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MULTIPURPOSE LAMINAR-FLOW ADHESION CELLS FOR THE STUDY OF BACTERIAL COLONIZATION AND BIOFILM FORMATION

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The ability to reproducibly colonize substrata with concomitant monitoring of biofilm development is essential to laboratory studies of microbial activities at surfaces. A test system was developed whereby on-line, non-destructive measurements (open circuit potential [OCP], direct cell counts, bioluminescence, oxygen) of bacterial colonization and metabolic activity could be obtained from a series of laminar-flow adhesion cells. The cells consisted of two high-density polyethylene blocks 32 mm H × 65 mm W × 178 mm L, with a 1 mm deep flow channel milled in the top block. A glass viewing window enabled direct observation of a removable, flush-mounted 25 × 50 mm coupon, which was recessed into the bottom block. Laminar-flow conditions were validated at linear flow rates up to 1·3 cm s^{-1} (20 ml min^{-1}). Reproducible colonization of *Pseudomonas fluorescens* monocultures was obtained, with 72 h direct-counts and viable counts ranging from between 5·1 to 10·4 × 10^7 and 1·4 to 2·2 × 10^7 cells cm^{-2}, respectively, for replicated flow cells (n = 20). *In situ* pulse-labeling of intact biofilms with ^{14}C-acetate resulted in reproducible incorporation of the radiolabel into cell membrane lipids, with 60 min uptake values ranging from 1·0 to 1·8 × 10^{-3} DPM $cell^{-1}$. On-line OCP measurements remained stable in sterile test systems, varying by less than 12 mV, for over 80 h. The flow cells provided a means for reproducibly colonizing various substrata under *in situ* environmental conditions without perturbing the developing biofilms.

KEY WORDS: On-line biofilm monitoring, flow-cells, colonization studies, *Pseudomonas fluorescens*.

INTRODUCTION

The study of biofilm development on inanimate substrata in natural environments, industrial distribution systems, wastewater treatment operations, and in laboratory- and pilot-scale test systems has been an active area of research for the past 50 years. Despite an intuitive recognition of their preeminent role in these environments, progress in understanding the dynamics of bacterial colonization and biofilm formation has not kept pace with other developments in environmental microbiology. The pioneering studies of Henrici (1933) and Zobell (1943) on bacterial colonization were carried out by exposing glass slides to freshwater and marine ecosystems. A serious gap exists between current analytical techniques for evaluating bacteria and consortial communities and relevant, workable systems for the reproducible development of bacterial biofilms. In many studies, the convenience of using glass substrata in static cultures has outweighed any relevance to the environment which is to be simulated.

Fletcher (1976) and Fletcher and Pringle (1985) have described a spectrophotometric-adhesion assay in which axenic cultures are placed onto polystyrene petri or tissue culture dishes, rinsed, then stained with crystal violet. Differential adhesion in this system is measured by absorbance at 590 nm. Marshall *et al.* (1971) suspended glass coverslips in natural stream systems and differentiated reversible from irreversible

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bacterial sorption based upon susceptibility of sorbed cells to a rinsing procedure. Pedersen (1982) utilized a low-flow seawater system to colonize glass coverslips contained within electrochemical cells. Colonization efficiency was determined using the spectrophotometric procedure described above. Much of the experimental variance described in this statistical study was ascribed to the rinsing and staining procedures used. van Pelt *et al.* (1985) studied differential adhesion of a *Streptococcus* sp. to surfaces of varying free surface energies in a stirred contactor cell. A series of external rinse and fixation procedures was also required prior to cell enumeration.

Rosenberg and Kjelleberg (1986) have stressed the importance of hydrophobic interactions as determinants of bacterial adhesion. Accordingly, their study systems have utilized substrata with varying degrees of hydrophobicity as colonization surfaces. For example, hydrophobic interaction chromatography (HIC) using octyl- or phenyl-sepharose beads as the stationary phase (Dahlback *et al.*, 1981) and the bacterial adhesion to hydrocarbons (BATH) assay (Rosenberg *et al.*, 1980) have been widely applied. In both of these batch-type adhesion test systems, a suspension of the bacterial culture of interest is brought into contact with the hydrophobic substratum, and relative adhesiveness determined *via* endpoints such as turbidity and retention of radiolabeled substrates.

