strength increases above 0.1, floc stability decreases and flocs become detached (Zita & Hermansson, 1994). This theory has been demonstrated with *Achromobacter* sp. and *Pseudomonas* sp., where reversible adsorption was shown when cells were displaced by gentle rinsing or fluid shear (Marshall *et al.*, 1971). In severe cases, increases in the ionic strength lead to heavy loss of micro-organisms (deflocculation) into the treated effluent. This in turn causes the release of toxic xenobiotic and nitrogenous compounds into the water supply.

Deflocculation or 'wash-out' can also be caused by the presence of low levels of dissolved oxygen, shock toxic loads, low pH, floc age and turbulence in waste water reactors. Many laboratory studies support the role of abiotic factors in determining the extent of biofilm detachment. For example, when an *A. hydrophila* biofilm was exposed to nutrient-limiting conditions there was an increase in detachment rate (Sawyer & Hermanowicz, 1998). In addition, Melo & Vieira (1999) demonstrated that high liquid velocity and turbulent flow in waste water reactors induced detachment due to the hydrodynamic forces.

The fluidized-bed system (Fig. 6) comprises a combination of both fixed and suspended bacterial cells where, through a constantly formed layer of extracellular polymer

Fig. 6. S

substan
advant
and, di
associa
such as
prevent
1980). I
the rate
detachn
consec

Biofil
the cor

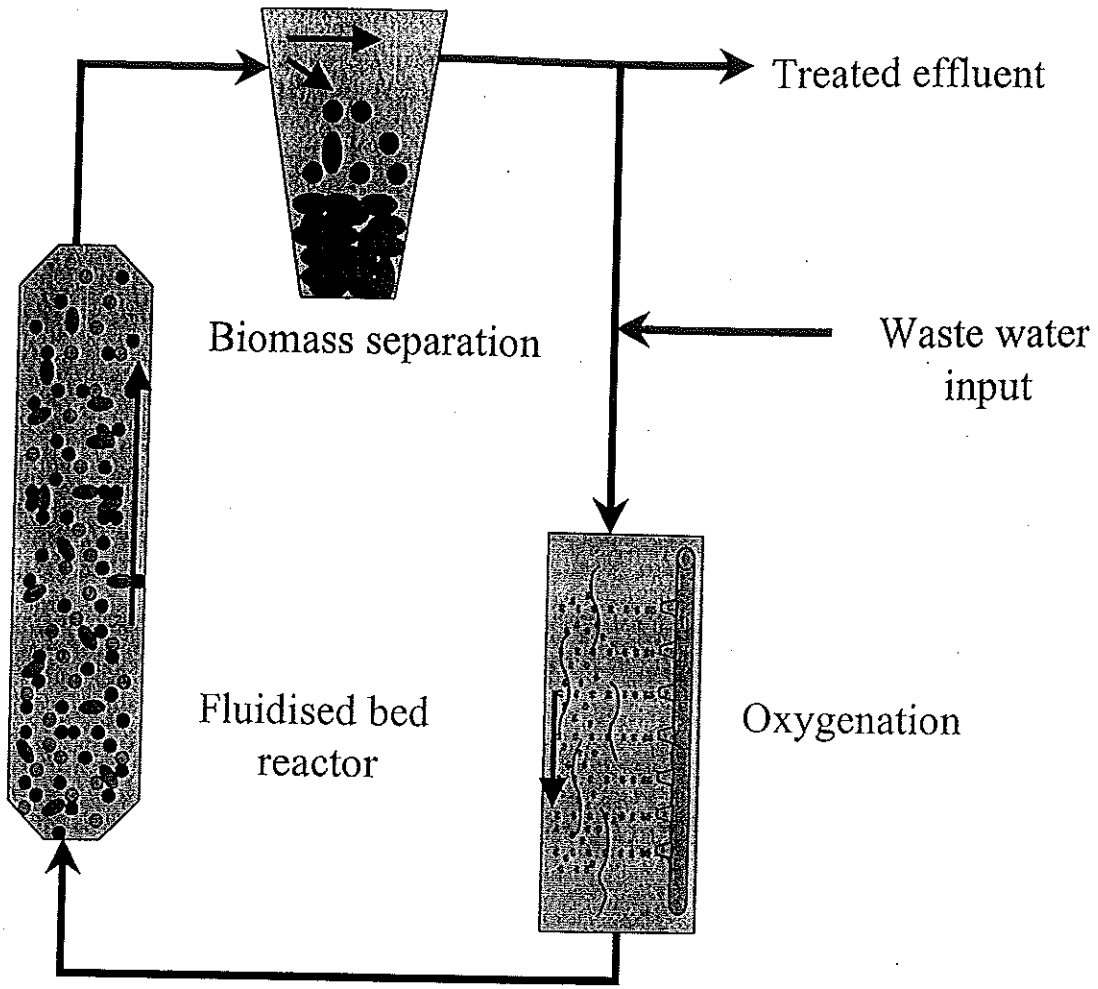

Fig. 6. Schematic representation of a fluidized-bed reactor.

substances, an upward flow of waste water can be effectively treated. This system has advantages over the floc growth system as a very high density of biomass can be formed and, due to the free movement of particles, the system does not have the blockages associated with the fixed-bed process. In addition, the use of solid support materials such as sand, carbon, glass or anthracite particles, ranging in size from 0.2 to 3 mm, prevents 'wash-out' as encountered in the floc growth system (Cooper & Wheeldon, 1980). Finally, the rate of biomass removal through the effluent stream never exceeds the rate at which biomass accumulates, thus the flow rate is low, leading to minimal detachment through erosion. However, there is a small degree of biofilm detachment as a consequence of abrasive forces.

Biofilm control in water systems

The control of biofilms in industrial water distribution systems is very challenging (Holt, 1995) as it is essential to optimize chemical or physical treatments to gain

