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Biofilms and the technological implications of microbial cell adhesion

James D. Bryers

Center for Biofilm Engineering, National Science Foundation Engineering Research Center, Montana State University, Bozeman, MT 59717-0398, USA

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Abstract

Biofilms are a collection of cells entrapped within a gelatinous matrix comprising mostly insoluble extracellular polymers that the cells secrete. Although the term is applied mostly to bacterial cells and their secreted insoluble exopolymers, any biologically active layer of cells (microbial, plant, or mammalian cells) can be considered a biofilm. Any surface in contact with a biological fluid is a potential target surface for microbial cell adhesion. Once cells are attached, subsequent growth and replication of surface-attached cells, cell exopolymer production, further recruitment of planktonic cells from the fluid phase, and various biofilm detachment processes constitute what is collectively known as biofilm formation and persistence.

Biofilms can play both beneficial or detrimental roles depending on whether their formation within a specific system is intentional or inadvertent. This article will review both the current and emerging technological implications of bacterial cell adhesion and biofilm formation including biomaterials preparation to prevent bacterial infections of medical implants; development of novel antibiotic therapies to control biofilm-bound bacteria; designer nanocrystalline filaments called "bionites", fabricated from strands of bacteria, that possess unusual magnetic, optical and biocatalytic properties; specific hazardous waste detoxification by immobilized recombinant bacteria; improved recombinant plasmid retention within biofilm populations, and stable biosensors.

Key words Biofilm applications, Biofilm engineering, Biofilm technologies

Introduction

Biotechnology is the manipulation of cellular fragments (e.g. enzymes, organelles) or entire cells to mediate desired biological conversions and the subsequent development of those reactions from laboratory scale to commercial reality. Man has exploited biological processes for centuries in such activities as brewing, wine making, bread making, food preservation, and waste treatment. However, until the 1970s, the performance, reactivity, and product specificity of a biological process was inherent to the specific biocatalyst (enzyme or whole cell) used. The emergence of genetic manipulation, recombinant DNA technology, cell fusion, and advances in immunology now permit the orchestration of desired metabolism, production of exotic molecules, and the improvement of process selectivity.

Biofilms or immobilized cell systems are pertinent within biotechnology because of (1) reactor performance advantages that immobilized cells provide over freely suspended cultures, (2) specific metabolic improvements or products created upon immobilization, (3) their ability to localize a specific biological response which can be exploited in biosensor design, and (4) critical physiological requirements of certain cell lines (plant and animal cells) for adhesion and anchorage.

This article will review both the current and emerging technological implications of bacterial cell adhesion and biofilm formation including bio-

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materials preparation to prevent bacterial infections of medical implants; development of novel antibiotic therapies to control biofilm-bound bacteria; designer nanocrystalline filaments called 'bionites', fabricated from strands of bacteria, that possess unusual magnetic, optical and biocatalytic properties; specific hazardous waste detoxification by immobilized recombinant bacteria; improved recombinant plasmid retention within biofilm populations; and stable biosensors.

Biofilm formation

Microbial biofilms, especially bacterial, accumulate as a consequence of the microbes' ability to adsorb to a surface (termed a substratum), replicate, produce extracellular polymers, and metabolize nutrients and substrates dissolved in the surrounding fluid phase. As illustrated in Fig. 1, the initial colonization of a surface — whether it be a rock in an alpine stream, a plastic surface in a wastewater treatment system, or an endoprosthetic medical implant — and the subsequent biofilm formation comprise a complex series of chemical, physical, and biological processes. For details regarding the system parameters governing these individual processes, the reader should consult Bryers and Characklis [1], Bryers [2], Characklis and Marshall [3], Costerton et al. [4].

Naturally formed bacterial biofilms can serve

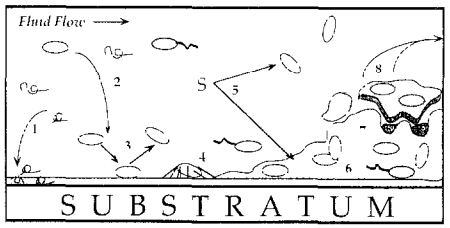

Fig 1. Processes contributing to bacterial biofilm formation.

both beneficial and detrimental roles depending upon whether their development is controlled or unintentional. The technological ramifications of both the benefits and detriments of cell adhesion and biofilm formation are discussed in this paper.

Beneficial uses of microbial biofilms and captured cell systems

Natural biofilms

Examples of microbial processes that have traditionally derived economic or process advantage by being carried out in a natural biofilm mode are detailed in this section.

Acetic acid production

"Vin aigre" in French is sour wine and, by law, the acetic acid solution must be produced by the biological oxidation of ethanol. In the current "quick vinegar" process, alcoholic solutions are trickled over *Acetobacter spp.* biofilms formed on large columns (55 m^3) of packed beechwood chips (50 mm diameter). Similar to a cooling tower, this reactor configuration is necessary to provide the substantial amounts of oxygen required and to dissipate the heat generated in the highly exothermic ethanol oxidation.

Microbial leaching

Today, approximately, 10–20% of the copper mined in the US is extracted by microbial-assisted processing of low-grade ores or tailings [5,6]. Research has also extended microbial leaching to the recovery of other metals such as uranium, silver, cobalt, molybdenum, nickel, and gold [7] as well as the desulfurization of coal [8,9]. Most microbial leaching depends on microbial oxidation of the metal sulfides. Aqueous environments in association with spent mineral ores produce very harsh conditions of low pH, high metal concentrations, and high temperatures which become enriched in microbes of very discriminating nutritional requirements (e.g. *Thiobacillus ferrooxidans, Leptospirillum ferrooxidans, Acidophilium cryptum,*

and *Sulfolobus spp.*). To carry out their oxidative liberation of bound metals, these bacteria must first form biofilms on the metal ore particles. The most common operation is similar to the vinegar reactor above in that ore is piled to form a packed bed over which acidified water (pH, 1.5–3.0) is sprayed. For example, the acidophilic bacterium, *Thiobacillus ferrooxidans*, actively oxidizes the soluble ferrous iron and attacks the sulfide minerals, releasing the soluble cupric ion. Elemental sulfur would accumulate on the ore, limiting the extent of leaching, if not for *Thiobacillus thiooxidans* which oxidizes the reduced sulfur, maintaining the acidic environment. Similar processes have been reported for the extraction of uranium ores.

Process engineering of microbial leaching is sorely lacking. Biological reaction(s) and mass transfer rates in current systems most likely limit the efficiency of the overall process, although recent work in the fluidized bed process looks promising [10].

Wastewater treatment systems

Microorganisms grow at a rate dictated by their physiological status and prevailing growth conditions. If the microbes are growing in suspension in a continuously fed reactor (i.e. a chemostat), the growth rate is dictated by the reactor residence time. Cell concentration at a fixed residence time is a function of the inlet concentration of the limiting substrate(s) and the cell's efficiency to convert that substrate to biomass. Should the reactor residence time decrease below the maximum generation time of the culture, cells will be washed out of the reactor. Washout thus limits the maximum substrate removal rate attainable in a chemostat. The objective of a wastewater treatment reactor is to remove the maximum amount of a contaminant (the substrate) within the minimum residence time. Consequently, wastewater treatment using suspended cultures is seldom carried out in chemostats but rather in activated sludge systems where biomass in the effluent is separated, concentrated and recycled. This cell recycle serves to maintain elevated levels of the culture so that substrate loading rates can be increased.