The application of dynamic-flow adhesion cells to studies of bacterial colonization and biofilm formation provides two distinct advantages. Firstly, hydrodynamic parameters of flow velocity, Reynolds number, and shear force can be defined and controlled. Rational comparisons between different treatment effects (surface energy, substratum topology, *etc.*) and among other model systems can thus be made. Secondly, problems associated with inconsistent and ill-defined rinsing of "reversibly" bound cells, culture media, radiolabeled substrates, or vital stains can be obviated; exposure of the biofilm to interfacial forces during passage through the medium-air interface is thereby limited.

Real efforts have been made in recent years to develop test systems which enable direct and real-time evaluation of bacterial colonization on inanimate substrata exposed to dynamic flow conditions. Duxbury (1977) provides information on the construction and operation of a perfusion microculture chamber for studying cell growth. The system allows changes in bulk phase environmental conditions to be made during real-time observation of bacteria. The radial flow cells of Fowler and McKay (1980) enable development and monitoring of colonization in a shear gradient. The application of these flow cells to on-line monitoring of bacterial adhesion is described in Mittelman *et al.* (1992). Sjollema *et al.* (1989a) enumerated *Streptococcus sanguis* cells adhering to a parallel-plate cell using on-line image analysis. The cell was relatively simple, consisting of two machined polymethylacrylate plates separated by a 0·06 cm flow channel. The rectangular cells contained fixed lower and upper glass windows for illumination and observation, respectively. Lock and Ford (1983) utilized a jacketed flow cell surrounded by extremely sensitive thermistors to measure heat production to riverine epilithic biota. Bacterial biofilms have also been developed on germanium crystal surfaces and living biomass constituents monitored on a real-time basis using FTIR in the attenuated total reflectance (ATR) mode (Nichols *et al.*, 1985).

Berg and Block (1984) and Caldwell and Lawrence (1988) have designed small-volume flow cells for continuous monitoring of bacterial colonization under well-defined hydrodynamic parameters. Their cells are roughly based upon Schwobel's (1954) early slide culture flow cell, which consisted of a set of glass coverslips separated by a thin steel support. Exact control over the bulk phase environment, coupled with the ability to rapidly change biotic communities within the flow stream, enable the

study of bacterial colonization at the level of the *in situ* microenvironment. While these flow cells do offer some advantages over previous designs, they, as with most of their predecessors, are designed for on-line microscopic evaluation of bacterial colonization on glass substrata. Provisions for monitoring biofilm development on other, non-glass substrata, electrochemical methods for studying adhesion, and quantitative assessments of biofilm biomass and metabolic activity have not been applied.

This work describes the design, construction, and validation of laminar-flow adhesion cells with provisions for on-line monitoring of microbial colonization and biofilm development. Provisions have been made for the incorporation of on-line electrochemical monitoring of surface potential changes as a function of colonization. The flow cells can be used for adhesion studies simulating *in situ* environmental conditions with minimal perturbations of the planktonic or sessile populations.

MATERIALS AND METHODS

Design and Hydrodynamic Considerations

The following design criteria were utilized in the development of the flow cells. Flow through the cells should be laminar over the coupon surface; the coupons should be easily replaceable; the cells should be constructed of high-quality materials which are low in extractables, highly corrosion resistant, easy to clean, and offer resistance to repeated chemical and/or gaseous sterilization. In addition, the cells should have provisions for continuous macro- and microscopic monitoring of the coupons; they should enable continuous monitoring of bulk phase electrochemical parameters such as pO^2, pH, and Eh; the cells should provide for continuous monitoring of the open circuit potential (OCP) developed at the coupon surface. It is also desirable that these design criteria be consistent with production costs of less than $300.00 per cell.

Inlet and outlet ports to the flow channel were designed to provide constant velocity across their respective cross sections. This was accomplished by designing the entry and exit sections to flare into a trumpet-shape (Goldstein, 1965). In addition to entry/exit design, another important determinant of fluid dynamics is entry length. Davies and White (1928) demonstrated that for flow in a channel an entry length >54 times the channel depth was sufficient to allow transition from turbulent to laminar flow. Based upon their calculations, and a 1 mm deep by 25 mm wide flow channel, and entry length of 55 mm between the bottom flare of the inlet and the upstream side of the coupon recess was selected.