complete depletion of the biofilm, without compromising water quality. There are many strategies employed in treating contamination by biofilms (biofouling) in industrial water processes; most of these involve the detachment of the biofilm from its surface. Biocide regimes using chlorine dioxide and ultraviolet irradiation are two such methods that have proved successful in biofilm detachment and control in water systems (Walker *et al.*, 1995). Shear force is another technique and involves the physical removal of the biofilm from its surface under many conditions, including alterations in fluid flow and changes in hydrodynamic conditions (Stoodley *et al.*, 1999b). Such erosive methods to combat and thus deform biofilms can also be achieved by many other processes, including flushing, backwashing, sand scouring and the use of non-abrasive or abrasive sponge balls (Rittmann, 1989).

In summary, it is evident that there are many modes of biofilm detachment in the water industry. The influence of factors such as the system used, nutrient limitations and physical stresses all have a role in influencing detachment rates. This is of significance in terms of both biofilm control and the potential hazardous implications that may arise. Of the limited research conducted on biofilm detachment, most studies focus on laboratory observations but there are a few studies of the natural detachment processes in water systems.

CONCLUSION

This chapter has highlighted the importance of understanding the detachment process in biofilms and indicated that this is, as yet, a relatively new but crucial area of research. However, through new experimental designs and methodology, progress is being made with establishing the mechanisms of biofilm detachment. Understanding detachment mechanisms is crucial for controlling biofilms. In the water industry, this knowledge is fundamental for the optimum performance of biofilm reactors to stabilize beneficial biofilms. In clinical situations, it is imperative to minimize biofilm formation and understand factors influencing the detachment of detrimental biofilms. Studies have shown that regulatory systems such as quorum sensing are involved in detachment and further research in this area could lead to the production of novel agents for biofilm control.

REFERENCES

Adams, J. L. & McLean, R. J. C. (1999). Impact of *rpoS* deletion on *Escherichia coli* biofilms. *Appl Environ Microbiol* 65, 4285–4287.