Biofilm or fixed film reactors are another means of retaining biomass within a flow-through reactor independently of the liquid phase residence time. Reactor geometries employed in biofilm treatment systems include:

(1) completely mixed reactors, such as rotating biological contactors (RBCs);

(2) fixed- or packed-bed reactors; conventionally known as trickling filters, these systems can either employ rocks or, more recently, reticulated or corrugated plastic packing as biofilm support material;

(3) fluidized beds of either non-porous sand or plastic (0.2–2 mm diameter) or porous biomass support particles (e.g. activated carbon, ceramics, three-dimensional stainless-steel wire "webs", polyester sponge cubes).

Wastewater treatment is probably the largest single application of natural biofilm reactors. Such biofilm wastewater treatment systems have been applied to carbon oxidation, nitrification, denitrification, anaerobic methane generation, hazardous or xenobiotic detoxification, and heavy metals removal. The reader is directed to several excellent reviews on the design and operation of biofilm wastewater treatment systems [11–13]. Recent examples of biofilm reactors employed for wastewater treatment reported in the literature include: polychlorinated hydrocarbon degradation [14], toluene degradation [15], denitrification [16,17], cadmium removal [18], anaerobic butyrate degradation [19], nitrification [20–22], glyphosphate degradation [23], anaerobic propionate degradation [24], phenolic wastewaters [25], uranium removal [26], and anaerobic carbon removal [27,28].

Captured cell systems

Natural biofilms of any cell type suffer from a lack of control over the development of the biofilm. In microbial cell systems, excessive biofilm formation can lead to such problems as: (1) unstable

effluent biomass concentrations; (2) excessive mass transfer limitations; (3) hydraulic instabilities and excessive pressure drops in porous media systems; (4) uncontrolled biofilm particle buoyancy in fluidized-bed systems, (5) creation of spatially distinct microcolonies.

To alleviate these problems of uncontrolled biofilm formation, several alternative biomass supports or immobilization methods have emerged. Biomass support systems are classified as that group of porous inert support materials that provide extremely high internal surface areas for cell attachment and subsequent cell colonization, or complete biofilm formation. Cell immobilization methods comprise a series of different methods that either bind a cell chemically or physically to a substratum, capture the cell within a polymer gel or an inorganic hydroxide metal precipitate matrix, or surround a cell suspension within a semipermeable membrane. Biomass support and immobilized cell systems can be fabricated to allow reactor operation in either the chemostat or plug flow mode.

In biomass support systems, adhesion, cell growth, and biofilm formation are promoted. Biomass is contained within the confines of the support medium which dictates a known diffusion path and predictable particle reactivity. Any biomass that exceeds the boundaries of the support matrix is swept away by either prevailing shear stresses or particle–particle abrasion. Examples of biomass support systems include biomass capture particles [13] shown in Fig. 2, porous ceramic matrices, hollow fiber membranes (Fig. 3), and collagen sponge beads (Fig. 4). Selective inoculation of biomass supports can provide the ability to stage sequential biological conversions in a series of reactor units.

Artificially immobilized whole cell systems differ from natural biofilms or biomass support systems in at least two ways: (1) immobilization is an engineered process with cells captured within or bound to a support using any one of a variety of chemical or physical techniques; (2) growth and replication of the immobilized cells often is not

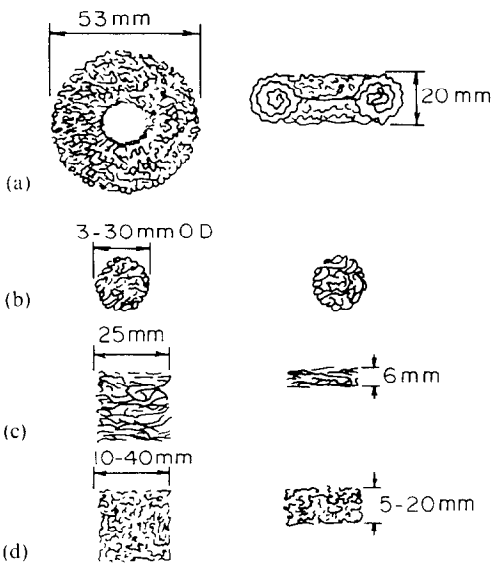

Fig 2 Example of biomass support particles. (a) plastic mesh toroids, (b) stainless steel wire mesh spheres; (c) polyethylene foam pads, (d) polyester foam sponge

necessary or desired. Figure 5 summarizes the various immobilization techniques available. For details regarding these immobilization procedures, the reader is directed elsewhere [29–31].

Specific biotechnological applications of biomass support and immobilized cell systems are illustrated in the remaining sections.

Ethanol production

Black et al. [32] reported using stainless steel wire "webs" and polyester foam sponges to immobilize the yeasts *Saccharomyces cerevesiae* and *S. uvarium* within fluidized particle reactors operated at dilution rates of 0.78 and 1.35 h^{-1}, respectively. Overall ethanol production rates were approximately 12 kg m^{-3} h^{-1} in both systems with the ethanol yield being, respectively for each species, 80% and 98% of that theoretically possible. Total biomass per support particle was 16 mg for the stainless steel webs and 30 mg for the polyester sponges.

Cellulase production

Webb et al. [33] report the continuous production of the enzyme cellulase by the filamentous