Another consideration was the effect of surface roughness on laminar flow, which is described by

$$R_\varepsilon = .75R(\varepsilon/a)^2 \qquad (1)$$

where RF_ε = Reynolds number due to surface roughness; $R = 2au_m/\mu$; a = channel depth; u_m = average velocity; μ = kinematic viscosity; ε = maximum height of protuberance.

A critical R_ε, Rc, can be defined such that for $R_\varepsilon > Rc$, a vortex wake forms behind the protuberance and for $R_\varepsilon < Rc$, flow closes up behind the protuberance and the effect is negligible. Goldstein (1965) gives the Rc for a flat plate normal to flow at ≈ 30. Therefore, $\varepsilon/a < 4/R^{1/2}$ defines the limit for roughness. It can be seen from this derivation that both channel depth and velocity influence turbulence at the surface imparted by surface inhomogeneities. Since coupons of substrata with varying topologies might be utilized

in adhesion assays, selection of a deeper channel would enable use of a broader range of topological heterogeneities which would not effect transition from laminar to turbulent flow.

Based upon a channel depth of 1 mm and the assumption that the limit for laminar flow transition to turbulent flow for a rectangular flow channel is on the order of Reynolds number (Re) ≈ 1,000–2,000 (Davies & White, 1928), an upper limit on linear fluid velocities could be calculated by

$$u_m = \mu(Re/d) \quad (2)$$

where Re = Reynolds number; u_m = average fluid velocity over the coupon; d = channel depth; μ = kinematic viscosity.
Therefore, for an Re < 1,000, u_m must be $< 100\ cm\ s^{-1}$. Since u_m = volumetric flow rate/cross sectional area, an upper limit on the volumetric flow rate can be calculated as 1500 ml min^{-1} for the flow cell.

Materials of Construction

High density polyethylene (HDPE) was utilized for all wetted surfaces, with the exception of the viewing window. The glass viewing window consisted of a 24 × 50 mm #1 Corning glass coverslip (Corning, New York). The viewing window was fixed into position with a pliable, non-acetic acid containing, silicone sealant (Mexel Company, Lille, France). Various stainless steel coupons with dimensions of 2·5 mm H × 25·4 mm W × 50 mm L were obtained from Metal Samples (Mumford, AL). As with the glass viewing windows, coupons were fixed into position with the silicone sealant. Viton or n-Buna type o-rings (Knoxville Rubber & Gasket Company, Knoxville, TN) were utilized to ensure a water-tight seal. Thumbscrews and alignment pins were all of 316-type stainless steel. Inlet and outlet connections were threaded 5 mm polypropylene compression- or slip tube-type fittings.

Fittings for electrochemical measurements, *e.g.* open circuit potential (OCP) consisted of 12·5 mm W × 38·1 mm L polypropylene screws, drilled through the center to form a 4 mm diameter lumen. A 4 mm × 4 mm Vycor glass frit (EG & G, Princeton, NJ) was used as a salt bridge at the screw tip.

Construction of the Flow Cells

Schematics of the completed flow cell are shown in Figures 1–4. Figure 1 illustrates the major components of the cell, including the three electrochemical fittings. The fittings were screwed into the top block flush with the flow channel surface. A 3 mm diameter hole was drilled adjacent to the upstream electrochemical fitting to enable insertion of a semimicro oxygen electrode. Figure 2 shows a cross-section of the top block entry-exists and the viewing window recesses. The flow channel is also illustrated, showing the trumpet-shaped entry and exit ports. Figures 3 and 4 are detailed machining diagrams for the top and bottom blocks respectively.

The coupons and glass viewing window were inserted into 3·2 mm and 0·3 mm milled recesses respectively. The recesses were milled slightly deeper than the actual coupon and glass thicknesses to allow for sealant addition. Following insertion, both surfaces were flush with the flow channel. A thumbscrew inserted through a tapped hole in the bottom block served both as a connection to the working electrode (the coupon) and to facilitate coupon removal.