Allison, D. G., Evans, D. G., Brown, M. R. W. & Gilbert, P. (1990). Possible involvement of the division cycle in dispersal of *Escherichia coli* from biofilms. *J Bacteriol* 172, 1667–1669.

Allison, D. G., Ruiz, B., Sanjose, C., Jaspe, A. & Gilbert, P. (1998). Extracellular products as

mediators of the formation and detachment of *Pseudomonas fluorescens* biofilms. *FEMS Microbiol Lett* **167**, 179–184.

Allison, D. G., Heys, S. J. D., Willcock, L., Holah, J. & Gilbert, P. (1999). Cellular detachment and dispersal from bacterial biofilms: a role for quorum sensing? In *Biofilms – The Good, the Bad and the Ugly*, pp. 279–286. Edited by J. Wimpenny, P. Gilbert, J. Walker, M. Brading & R. Bayston. Cardiff: BioLine.

Altman, M., Semiat, R. & Hasson, D. (1999). Removal of organic foulants from feed waters by dynamic membranes. *Desalination* **125**, 65–75.

Applegate, D. H. & Bryers, J. D. (1991). Effects of carbon and oxygen limitations and calcium concentrations on biofilm removal processes. *Biotechnol Bioeng* **37**, 17–25.

Austin, J. W., Sanders, G., Kay, W. W. & Collinson, S. K. (1998). Thin aggregative fimbriae enhance *Salmonella enteritidis* biofilm formation. *FEMS Microbiol Lett* **162**, 295–301.

Backo, M., Gaenger, E., Burkart, A., Chai, Y. L. & Bayer, A. S. (1999). Treatment of experimental staphylococcal endocarditis due to a strain with reduced susceptibility in vitro to vancomycin: efficacy of ampicillin-sulbactam. *Antimicrob Agents Chemother* **43**, 2565–2568.

Bos, R., van der Mei, H. C. & Busscher, H. J. (1999). Physico-chemistry of initial microbial adhesive interactions – its mechanisms and methods for study. *FEMS Microbiol Rev* **23**, 179–230.

Boyd, A. & Chakrabarty, A. M. (1994). Role of alginate lyase in cell detachment of *Pseudomonas aeruginosa*. *Appl Environ Microbiol* **60**, 2355–2359.

Brading, M. G., Boyle, J. D. & Lappin-Scott, H. M. (1995). Biofilm formation in laminar flow using *Pseudomonas fluorescens* EX101. *J Ind Microbiol* **15**, 297–304.

Bremere, I., Kennedy, M., Michel, P., vanEmmerik, R., Witkamp, G. J. & Schippers, J. (1999). Controlling scaling in membrane filtration systems using a desupersaturation unit. *Desalination* **124**, 51–62.

Bryers, J. D. (1988). Modeling biofilm accumulation. In *Physiology Models in Microbiology*, vol. 2, pp. 109–144. Edited by M. J. Bazin & J. I. Prosser. Boca Raton, FL: CRC.

Buhrmann, F., VanderWaldt, M., Hanekom, D. & Finlayson, F. (1999). Treatment of industrial wastewater for reuse. *Desalination* **124**, 263–269.

Callow, M. E., Heath, C. R., Hartley Barry, A., Leadbeater, S. C. & House, W. A. (1995). Calcification of algal biofilms in hard waters. In *The Life and Death of Biofilm*, pp. 113–117. Edited by J. Wimpenny, P. Handley, P. Gilbert & H. Lappin-Scott. Cardiff: BioLine.

Chang, H. T., Rittman, B. E., Amar, D., Heim, R., Ehlinger, O. & Lesty, Y. (1991). Biofilm detachment mechanisms in a liquid-fluidized bed. *Biotechnol Bioeng* **38**, 499–506.

Characklis, W. G. (1990). Biofilm process. In *Biofilms*, pp. 195–231. Edited by W. G. Characklis & K. C. Marshall. New York: Wiley.