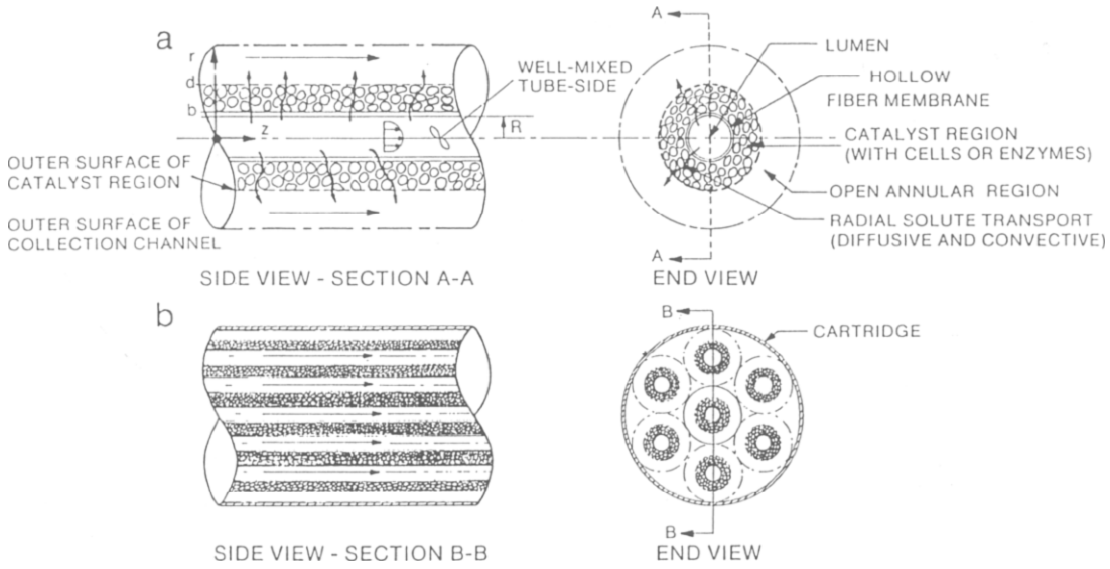

Fig. 3 Schematic of hollow fiber membrane bioreactor

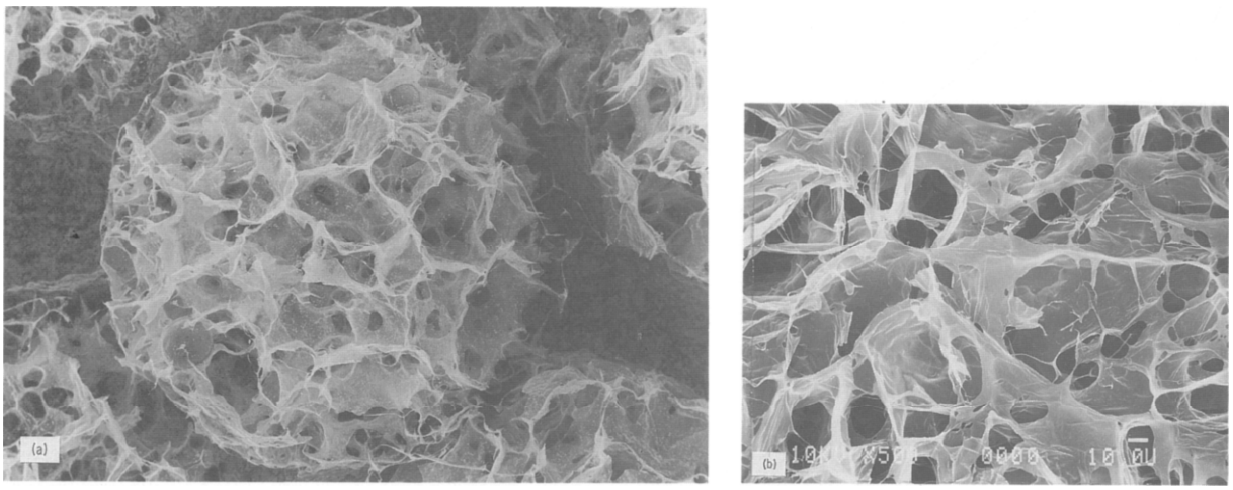

Fig. 4. Scanning electron microscopy photographs of a clean collagen porous sponge bead for mammalian cell cultivation: (a) low magnification, bead diameter 500 μm; (b) high magnification of bead internal structure.

fungus *Trichoderma viride* QM 9123 captured within stainless steel wire "webs" approximately 6 mm in overall diameter. The steel web beads were fluidized in a 10 l spouted bed reactor operated well past the washout dilution rate (0.012 h^{-1}) of the fungus. At an operating dilution rate of 0.18 h^{-1}, a maximum productivity of cellulase of 30 specific activity units (SPU) $l^{-1}\,h^{-1}$ was reported; specific productivity, 6.0 SPU $g^{-1}\,h^{-1}$ immobilized vs. 1.1 SPU g^{-1} in free suspension.

Recombinant gene expression

Inloes et al. [34] reports the growth of a recombinant *E. coli* C600 (pBR322), which had been cloned to overproduce β-lactamase, within a hollow fiber membrane reactor. Suspension culture perfor-

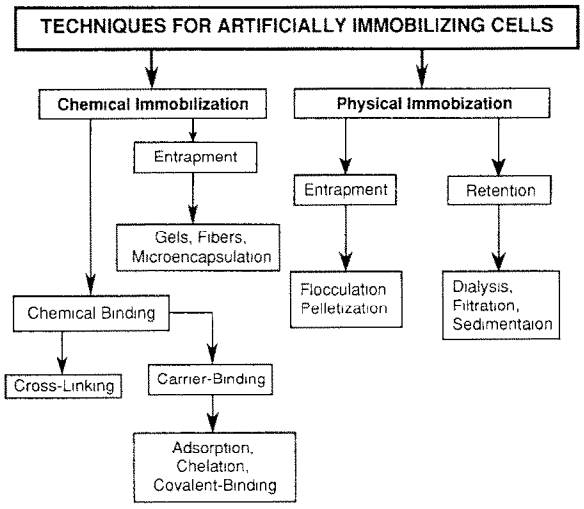

Fig. 5 Methods of artificial whole cell immobilization.

mance was 1×10^{-10} SPU per cell h^{-1} and 0.84 SPU ml^{-1} h^{-1} while for the hollow fiber immobilized system results were reported as 2×10^{-11} SPU per cell h^{-1} and 10.0 SPU ml^{-1} h^{-1}.

The effects of cell immobilization on plasmid stability has been investigated by de Taxis du Poët et al. [35]. Plasmid-bearing *E. coli* BZ18 (pTG201) were immobilized in κ-carrageenan beads that were subsequently fluidized in a chemostat operated at a volumetric residence time of 15 min. Immobilized reactors were operated without antibiotic selection pressure and performances compared to suspended cultures under similar conditions (dilution rate below washout). The plasmid encoded for expression of catechol 2,3-dioxygenase. Plasmid-bearing populations were selectively washed out of the chemostat cultures if operated without antibiotics. Immobilized cultures exhibited continuous expression of the enzyme over several hundred generation times and cells extracted from the beads exhibited increased copy numbers, without antibiotic selection pressure. Nasri et al. [36] extended this analysis to three genetically different *E. coli* hosts using the same plasmid and again reported that the fraction of plasmid-bearing cells immobilized was greater than in suspension. Sayadi et al. [37] employed an *E. coli* W3110 (pTG201) again immobilized within κ-carrageenan beads and fluidized within a chemostat at a dilution rate of 1.3 h^{-1}. They found that immobilization increased the stability of pTG201 even under glucose-, nitrogen-, or phosphate-limited growth conditions without the use of antibiotics.

Wastewater treatment

Nesaratnam and Ghobrial [38] compared the performance of a conventional activated sludge system to that of a system of fluidized polyester sponges, both containing a mixed culture of microbes, for the treatment of oil refinery petrochemical wastes. Both systems were operated at residence times of 12 and 7 h. Removal of carbon and nitrogen oxygen demand in the biomass support system was comparable to that of the suspended culture system. The process advantage of the polyester sponges is that the clarifier–sedimentation stage in the activated-sludge process is not necessary, thus reducing overall system volume.

Messing [39] captured methanogenic cultures within the porous structure of ceramic cartridges to convert sewage organics to methane. The cell support was Cordierite™ (Corning Glass Co., average pore diameter 3 μm, pore volume 4400 mm^3 g^{-1}, porosity 57%). COD reduction varied between 60 and 90%, with the resultant gas 90% CH_4 and 10% carbon dioxide.

Karube et al. [40] compared methane gas production from various wastewaters treated by methanogenic bacteria immobilized in three different gels; agar, polyacrylamide, and collagen membranes. Agar membranes exhibited the highest methane production activity (450 μmol CH_4 g^{-1} (dry weight) h^{-1}) of the three carrier membranes.