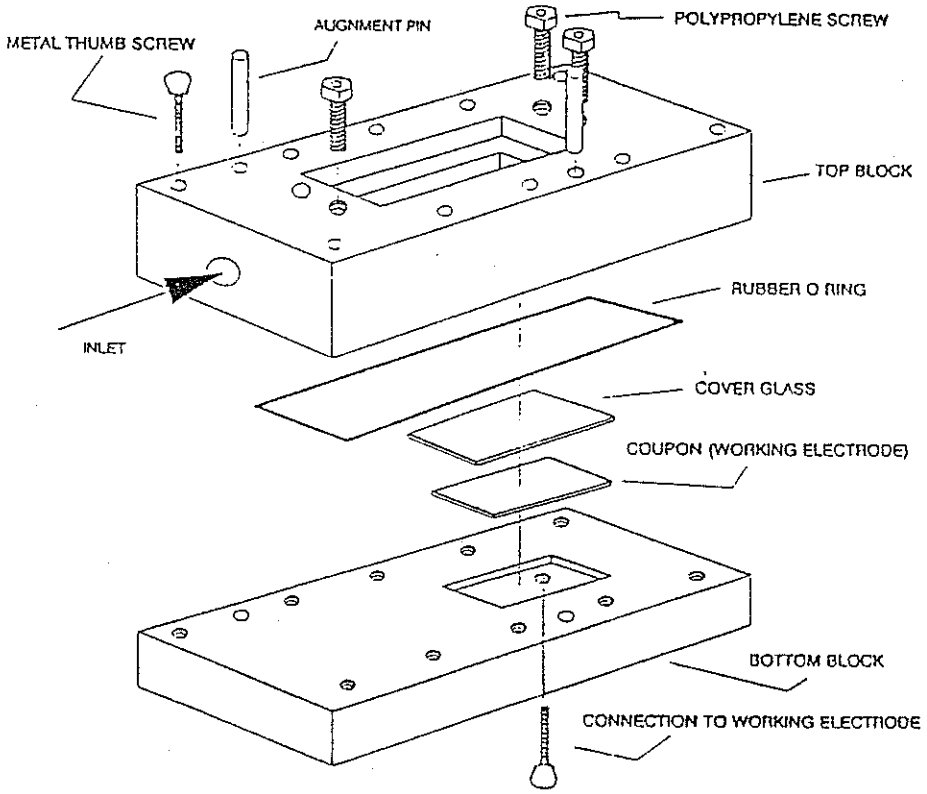

Fig. 1 Exploded view of laminar-flow adhesion cell illustrating cell assembly.

A machine tolerance of $\pm 0{\cdot}075$ mm was specified for all wetted surfaces. The machine work on the flow cells was performed by J & J Machine and Tool Company (Knoxville, TN).

Reagents

All chemicals used were of analytical reagent grade or better, unless otherwise specified.

Reference Electrode Preparation

Instructions for preparing the Ag/AgCl reference electrodes are given in Uhlig and Revie (1985). Alternatively, saturated calomel reference electrodes (Corning, Ithaca, NY) can be utilized. The completed electrodes were inserted into a saturated KCl solution contained within the electrochemical fittings.

Preparation of Flow Cells and Colonization Substrata

Prior to initiation of validation experiments, the flow cells were disassembled and critically cleaned in an Alconox soap (Alconox, New York) solution, followed by a