Characklis, W. G., McFeters, G. S. & Marshall, K. C. (1990). Physiological ecology in biofilm systems. In *Biofilms*, pp. 341–394. Edited by W. G. Characklis & K. C. Marshall. New York: Wiley.

Christensen, B. E. & Characklis, W. G. (1990). Physical and chemical properties of biofilms. In *Biofilms*, pp. 93–130. Edited by W. G. Characklis & K. C. Marshall. New York: Wiley.

Cooper, P. F. & Wheeldon, D. H. V. (1980). Fluidised and expanded bed reactors for wastewater treatment. *Water Pollut Control* **79**, 286–306.

Costa, G. M., Pizzi, C., Leone, C., Borghi, A., Cordioli, E. & Bugiardini, R. (1999). Thrombosis of a mitral valve prosthesis resulting from *Staphylococcus epidermidis* endocarditis. *Cardiologia* **44**, 675–678.

Costerton, J. W. (1999). The role of bacterial exopolysaccharides in nature and disease. *J Ind Microbiol Biotechnol* 22, 551–563.

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

Daly, B., Betts, W. B., Brown, A. P. & O'Neil, J. F. (1998). Bacterial loss from biofilms exposed to free chlorine. *Microbios* 96, 7–21.

Davies, D. G., Parsek, M. R., Pearson, J. P., Iglewski, B. H., Costerton, J. W. & Greenberg, E. P. (1998). The involvement of cell-to-cell signals in the development of a bacterial biofilm. *Science* 280, 295–298.

Douglas, C. W. I., Heath, J., Hampton, K. K. & Preston, F. E. (1993). Identity of viridans streptococci isolated from cases of infective endocarditis. *J Med Microbiol* 39, 179–182.

Eginton, P. J., Gibson, H., Holah, J., Handley, P. S. & Gilbert, P. (1995). Quantification of the ease of removal of bacteria from surfaces. *J Ind Microbiol* 15, 305–310.

Elvers, K. T., Leeming, K., Moore, C. P. & Lappin-Scott, H. M. (1998). Bacterial-fungal biofilms in flowing water photo-processing tanks. *J Appl Microbiol* 84, 607–618.

Entenza, J. M., Vouillamoz, J., Glauser, M. P. & Moreillon, P. (1999). Efficacy of trovafloxacin in treatment of experimental staphylococcal or streptococcal endocarditis. *Antimicrob Agents Chemother* 43, 77–84.

Gabriel, M. M., Schultz, C. L., Wilson, L. A. & Ahearn, D. G. (1996). Effect of *Staphylococcus epidermidis* on hydrogel contact lens retention on the rabbit eye. *Curr Microbiol* 32, 176–178.

Gavin, J., Button, N. F., Watson-Craik, I. A. & Logan, N. A. (2000). Observation of soft contact lens disinfection with fluorescent metabolic stains. *Appl Environ Microbiol* 66, 874–875.

Gilbert, P., Evans, D. J., Evans, E., Duguid, I. G. & Brown, M. R. W. (1991). Surface characteristics and adhesion of *Escherichia coli* and *Staphylococcus epidermidis*. *J Appl Bacteriol* 71, 71–77.

Gopinathan, U., Stapleton, F., Sharma, S., Willcox, M. D., Sweeney, D. F., Rao, G. N. & Holden, B. A. (1997). Microbial contamination of hydrogel contact lenses. *J Appl Microbiol* 82, 653–658.

Gorlin, A. I., Gabriel, M. M., Wilson, L. A. & Ahearn, D. G. (1996). Binding of Acanthamoeba to hydrogel contact lenses. *Curr Eye Res* 15, 151–155.

Governal, R. A. & Gerba, C. P. (1999). Removal of MS-2 and PRD-1 bacteriophages from an ultrapure water system. *J Ind Microbiol Biotechnol* 23, 166–172.