Nilsson et al. [41] employed cells of *Pseudomonas denitrificans* immobilized in an alginate gel to denitrify water. In the absence of an exogenous carbon source, the immobilized cells enzymatically converted nitrate to nitrogen gas. Cells retained 75% of their initial nitrate reducing capacity after 21 days of storage at 4 °C. A column packed with gel beads (2 mm diameter) reportedly

[41] produced 0.07 m^3 denitrified water kg per gel h^{-1} for 2 months from a high nitrate feed stream (22 g NO_3^-–N m^{-3}). Cell activity could be regenerated by periodically feeding an organic carbon nutrient solution to the column but eventually this resulted in sufficient cell growth to rupture the gel beads and contaminate the effluent.

Nakajima et al. [42] report recovering uranium from seawater and freshwater using both *Streptomyces uiridochromogenes* and *Chlorella regularis* within polyacrylamide gels. Adsorbed uranium was recovered by a caustic wash cycle.

Klein et al. [43] illustrated phenol degradation by *Candida tropicalis* immobilized in ionic polymer networks fabricated from styrene–maleic acid copolymer. A fixed bed reactor containing 140 g of beads (3 mm diameter) eliminated 99% of the influent phenol for approximately 20 days prior to losing capacity; activity could be regenerated periodically using a nutrient-rich medium.

Suzuki et al. [44] immobilized *Clostridium butyricum* cells within a polyacrylamide gel to produce hydrogen gas anaerobically from the degradation of an industrial alcoholic wastewater (BOD, 660 g m^{-3}). Hydrogen produced in the packed column passed to a liquid fuel cell generating a current of 8–9 mA for 20 days.

Recently, captured cell systems have been reportedly used in the production of acetic acid [45], L-lysine [46], gluconic acid [47], kojic acid [48,49], ethanol [50,51], and in the epoxidation of propene [52].

Biosensor applications

Cells artificially immobilized within gel membranes provide the ability to 'locate' a cellular or enzymatic reaction at a specific point, thus leading to the evolution of specific biosensor probes. A biosensor consists of a biologically sensitive material (cells, enzymes, antibodies, organelles) in direct contact with an appropriate transducing device that converts the biochemical reaction into an electric signal. The biological component is responsible for the 'recognition' of a specific component in the system in question which in turn triggers a biochemical reaction at the sensor surface. The types of transducers available and their application to biosensors are summarized below.

Karube and co-workers [53–57] and Blenkinsopp and Costerton [58] detail the construction of microbial biosensors for detecting the organic pollutant load in a receiving water, correlating biosensor output to sample BOD. Either yeast or bacteria were immobilized within acetylcellulose membranes then fixed to a conventional dissolved oxygen probe. Response of the probe was linear below a sample BOD concentration of 60 g m^{-3}. A similar design employing nitrifying autotrophic bacteria immobilized on an oxygen probe allows the detection of dissolved ammonia below 1.5 ppm [59].

Technological impacts of detrimental biofilms

Uncontrolled biofilm formation within natural, engineered, and biomedical systems can create numerous detriments as detailed in several recent definitive reviews [3,58,59]. Detriments arise by way of a biofilm's influence on the transport of mass, momentum, and energy. Detriments attributed directly to bacterial biofilms, documented in recent literature, include: (a) material deterioration and corrosion [60–68]; (b) increases in both frictional and heat transfer resistances [66,69]; (c) attachment to and infection of biomedical implant devices [70–77]; (d) operational problems in bioreactors that range from nuisance to catastrophe [58,59,78].

Control of detrimental biofilms

Control of biofilm formation can be either a positive or negative exercise depending on whether the biofilm is detrimental or beneficial to the operation of the system in question. Methods to control unwanted, detrimental biofilms attempt to either prevent bacterial adhesion, retard biofilm formation, or eradicate existing biofilm.

Recent articles investigating the prevention of cell adhesion to biomaterials have focused on

Table 1
Biosensor transducers and their applications

Transducer	Measurement mode	Application
Ion-selective	Potentiometric	Ions in biological media, enzyme sensors
Gas-sensing electrode	Potentiometric	Gases, enzymes, substrates
Field-effect transistors	Potentiometric	Ions, gases, substrates
Optoelectronic, fiber-optic, waveguide devices	Optical, evanescent wave distortion	pH, immunological compounds, substrates
Thermistors	Calorimetric	Enzyme sensors, whole-cell sensors for substrates, products, gases, pollutants, antibiotics
Enzyme electrodes	Amperometric	Enzyme substrates
Conductivity probes	Conductance	Enzyme substrates

modification of surface chemical properties of the substratum by: photochemical coupling of benzophenone derivatives of polyethylene glycol, polyacrylamide, and polyvinyl pyrrolidone [79]; passive adsorption of pluronic surfactants (copolymers of polyethylene oxide and polypropylene oxide) to polystyrene [80]; incorporation of polyethylene oxide into the upper layers of polyethylene terephthalate by solvent swelling [81]; and a series of neutral, anionic, and cationic surfactants adsorbed onto stainless steel or glass [82]. Rather than dissuade adhesion, one alternative control approach for biomedical materials is to retard surface microbial activity by incorporating within the substratum a slowly released antibiotic agent, such as gentamicin within polymethyl methacrylate [72,83], methyl and propylparaben (*p*-hydroxybenzoic acid esters) in polyurethane [84], cefazolin adsorbed onto intravascular catheters [85], silver-coated stent material [86] and antiseptic (chlorohexidine, mandelic acid, and mandelic and lactic acids combined) rinses of silicone surfaces [87,88].

Remedial approaches to eliminate or eradicate biofilms existing in engineered systems consist of either mechanical cleaning, materials or unit replacement, or chemical biocide challenges [89]. One novel alternative to chemical antagonism of a biofilm has been to eliminate a nutrient essential for microbial growth. Bakke et al. [90] chose to control microbial oil 'souring', mediated by sulfate reducing bacterial biofilms, by limiting the sulfate concentrations dissolved in the water used in flooding operations. In the petrochemical, refining, and the power industries, either oxidants (chlorine, ozone, bromides) or biocides are employed regularly to suppress biofouling of water cooled heat exchangers; biocides prove mostly ineffective in that they inactivate the bacterial cells but, unlike oxidants, are incapable of eliminating the source of the system inefficiencies, the biofilm matrix. Blenkinsopp et al. [91] report that, when applied within a low strength electrical field (≈ 12 V cm^{-1}) with a low current density (≈ 2.1 mA cm^{-2}), several industrial biocides exhibited enhanced killing action against *Pseudomonas aeruginosa* biofilms.

Elimination of bacterial infections of in-dwelling prostheses, orthopedic implants, or various tissue infections consists specifically of antibiotic challenges including gentamicin [92], rifampin alone and rifampin plus combinations of cefazolin and vancomycin [93], clindamycin and trospectomycin [94], and ciproflaxin [95] although biofilm-bound cells prove quite resilient to such chemical antagonism [96,97]. Regrettably, in studies on antibiotic challenges and the substratum chemistry modification, all too often experiments are carried out with very little attention paid to the mechanism controlling bacterial accumulation at the substratum. Fluid conditions are often quiescent, leaving the system susceptible to mass transfer limitations. Both fluid and liquid phase concentrations of system variables (cell concentration, nutrients, oxygen) are often monitored but once in an experiment, if at all. Care should be taken in any

extrapolation of 'adhesion kinetics' determined from such experiments to 'real world' conditions.