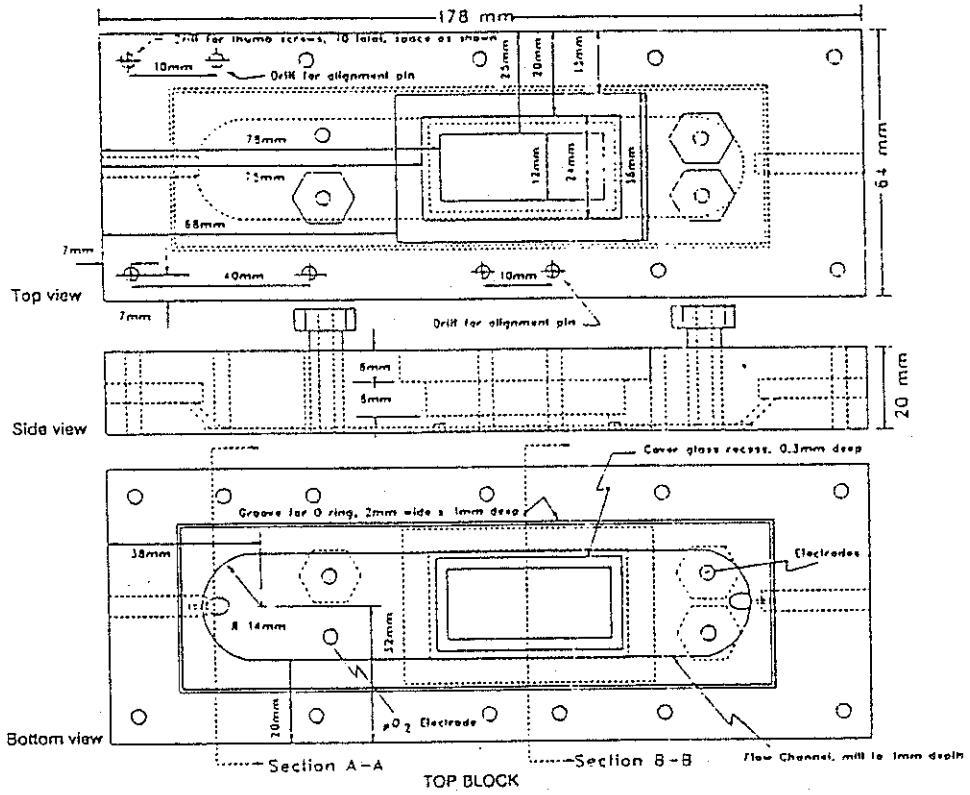

Fig. 2 Schematic diagram of top portion of flow cell containing the flow channel. Top, side, and bottom views of the cell are illustrated.

solvent rinsing series in the order chloroform, acetone, methanol, deionized water. Coupons for biofilm development were polished to a 600-grit finish, then placed in a sonicator bath and critically cleaned in the same manner as the flow cells. The flow cells were then assembled, wrapped in two layers of Bioshield sterilization material (Baxter, Valencia, CA), and ethylene oxide gas sterilized. All tubing connections to the flow cells were made under aseptic conditions in a laminar-flow hood.

Bulk Phase Feed System

Sterile, oligotrophic culture medium was pumped through the flow cells at a constant volumetric flow rate of 20 ml min^{-1} *via* a series of pulse-dampened Masterflex peristaltic pumps (Cole Parmer, Chicago). The medium composition was (in g l^{-1}): sodium lactate, 0·05; sodium succinate, 0·05; NH_4NO_3, 0·05; Na_2SO_4, 0·12; KH_2PO_4, 0·19; K_2HPO_4, 0·63; $FeCl_3$, 0·33 ml of a 10 mM solution; Hutner's salt solution, 1·0 ml. The completed medium was steam sterilized. The final pH of the culture medium was 7·2. Tubing and connections were of silicone and polypropylene, respectively, throughout the feed system. Effluent from the flow cells was shunted to drain. Injection ports for addition of dye and bacteria were located approximately 50 mm upstream from the