Hammer, M., Arnqvist, A., Bian, Z., Olsen, A. & Normark, S. (1995). Expression of two *csg* operons is required for production of fibronectin- and congo red-binding curli polymers in *Escherichia coli*. *Mol Microbiol* 18, 661–670.

Hawthorn, L. E. & Reid, G. (1990). Exclusion of uropathogen adhesion to polymer surfaces by *Lactobacillus acidophilus*. *J Biomed Mater Res* 24, 39–46.

Holt, D. (1995). Challenge of controlling biofilms in water distribution systems. In *The Life and Death of Biofilm*, pp. 161–166. Edited by J. Wimpenny, P. Handley, P. Gilbert & H. Lappin-Scott. Cardiff: BioLine.

Houry, D. & Crisman, T. (1999). Bivalve polymicrobial infective endocarditis. *South Med J* 92, 1098–1099.

Jaouen, P., Lepine, B., Rossignol, N., Royer, R. & Quemeneur, F. (1999). Clarification and concentration with membrane technology of a phycocyanin solution extracted from *Spirulina platensis*. *Biotechnol Tech* 13, 877–881.

Kelly, J. & Barnass, S. (1999). *Staphylococcus aureus* endocarditis presenting as meningitis and mimicking meningococcal sepsis. *Int J Clin Pract* 53, 306–307.

Koyuncu, I., Turan, M., Topacik, D. & Ates, A. (2000). Application of low pressure nanofiltration membranes for the recovery and reuse of dairy industry effluents. *Water Sci Technol* 41, 213–221.

Kwok, W. K., Picioreanu, C., Ong, S. L., Van Loosdrecht, M. C. M., Ng, W. J. & Heijnen, J. J. (1998). Influence of biomass production and detachment forces on biofilm structures in a biofilm airlift suspension reactor. *Biotechnol Bioeng* 58, 400–407.

Ladd, T. I. & Costerton, J. W. (1990). Methods for studying biofilm bacteria. *Methods Microbiol* 22, 285–310.

Lamed, R. & Bayer, E. A. (1986). Contact and cellulolysis in *Clostridium thermocellum* via extensile surface organelles. *Experientia* 42, 72–73.

Landa, A. S., van der Mei, H. C., van Rij, G. & Busscher, H. J. (1998). Efficacy of ophthalmic solutions to detach adhering *Pseudomonas aeruginosa* from contact lenses. *Cornea* 17, 293–300.

Lappin-Scott, H. M. & Costerton, J. W. (1989). Bacterial biofilms and surface fouling. *Biofouling* 1, 323–342.

Lappin-Scott, H. M., Brading, M. G. & Jass, J. (1994). How do bacteria reach surfaces? In *Bacterial Biofilms*, pp. 19–24. Edited by J. Wimpenny, W. Nichols, D. Stickler & H. M. Lappin-Scott. Cardiff: BioLine.

Lee, S. F., Li, Y. H. & Bowden, G. H. (1996). Detachment of *Streptococcus mutans* biofilm cells by an endogenous enzymatic activity. *Infect Immun* 64, 1035–1038.

Loewen, P. C., Hu, B., Strutinsky, J. & Sparling, R. (1998). Regulation in the *rpoS* regulon of *Escherichia coli*. *Can J Microbiol* 44, 707–717.

Lynch, M. J., Swift, S., Kirke, D., Dodd, C. E. R., Keevil, C. W., Stewart, G. S. A. B. & Williams, P. (1999). Investigation of quorum sensing in *Aeromonas hydrophila* biofilms formed on stainless steel. In *Biofilms – The Good, the Bad and the Ugly*, pp. 209–223. Edited by J. Wimpenny, P. Gilbert, J. Walker, M. Brading & R. Bayston. Cardiff: BioLine.