Technologies for biofilm detection and analysis

Historically, the study of biofilms has required the evolution of both (1) reactor systems in which to cultivate biofilms under controlled fluid phase and surface conditions and (2) the analytical methods to assess biofilm presence, reactivity, and resultant system response. Twenty years ago, design and operation of a biofilm reactor or the analysis of microbial activity in an alpine stream or heat exchanger were satisfied by measurements of attached biomass concentrations (in mass dry weight or total cell numbers) and indirect biofilm activity measurements (dissolved oxygen uptake rates, ATP, heterotrophic potentials). All such diagnoses were, and for the most part still are, dependent upon destructive sampling of the biofilm.

Biofilms can be pure or mixed strain, pure or mixed species, or mixed cell (bacteria, platelets, neutrophils, endothelial cells) systems. Recently, research has recognized the need to focus on the heterogeneous nature of biofilm ecology with regard to: (1) temporal changes and spatial distributions in cell populations; (2) local concentrations of electron donor, acceptor, and pH; (3) spatial differences in biofilm physical, chemical, viscoelastic, and mass transport properties. Fortunately, the advent of several molecular and cellular probes and diagnostic tools has had a tremendous impact on our ability to probe biofilm ecology in situ, non-invasively.

Huang et al. [98], Buret et al. [99], and Mittleman et al. [100] all describe the utility of a rectangular fluid flow cell in which to study bacterial adhesion and the early stages of biofilm formation under flow conditions. These flow cells provide for microscopic surveillance of the substratum, access for microelectrode probes, and removable portions of substratum for destructive sampling of the biofilm. Advantages of flow cells are that they mimic the fluid flow hydrodynamics inherent to many systems plagued by biofilm formation, provide controlled well-defined hydrodynamic conditions, and access for visual observation of biofilm formation. Disadvantages of such flow cell devices include limited sample surface area and, if operated on a once-through flow basis, the potential exists for gradients in biofilm amount and fluid phase concentrations to develop in the longitudinal direction. Flow cell reactors are not novel, having been reported on numerous occasions since the 1970s, and they are by no means the only reactor configuration suitable for biofilm studies [3]. What makes these articles distinctive is their emphasis on the need for bacterial adhesion studies to be carried out under defined fluid flow conditions at known substratum conditions rather than the arcane use of quiescent liquid at undefined conditions.

Kinniment and Wimpenny [101] describe a different study system in which biofilms are cultivated, under controlled fluid phase conditions, receiving a continuous supply of substrate, within recessed 'wells' in the bottom plate of a laboratory reactor. A wiper blade constantly stirs the fluid phase above the plate thus eliminating biofilm everywhere in the reactor except within the recesses. Removable test surface samples from the bottom of each recess allow periodic sampling of biofilm of known thickness. In this study, *Pseudomonas aeruginosa* biofilm samples were periodically removed, quick frozen, and thin sectioned parallel to the substratum. Thin sections of *Pseudomonas aeruginosa* biofilm were then analyzed for adenylate concentrations and adenylate energy charge as a function of biofilm depth.

Analysis of the heterogeneity of biofilms is concerned with spatial gradients of: (1) total cell concentrations; (2) individual species concentrations; (3) local concentrations of various dissolved nutrients, and selected ions. Kuhn et al. [102] used mithramycin to stain fluorescently total DNA of *E. coli* immobilized with Sr-alginate beads. Cell concentration profiles as a function of radial position in the bead were determined at different lifetimes of operation by microfluorimetry on thin sections of the beads. Stewart et al. [103] applied a pulse-chase of $^{35}SO_4^{2-}$ and subsequent liquid

emulsion autoradiography of thin sections of hollow fiber membranes containing immobilized *E. coli*. Sections illustrate viable cell concentrations as a function of radial position at different locations and under different operating conditions of the hollow fiber reactor.

Rogers and Keevil [104] report the destructive sampling of a multispecies biofilm, intentionally inoculated with *Legionella pneumophilia*, followed by both immunogold and fluorescein immunolabeling for this species. Episcopic differential interference contrast microscopy was employed to visualize simultaneously the total biofilm community and the labeled *Legionella* species. This technique was able to provide observations of variations in the biofilm community from one position to another (parallel to the substratum) but was unable to discern such variations with depth (perpendicular to the substratum).

Similarly, Rodriguez et al. [105] exploited the potential of a fluorescent redox probe, 5-cyano-2,3-ditolyl-tetrazolium chloride (CTC), for direct observation of actively respiring bacteria. Oxidized CTC is colorless but upon reduction by electron transport activity, the insoluble CTC-formazan fluoresces at 365 nm excitation. Respiring marine bacteria within thin (10–50 μm) biofilms attached to optically non-transparent polysulfone substrata were rapidly and easily enumerated. Respiring cell counts via CTC were also useful in directly assessing the efficacy of various biocidal control agents.

The advent of modern molecular biology has also provided useful diagnostic methods with which to analyze biofilm ecology. Amann et al. [106] characterize the population structure of sulfidogenic biofilms, established in anaerobic bioreactors, by selective polymerase chain reaction (PCR) amplification and fluorescent microscopy. 16S rRNA common to the sulfate reducing bacteria in the biofilm was selectively amplified by PCR and used to design both general and specific fluorescent hybridization probes. Biofilms (5–10 μm thick) on glass cover slips immersed within laboratory anaerobic biofilm reactors, were fixed in formaldehyde and dehydrated prior to hybridization with the RNA probes. Hybridized biofilm samples were then viewed by epifluorescent microscopy.

McCarter et al. [107] report a very elegant series of genetic techniques to investigate bacterial responses to various stimuli, one being the adhesion to a surface. Using a transposon (mini-Mu*lux*), the authors are able to incorporate recombinant reporter gene insertions adjacent to target gene promoter sites that then encode for light production as a function of the target gene expression. Light production is conveniently measured by exposure of X-ray or photographic film, visual examination, chemiluminescence or photometry. Such techniques also obviate the need to disrupt biofilms prior to quantification. McCarter et al. employed a *lux* reporter gene adjacent to the chromosomal gene encoding for swarmer cell differentiation that *Vibrio parahaemolyticus* experiences upon association with a solid surface. Resultant mutants thus emit light upon growth at a surface but not in suspension. Dagostino et al [108] also employed transposon mutagenesis to insert into an appropriate recipient bacterium, a marker gene that lacks its own promoter. The premise is that if a suitable target gene is 'on' at the surface, then expression of the marker will be observed only in the presence of the surface. Dagostino et al. employed *E. coli* C600 (pRK2013: mini-MuTetr *lacZ*) as donor and two marine bacteria as recipients. The authors were able to isolate successfully a transposon generated mutant in which the *lacZ* gene was not expressed in either liquid or agar but was when growth was associated on a polystyrene substratum.