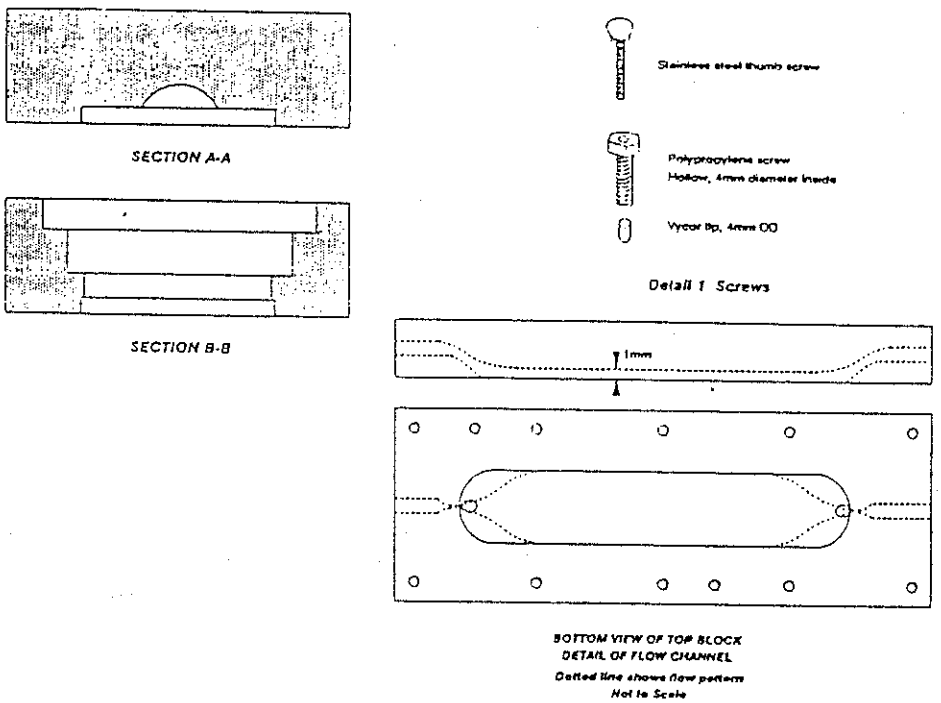

Fig. 3 Schematic of flow channel pattern showing details of electrochemical fittings and channel geometry.

inlet fittings. The ports consisted of polypropylene 'y' connectors (Cole Parmer, Chicago).

Validation of Flow Laminarity

Two qualitative techniques were employed to validate laminar flow conditions after Goldstein (1965) and Berg and Block (1984). A red silk thread was fixed to the inlet fitting, passed through the flow channel, and observed through the viewing window. Approximately 10 µl bromophenol blue was injected just upstream of the inlet port and the dye front observed through the viewing window. All flow cells were tested for flow laminarity.

Bacterial Biofilm Development and Analysis

Medium flow was maintained for 18 h prior to addition of bacteria and the sterility of the bulk phase was determined by acridine orange direct counts (AODC). Axenic cultures of *Pseudomonas fluorescens* (*lux*) kindly provided by Dr Gary Sayler, hereafter referred to as 5RL, were maintained in continuous culture at a viable cell density of approximately 10^7 cells ml^{-1}. Details on the phenotypic and genotypic characteristics of this organism may be found in King *et al.* (1990). The culture was continuously injected at 0·5 ml min^{-1} for a period of 72 h. Biofilm development on the stainless steel coupon was observed macroscopically through the viewing window. Following establishment of a 72 h biofilm, culture addition was discontinued and the biofilm exposed to