Mackerness, C. W., Colbourne, J. C., Dennis, P. J., Rachwal, A. J. & Keevil, C. W. (1993). Formation and control of coliform biofilms in drinking water distribution systems. *Society for Applied Bacteriology Technical Series: Microbial Biofilms*. pp. 217–226.

Mai, G. T., McCormack, J. G., Seow, W. K., Pier, G. B., Jackson, L. A. & Thong, Y. H. (1993). Inhibition of adherence of mucoid *Pseudomonas aeruginosa* by alginase specific monoclonal antibodies and antibiotics. *Infect Immun* 61, 4338–4343.

Marciano-Cabral, F., Puffenbarger, R. & Cabral, G. A. (2000). The increasing importance of Acanthamoeba infections. *J Eukaryot Microbiol* 47, 29–36.

Marshall, K. C., Stout, R. & Mitchell, R. (1971). Mechanism of the initial events in the sorption of marine bacteria to surfaces. *J Gen Microbiol* 68, 337–348.

Melo, L. & Vieira, M. J. (1999). Physical stability and biological activity of biofilms under turbulent flow and low substrate concentration. *Bioprocess Eng* 20, 363–368.

Moore, G. F., Humphrey, T. J., Holah, J., Woodward, M. J. & Lappin-Scott, H. M. (1999). The effect of adverse conditions on the biofilm formation of *Salmonella enteritidis*. In *Biofilms – The Good, the Bad and the Ugly*, pp. 15–22. Edited by J. Wimpenny, P. Gilbert, J. Walker, M. Brading & R. Bayston. Cardiff: BioLine.

Nicolella, C., DiFelice, R. & Rovatti, M. (1996). An experimental model of biofilm detachment in liquid fluidized bed biological reactors. *Biotechnol Bioeng* 51, 713–719.

Nicolella, C., Chiarle, S., DiFelice, R. & Rovatti, M. (1997). Mechanisms of biofilm detachment in fluidized bed reactors. *Water Sci Technol* 36, 229–235.

Ohashi, A. & Harada, H. (1994). Characterization of detachment mode of biofilm developed in an attached-growth reactor. *Water Sci Technol* **30**, 35–45.

Padilla, A. P. & Tavani, E. L. (1999). Treatment of an industrial effluent by reverse osmosis. *Desalination* **126**, 219–226.

Patron, R. L., Climo, M. W., Goldstein, B. P. & Archer, G. L. (1999). Lyostaphin treatment of experimental aortic valve endocarditis caused by a *Staphylococcus aureus* isolate with reduced susceptibility to vancomycin. *Antimicrob Agents Chemother* **43**, 1754–1755.

Peyton, B. M. & Characklis, W. G. (1993). A statistical analysis of the effect of substrate utilization and shear-stress on the kinetics of biofilm detachment. *Biotechnol Bioeng* **41**, 728–735.

Puckas, M. R., Greenberg, E. P., Kaplan, S. & Schaefer, A. L. (1997). A quorum sensing system in the free-living photosynthetic bacterium *Rhodobacter sphaeroides*. *J Bacteriol* **179**, 7530–7537.

Reid, G., McGroarty, J. A., Domingue, P. A. G., Chow, A. W., Bruce, A. W., Eisen, A. & Costerton, J. W. (1990). Coaggregation of urogenital bacteria *in vitro* and *in vivo*. *Curr Microbiol* **20**, 47–52.

Rioufol, C., Devys, C., Meunier, G., Perraud, M. & Goullet, D. (1999). Quantitative determination of endotoxins released by bacterial biofilms. *J Hosp Infect* **43**, 203–209.

Rittmann, B. E. (1989). Detachment from biofilms. In *Structure and Function of Biofilms*, pp. 49–58. Edited by W. G. Characklis & P. A. Wilderer. New York: Wiley.

Roberts, S. K., Lappin-Scott, H. M. & Leeming, K. (1999a). The control of bacterial-fungal biofilms. In *Biofilms – The Good, the Bad and the Ugly*, pp. 93–104. Edited by J. Wimpenny, P. Gilbert, J. Walker, M. Brading & R. Bayston. Cardiff: BioLine.