Several citations exist that report the use of invasive but non-destructive analysis of solute concentration profiles, by microsensor chemical probes, as a function of spatial dimension within the developing biofilm. Microsensors (tip size $\leqslant$ 15 μm) exist that can detect various dissolved solutes including glucose, oxygen, pH, sulfide, and ammonia. Lewandowski et al. [109] report using micro-oxygen sensors (tip size, 15 μm) to estimate oxygen profiles, local oxygen uptake rates, and oxygen diffusion coefficients in a mixed culture

biofilm community cultivated within a laboratory biofilm reactor. Kühl and Jørgensen [110] employed O_2, S^{2-}, and pH microsensors (tip size, 20–25 μm) to study O_2 respiration, H_2S oxidation, and SO_4^{2-} reduction in compact aerobic trickling filter biofilms. O_2 respiration was found in only the upper 0.2–0.4 μm of the biofilm whereas SO_4^{2-} reduction occurred in the deeper anoxic parts of the biofilm. All H_2S produced in these anoxic depths was reoxidized by O_2 in a narrow zone in the biofilm. Cronenberg and van den Heuvel [111] report the use of glucose oxidase immobilized to a platinum electrode (tip size, 20 μm) to determine in situ the glucose diffusion coefficient and glucose uptake rates by yeast cells immobilized within agar beads.

The greatest advances in biofilm analyses have arisen in the area of non-invasive non-destructive diagnosis. Lewandowski et al. [112] employed spin-echo and dual spin-echo nuclear magnetic resonance imaging (MRI) to 'visualize' laminar flow fluid velocity profiles within an artificial porous media system cultivating bacterial biofilms. Both MRI techniques combined to provide estimates of local fluid velocities as well as indications of 'dead' water (protons) where nuclei relax at a faster rate than free water; indirectly indicating a biofilm colony.

Bremer and Geesey [63, 113] cultivated biofilms of a fresh water copper-corroding bacterium on attenuated total reflectance (ATR) waveguides of germanium within which an evanescent IR wave is transmitted. Spectra of materials that adsorb in the IR at the interface of the ATR crystal are collected, fast Fourier series transformed and the IR spectra of water subtracted to provide a continuous on-line measure of the molecular chemistry occurring at the cell–substratum interface. Accumulation of IR spectral intensities at these wavenumbers suggests the accumulation of several proteins and polysaccharides on the substratum. Since the IR wave can not penetrate more than 0.6 μm above the crystal interface, this accumulation should not imply an increase in biofilm thickness.

Perhaps the single most significant advance to affect our understanding of biofilm ecological processes will undoubtedly be the confocal laser scanning microscope (CLSM) coupled with digital image analysis and a variety of fluorescent stains. Lawrence et al. [114] illustrate the ability to section 'optically' fully hydrated biofilms, both horizontally and sagitally; producing 'optical sections' of undisturbed biofilms with a spatial thickness of 2 μm. Any fluorescent probe (cellular DNA stain; immunofluorescent stain; pH, ion, and redox sensitive stains; viability redox stains) or any combination of probes can be simultaneously recorded by the CLSM with great clarity and little background interference, due to the confocal exclusion of any fluorescence originating from excited fluorochrome above and below the focal point. Three-dimensional reconstructions of biofilms are possible by displaying as stereo pairs several biofilm optical thin sections. CLSM is a critical tool in population community structure analysis of mixed strains, species, and cell lines and will allow detailed examination of the relationships between biofilm structure, adaptation, reactivity and response to external stress.

Biofilms: the next generation?

Bacterial whole-cell adsorbents

Recent development of cell systems for the expression of heterologous proteins on the surface of Gram negative bacteria has stimulated considerable interest in practical applications [115] one of which may be facilitated by a biofilm mode of growth. Expression of polypeptides on the surface of Gram negative bacteria has been pursued for years partly because of interest in vaccine production. Gram negative bacteria exhibit two membranes both of which consist of a phospholipid inner layer and a liposaccharide outer layer. Translocation through the outer membrane is the major obstacle in the expression of cell surface proteins. Except for certain enzymes transported by bacterial pathogens, extracellular proteins are

exported via specialized systems requiring several auxiliary components. Native proteins that have been used as surface-expression vehicles include the *E. coli* maltoporin, the K88ac and K88ad pilin proteins, the *S. typhimurium* flagellin, the TraT lipoprotein, and other *E. coli* outer membranes such as PhoE and OmpA.

Immobilization of specific antibodies and other polypeptides for use in affinity separations requires that the proteins are first produced by cell culture fermentation, purified, and the specific antibody covalently linked to a solid-phase support. These processes are extortionately expensive and, as a result, are appropriate only for the purification of high-value products. Expression of proteins on the surface of a bacterium could be an inexpensive alternative for preparing affinity adsorbents. Proteins anchored to the cell are in effect produced in an immobilized form on microscopic particles (i.e. the cells). For 20 years, wild-type bacteria carrying native surface receptors have been used as whole-cell adsorbents. *Staphylococcus aureus* produces surface bound protein A, which has a high affinity for the Fc domain of certain immunoglobulins. Heat killed and stabilized *S. aureus* cells have been used for purification and immobilization of antibodies for both analytical and preparative purposes [116]. Expression of single-chain antibodies and protein receptors on the surface of bacteria makes virtually an unlimited range of whole-cell adsorbents possible. Adhesion or immobilization of the recombinant cells to inert supports would allow fabrication of speciality columns for separation of specific proteins or speciality chemicals from mixtures, or pollutants from dilute aqueous streams.

Biological materials fabrication

Mendelson [117] reports that the addition of soluble metal salts of calcium, iron, or copper to cultures of *Bacillus subtilis* grown in web form nucleated precipitation at the surface of the bacterial cell walls. Mineralized cell filaments could be drawn into a fiber that, when dried, consisted of a bacterial cell thread backbone carrying an inorganic solid. This is the first report on the application of biotechnology to materials synthesis where bacterial cell walls serve as a template for metal fiber formation. The resultant materials, termed 'bionites', form inorganic fibers with unusual magnetic and optical properties and reported dimensions 12 cm in length by 1 mm diameter. Using a genetically engineered strain of *Bacillus subtilis* carrying the *E. coli lacZ* gene to express β-galactosidase, Mendelson was also able to fabricate a calcium based filament, 'calbactonite', that exhibited immobilized β-galactosidase activity. One can envision a new route for fabricating either biocompatible biomedical composites or stable biosensors on the nanoscale by first (a) employing specific ligand–receptor binding to 'seduce' designer bacteria, with specific enzymatic activities, to attach to a surface in a desired geometric pattern followed by (b) deposition of the inorganic fiber material.

Summary

Biofilms are a collection of microorganisms attached to a surface and bound within a gelatinous matrix composed of extracellular polymeric material which the cells secrete. Biofilms can form on any surface exposed to an aqueous environment containing microorganisms. Formed unintentionally, biofilms can create serious detriments in natural, engineered and biomedical systems. Alternatively, if their accumulation is controlled, biofilms are one form of immobilized cell system and, as such, they are pertinent within biotechnology because of (1) reactor performance advantages that immobilized cells provide over freely suspended cultures, (2) specific metabolic improvements or products created upon immobilization, (3) their ability to localize a specific biological response which can be exploited in biosensor design. This article has reviewed both the current and emerging technological implications of bacterial cell adhesion and biofilm formation. Such existing and emerging technologies can be associ-

ated with either the detection, control, or exploitation of the biofilm mode of microbial growth.