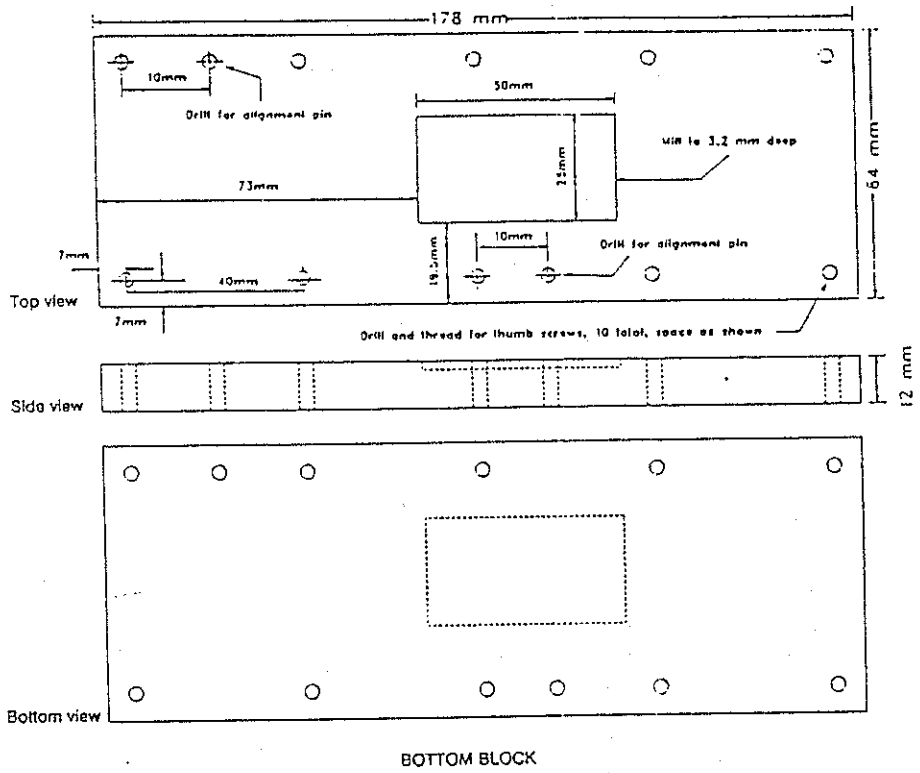

Fig. 4 Schematic diagram of bottom portion of flow cell containing the coupon/working electrode. Top, side, and bottom views of the cell are illustrated.

flowing sterile medium for 10 min. The coupons were removed and quantitatively extracted using 1·2 cm diameter glass o-ring fittings (Kontes Glass, Vineland, NJ). Following addition of 0·6 ml volumes of the ice-cold, sterile medium to the extractors, the coupon surfaces were sonicated to remove adherent cells using three 3 s pulses at 20% power (Heat Systems sonicator, Plainview, NJ). Each of the biofilm extracts was assayed for viable cell counts, AODC, and protein. Aerobic plate counts (APC) were performed with yeast extract peptone agar as the plating medium. Spread plates were incubated for 72 h at 25°C. AODC and micro-Lowry protein assays (Sigma Chemical, St Louis, MO) were performed on aliquots from each of the sonicate suspensions. Each coupon was sampled in three, equally spaced 1·13 cm^{-2} areas along its length.

Electrochemical Monitoring

Each electrochemical fitting was tested for continuity with both Ag/AgCl and saturated calomel electrodes (SCE) (Corning, New York) connected to a voltmeter (Sycopel Scientific, East Bolden, England). Vycor tips observed to be leaking, plugged, or yielding unstable voltage readings were discarded. Monitoring of OCP was performed at approximately 8 h intervals for 80 h in uninoculated and inoculated flow cells.

A semimicro dissolved oxygen polarographic electrode (Microelectrodes, Londonderry, NH), monitored by an Orion (Cambridge, MA) pH/ion monitor, was tested for stability and maintenance of calibration.

Bioluminescence Measurements

Biofilms continuously induced with sodium salicylate were monitored for bioluminescence using a liquid light pipe-photomultiplier tube-ammeter light monitoring system (Oriel, Stratford, CT). The 0·25 cm^2 detector head was manually moved across 9 different viewing window areas on each of nine flow cells. All measurements were performed in the dark.

RESULTS

Fluid Flow Observations

The silk thread observed at the center of the viewing window remained fixed in one location and did not demonstrate any lateral or vertical movement about its axis. One flow cell of the 24 tested failed this test. This failure resulted from a small machine tool recess (approximately 0·2 mm) which was found in the upstream flow channel.

At the flow rate used, the dye front moved very quickly across the viewing window area. However, after repeated dye additions, it was possible to determine that the velocity of dye front movement was constant across the coupon surface. A significant difference in flow pattern was observed in the failed cell described above.

Bubble formation in several of the flow cells created discontinuities within the biofilms. Bubbles appeared to form 2–4 h prior to the development of visible biofilms on the coupon surfaces. Dye injection demonstrated that flow appeared to be disturbed by the bubbles, which formed between the coupon and viewing glass window. In most instances, however, the bubbles dispersed upon elevation of the downstream end by approximately 45° from normal.

Reproducibility of Colonization

Results of the biofilm analyses for 4 flow cells, each colonized in triplicate, are shown in Figure 5. Each coupon was sampled in three locations; no significant effect of sample location on AODC, APC, or protein values was found. An analysis of variance for the AODC and APC data revealed that no significant differences in biofilm cell numbers existed among the 4 flow cells examined ($P < 0·05$). Cell counts ranged from between $5·1 \times 10^7$ and $10·4 \times 10^7$ cells cm^{-2} for AODC, and $1·4 \times 10^7$ and $2·2 \times 10^7$ cells cm^{-2} for APC.