Roberts, S. K., Bass, C., Brading, M., Lappin-Scott, H. M. & Stoodley, P. (1999b). Biofilm formation and structure: what's new? In *Dental Plaque Revisited – Oral Biofilms in Health and Disease*, pp. 15–35. Edited by H. N. Newman & M. Wilson. Cardiff: BioLine.

Sawyer, L. K. & Hermanowicz, S. W. (1998). Detachment of biofilm bacteria due to variations in nutrient supply. *Water Sci Technol* **37**, 211–214.

Sperandio, V., Mellies, J. L., Nguyen, W., Shin, S. & Kaper, J. B. (1999). Quorum sensing controls expression of type III secretion gene transcription and protein secretion in enterohemorrhagic and enteropathogenic *Escherichia coli*. *Proc Natl Acad Sci USA* **96**, 15196–15201.

Stamler, J. F. (1998). The complications of contact lens wear. *Curr Opin Ophthalmol* **9**, 66–71.

Stapleton, F. & Dart, J. (1995). Pseudomonas keratitis associated with biofilm formation on a disposable soft contact lens. *Br J Ophthalmol* **79**, 864–865.

Steinberg, J. P., Clark, C. C. & Hackman, B. O. (1996). Nosocomial and community-acquired *Staphylococcus aureus* bacteremias from 1980 to 1993: impact of intravascular devices and methicillin resistance. *Clin Infect Dis* **23**, 255–259.

Stewart, P. S. (1993). A model of biofilm detachment. *Biotechnol Bioeng* **41**, 111–117.

Stoodley, P., Lewandowski, Z., Boyle, J. D. & Lappin-Scott, H. M. (1999a). Structural deformation of bacterial biofilms caused by short-term fluctuations in fluid shear: an *in situ* investigation of biofilm rheology. *Biotechnol Bioeng* **65**, 83–92.

Stoodley, P., Dodds, I., Boyle, J. D. & Lappin-Scott, H. M. (1999b). Influence of hydrodynamics and nutrients on biofilm structure. *J Appl Microbiol* **85**, 19S–28S.

Stoodley, P., Jørgensen, F., Williams, P. & Lappin-Scott, H. M. (1999c). The role of

hydrodynamics and AHL signalling molecules as determinants of the structure of *Pseudomonas aeruginosa* biofilms. In *Biofilms – The Good, the Bad and the Ugly*, pp. 223–230. Edited by J. Wimpenny, P. Gilbert, J. Walker, M. Brading & R. Bayston. Cardiff: BioLine.

Stoodley, P., Boyle, J. D., DeBeer, D. & Lappin-Scott, H. M. (1999d). Evolving perspectives in biofilm structure. *Biofouling* **14**, 75–90.

Stoodley, P., Lewandowski, Z., Boyle, J. D. & Lappin-Scott, H. M. (1999e). The formation of migratory ripples in a mixed species bacterial biofilm growing in turbulent flow. *Environ Microbiol* 447–455.

Tijhuis, L., Hijman, B., van Loosdrecht, M. C. M. & Heijnen, J. J. (1996). Influence of detachment, substrate loading and reactor scale on the formation of biofilms in airlift reactors. *Appl Environ Microbiol* **45**, 7–17.

Walker, J. T., Mackerness, C. W., Mallon, D., Makin, T., Williets, T. & Keevil, C. W. (1995). Physico-chemical eradication in the field. In *The Life and Death of Biofilm*, pp. 167–171. Edited by J. Wimpenny, P. Handley, P. Gilbert & H. Lappin-Scott. Cardiff: BioLine.

Zita, A. & Hermansson, M. (1994). Effects of ionic-strength on bacterial adhesion and stability of flocs in a waste-water activated-sludge system. *Appl Environ Microbiol* **60**, 3041–3048.