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References

1 J D Bryers and W G. Characklis, in L. Melo, T.R. Bott, M. Fletcher and B. Capdeville, (Eds.), Biofilm — Science and Technology, NATO Advanced Studies Institute Series, Vol 223, Kluwer, Amsterdam, 1992, p. 221.
2 J.D. Bryers, Biotechnol. Prog., 3 (1987) 57.
3 W.G Characklis and K.C. Marshall (Eds.), Biofilms, Wiley-Interscience, New York, 1990.
4 J W. Costerton, T J. Marrie and K.-J. Cheng, in D.C. Savage and M. Fletcher (Eds.), Bacterial Adhesion: Mechanisms and Physiological Significance, Plenum, New York, 1985, pp. 3–44.
5 C.L. Brierley, Crit. Rev. Microbiol., 6 (1978) 207.
6 C.L. Brierley, Scientific American, 247, (1982) 42.
7 R.W. Lawrence and A. Bruynesteyn, CIM Bull., 76 (1983) 107.
8 D. Chandra, L.L. McCarthy, D.L. Dexter, F J. Cummings and B. Rotman, Fuel, 58 (1979) 549.
9 C.F. Gockay and R.N. Yurteri, Fuel, 62 (1983) 1223.
10 F Kargi and J.M Robinson, Biotechnol. Bioeng., 24 (1982) 2115.
11 J.D. Bryers and W.G. Characklis, in W.G. Characklis and K.C. Marshall (Eds.), Biofilms, Wiley-Interscience, New York, 1990, Chapter 17.
12 C.P.L. Grady and H.C. Lim, Biological Wastewater Treatment, Marcel Dekker, New York, 1980.
13 P.F. Cooper and B. Atkinson (Eds.), Biological Fluidized-Bed Treatment of Water and Wastewater, Ellis Horwood, Chichester, 1981, pp. 671–696.
14 B.Z. Eathepure and T.M. Vogel, Appl Environ Microbiol., 57 (1991) 3418.
15 J.P. Arcangeli and E Arvin, Appl Microbiol Biotechnol, 37 (1992) 510.
16 I. Coelhoso, R. Boaventura and A. Rodrigues, Biotechnol. Bioeng., 40 (1992) 625.
17 D. Lemoine, T. Jouenne and G.A. Junter, Appl. Microbiol. Biotechnol., 36 (1992) 257
18 J.A. Scott and A M. Karanjkar, Biotechnol. Lett., 14 (1992) 737.
19 G. Zellner, M. Gevcke, E.C Demacario and H. Dickmann, Appl Environ. Microbiol., 36 (1991) 404.
20 C.D Gooijer, R.H. Wijffels and J. Tramper, Biotechnol. Bioeng., 38 (1991) 224.
21 R.H. Wijffels, C.D Gooijer, S. Kortekaas and J Tramper, Biotechnol. Bioeng, 38 (1991) 232.
22 T. Sumino, H Nakamura, N. Mori, Y. Kawaguchi and M Tada, Appl. Microbiol Biotechnol, 36 (1992) 556.
23 L.E. Hallas, W.J Adams and M.A Heitkamp, Appl. Environ. Microbiol., 58 (1992) 1215.
24 B. Heppner, G. Zellner and H. Dickmann, Appl. Environ. Microbiol., 36 (1992) 810.
25 A.M. Anselmo and J M. Novais, Biotechnol. Lett, 14 (1992) 239
26 L E. Macaskie, P.J. Clark, J.D. Gilbert and M.R. Tolley, Biotechnol. Lett., 14 (1992) 525
27 G. Gonzalez, F. Ramirez and O Monroy, Biotechnol. Lett, 14 (1992) 149.
28 D.E Fennel, S E. Underhill and W J. Jewell, Biotechnol. Bioeng, 40 (1992) 1218.
29 J.F Kennedy and J.M.S. Cabral, Appl. Biochem. Bioeng., 4 (1983) 189.
30 J.F. Kennedy, ACS Symp Ser. Vol. 106, American Chemical Society, Washington, DC, 1979, p. 119.
31 C D. Scott, Enzyme Microb. Technol, 9 (1987) 66.
32 G.M. Black, C. Webb, T M. Matthews and B. Atkinson, Biotechnol Bioeng, 26 (1984) 134.
33 C. Webb, H. Fukunda and B. Atkinson, Biotechnol. Bioeng., 28 (1986) 41.
34 D.S. Inloes, W.J. Smith, D P. Taylor, S N. Cohen, A.S. Michaels and C.R. Robertson, Biotechnol Bioeng., 25 (1983) 2653.
35 P. de Taxis du Poet, P. Dhulster, J.N. Barbotin and D. Thomas, J Bacteriol., 165 (1986) 871
36 M. Nasri, S Sayadi, J.N. Barbotin, P. Dhulster and D Thomas, Appl. Environ. Microbiol., 53 (1987) 740.
37 S. Sayadi, M. Nasri, J.N. Barbotin and D. Thomas, Biotechnol. Bioeng., 33 (1989) 801.
38 S.T. Nesaratnam and F.H. Ghobrial, Recycling Conservation, 8 (1985) 135.
39 R.A. Messing, Biotechnol. Bioeng., 24 (1982) 1115.
40 I. Karube, S. Kuriyama, T. Matsunaga and S. Suzuki, Biotechnol. Bioeng., 22 (1980) 847.
41 I. Nilsson, S. Ohlson, L. Haggstrom, N. Molin and K Mosbach, Eur. J. Appl. Microbiol. Biotechnol., 10 (1980) 261.
42 A. Nakijima, T. Horiskosma and T. Sakaguichi, Eur. J. Appl. Microbiol. Biotechnol., 16 (1988) 88.
43 J. Klein, U. Hackel and F. Wagner, in Immobilized Microbial Cells, ACS Symp. Ser. Vol. 106, American Chemical Society, Washington, DC, 1979, Chapter 7.
44 S Suzuki, J. Karube and T. Matsunaga, Biotechnol. Bioeng. Symp., 8 (1978) 501.
45 Y S. Park and K. Toda, Biotechnol. Lett., 14 (1992) 609.
46 S.G. Velizarov, E.I. Rainina, A.P. Sinitsyn, S.D. Varfolomeyev, V.I. Lozinsky and A.L. Zubov, Biotechnol. Lett., 14 (1992) 291.
47 M. Moresi, E. Parente and A. Mazzatura, Appl. Microbiol. Biotechnol., 36 (1991) 320
48 M Y. Kwak and J S. Rhee, Appl. Microbiol. Biotechnol., 36 (1992) 578.

49 M Y Kwak and J.S Rhee, Biotechnol. Bioeng., 39 (1992) 903.
50 J W Tzeng, L S Fan, Y.R. Gan and T T Hu, Biotechnol Bioeng., 38 (1991) 1253.
51 B. Demuyakor and Y Ohta, Appl Microbiol Biotechnol., 36 (1992) 717.
52 G A Kovalenko and V.D Sokolovskii, Biotechnol Bioeng., 39 (1992) 522
53 I Karube, S. Suzuki, S Kinoshita and J J Mizuguchi, Ind. Eng. Chem. Prod Res Dev., 10 (1971) 160.
54 I Karube, T Matsunaga and S. Suzuki, J Solid Phase Biochem., 2 (1977) 97.
55 I Karube, T Matsunaga, S. Mitsuda and S Suzuki, Biotechnol Bioeng., 19 (1977) 1535
56 M. Hikuma, H Suzuki, T. Yasuda, I Karube and S. Suzuki, Eur. J. Appl Microbiol. Biotechnol., 8 (1979) 289
57 M Hikuma, T. Kabok, T Yasuda, I Karube and S. Suzuki, Anal. Chem., 52 (1980) 1020
58 S A. Blenkinsopp and J W Costerton, Trends Biotechnol., 9 (1991) 138
59 J.D Bryers, Trends Biotechnol., 9 (1991) 422
60 S. Deckena and K.H Blotevogel, Biofouling, 5 (1992) 287
61 N.J.E Dowling, J. Guezennec, J Bullen, B Little and D C. White, Biofouling, 5 (1992) 315
62 R.D. Bryant, W Jansen, J Boivin, E J. Laishley and J W Costerton, Appl Environ Microbiol., 57 (1991) 2804.
63 P J Bremer and G G Geesey, Appl. Environ Microbiol., 57 (1991) 1956.
64 I B Beech, C C Gaylarde, J.J. Smith and G G Geesey, Appl. Environ. Microbiol., 35 (1991) 65
65 M.J. Franklin and D.C White, Current Opinion Biotechnol., 2 (1991) 450
66 N D Benbouzid-Rollet, M. Conte, J. Guezennec and D J. Prieur, Appl. Bacteriol., 71 (1991) 244
67 R Boopathy and L Daniels, Appl Environ Microbiol., 57 (1991) 2104.
68 M B Deshmukh, I Akhtar, R B. Srivastava and A.A Karande, Biofouling, 6 (1992) 13
69 P. Vandevivere and P Baveye, Appl Environ Microbiol., 58 (1992) 1690.
70 G. Reid, H.S Beg, C.A K. Preston and L.A Hawthorn, Biofouling, 4 (1991) 171
71 I H. Pratt-Terpstra, J. Mulder, A H Weerkamp, J Feijen and H.J.J. Busscher, J Biomater. Sci. Polym. Ed., 2 (1991) 239
72 C C. Chang and K.J. Merritt, Biomed Mater. Res., 26 (1992) 197.
73 K Merritt and C.C Chang, J. Biomater. Appl., 5 (1991) 185
74 L. Passerini, K. Lam, J.W Costerton and E.G King, Crit. Care. Med., 20 (1992) 665.
75 C P. Virden, M K. Dobke, P. Stein, C.L. Parsons and D.H. Frank, Aesthetic Plast. Surg., 16 (1992) 173.
76 D.A Jennings, M J. Morykwas, W W. Burns, M E. Crook, W P. Hudson and L C. Argenta, Ann Plastic Surg., 27 (1991) 216.
77 J.C. Nickel, J Downey and J.W Costerton, Urology, 39 (1992) 93
78 R.L S Whitehouse, E Peters, J Lizotte and C. Lilge, J. Dentistry, 19 (1991) 290
79 S.G. Dunkirk, S.L. Gregg, L.W Duran, J D Monfils, J E Haapala, J A. Marcy, D.L Clapper, R A Amos and P E Guire, J Biomater. Appl., 6 (1991) 131
80 M.J. Bridgett, M C. Davies and S.P Denyer, Biomaterials, 13 (1992) 411
81 N.P Desai, S F.A. Hossainy and J.A Hubbell, Biomaterials, 13 (1992) 417.
82 W K. Whitekettle, J Ind Microbiol., 7 (1991) 105.
83 C C Chang and K J Merritt, J. Orthopedic Res., 9 (1991) 284
84 G Golomb and A. Shpigelman, J Biomed Mater Res., 25 (1991) 937.
85 G D Kamal, M.A. Pfaller, L.E. Rempe and P.J. Jebson, J Am Med Assoc., 265 (1991) 2364.
86 J.W. Leung, G.T. Lau, J J Sung and J.W. Costerton, Gastrointest Endosc., 38 (1992) 338
87 D Stickler and P. Hewett, Eur J Clin Microbiol Infect Dis., 10 (1991) 416.
88 D Stickler and P Hewett, Eur. J. Clin Microbiol Infect. Dis., 10 (1991) 157.
89 C.L Wiatr and O X Fedyniak, J. Ind Microbiol., 7 (1991) 7.
90 R Bakke, B Rivedal and S Mehan, Biofouling, 6 (1992) 53
91 S.A. Blenkinsopp, A E. Khoury and J W Costerton, Appl Environ Microbiol., 58 (1992) 3770
92 T.M. Bergamini, J.C Peyton and W.G Cheadle, J. Surg. Res., 52 (1992) 101
93 G K. Richards, R F Gagnon and J Prentis, Trans. Am. Soc Artif Intern Organs, 37 (1991) M160.
94 N. Khardori, E. Wong, H Nguyen, C Jeffery-Wiseman, E. Wallin, R.P. Tewari and G P Bodey, J Infect Dis., 164 (1991) 108.
95 D J. Evans, D G Allison, M.R. Brown and P Gilbert, J Antimicrob Chemother., 27 (1991) 177
96 B D Hoyle and J W. Costerton, Prog Drug. Res., 37 (1991) 91
97 R.F. Gagnon, G.K. Richards and L Wiesenfeld, L Trans Am Soc Artif Intern Organs., 37 (1991) M158
98 C-T Huang, S W Peretti and J D. Bryers, Biotechnol Lett., 6 (1992) 193
99 A Buret, K.H. Ward, M.E Olsen and J W. Costerton, J. Biomed. Mater Res., 25 (1991) 865.
100 M.W. Mittleman, L L Kohring and D.C. White, Biofouling, 6 (1992) 39
101 S L. Kinniment and J W T Wimpenny, Appl. Environ Microbiol., 58 (1992) 1629
102 R H. Kuhn, S W Peretti and D F Ollis, Biotechnol. Bioeng., 38 (1991) 340.
103 P S Stewart, S F Karel and C.R. Roberston, Biotechnol Bioeng., 37 (1991) 824.
104 J. Rogers and C.W. Keevil, Appl. Environ. Microbiol., 58 (1992) 2326

105 G G. Rodriguez, D. Phipps, K. Ishiguro and H.F. Ridgway, Appl. Environ. Microbiol., 58 (1992) 1801.
106 R.I. Amann, J. Stromley, R. Devereux, R. Key and D.A. Stahl, Appl. Environ. Microbiol., 58 (1992) 614
107 L.L McCarter, R.E. Showalter and M R. Silverman, Biofouling, 5 (1992) 163.
108 L. Dagostino, A E. Goodman and K.C Marshall, Biofouling, 4 (1991) 113.
109 Z Lewandowski, G. Walser and W.G Characklis, Biotechnol Bioeng., 38 (1991) 877.
110 M. Kühl and B.B. Jørgensen, Appl. Environ. Microbiol., 58 (1992) 1164.
111 C.C.H. Cronenberg and J.C van den Heuvel, Biosensors Bioelectronics, 6 (1991) 255.
112 Z. Lewandowski, S.A Altobelli, P.D. Majors and E. Fukushima, Water Sci. Technol., 26 (1992) 577.
113 P.J. Bremer and G.G. Geesey, Biofouling, 3 (1991) 89.
114 J R. Lawrence, D.R. Korber, B.D. Hoyle, J.W. Costerton and D.E. Caldwell, J. Bacteriol., 173 (1991) 6558.
115 G Georgiou, H.L. Poetschke, C. Stathopoulos and J.A. Francisco, Trends Biotechnol., 11 (1993) 6.
116 S W. Kessler, Methods Enzymol., 73 (1981) 442.
117 N.H. Mendelson, Science, 258 (1992) 1633.