Electrochemical Measurements

The open circuit potentials for an uninoculated flow cell were extremely constant over an 80 h time frame (Table I). OCP varied by approximately 12 mV for the duration of this control experiment. Following inoculation of a second flow cell run in parallel with the sterile control, a transient decrease in OCP was observed (Table I). This was followed by an increase through the 76 h test period. A net change of approximately

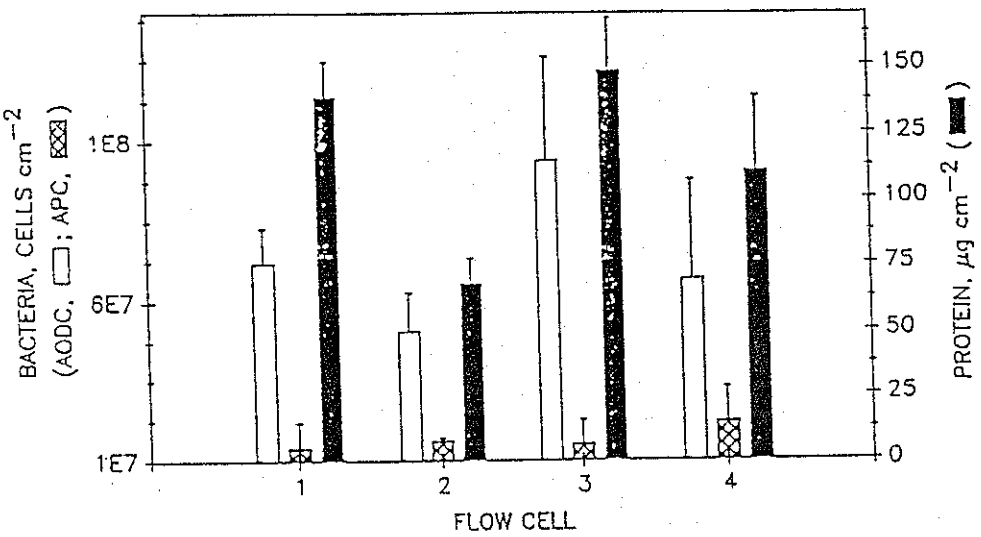

Fig. 5 Biomass measured on replica flow cells. Error bars represent ±1 SD.

Table 1 Open circuit potential of 316 stainless steel

Time (h)	*OCP, mV (Ag/AgCl/KCl)*	
	colonized[a]	*sterile*
0	40	52
12	42	59
24	41	60
36	38	59
48	30	52
60	42	53
70	62	60
72	73	60
76	76	61

[a]Addition of bacteria was begun following the 36 h measurement.

36 Mv was observed for the inoculated flow cell. Similar results (data not shown) were obtained for three other flow cells tested prior to and following colonization by 5RL.

Any physical or electrical (*e.g.* actuation of pump motors) perturbations tended to shift the OCP values substantially. Bubble formation at the Vycor tip created anomalies in OCP, which were quickly remedied by gently tapping the cells to dislodge bubbles. Inclination of the cells as described above significantly reduced the effect of bubble formation on OCP.

The oxygen electrode remained in calibration for the duration of a 72 h experiment. Oxygen levels in the flow cell monitored ranged from approximately 6·0 to 0·5 mg l^{-1};

Table 2 Bioluminescence of induced 5RL biofilms on 316 stainless steel[a]

Flow cell	*Average bioluminescence (namps)*[b]	*Standard deviation*	*Coefficient of variation*
1	4·7	0·77	16·0
2	7·9	1·4	18·0
3	9·2	1·6	17·0
4	8·0	1·0	13·0

[a]Sessile bacteria were continuously induced with sodium salicylate; the contribution of bulk-phase bioluminescent bacteria to the coupon readings ranged from approximately 0·05–0·10 namps.
[b]An average of 9 readings were taken from the upstream, central, and downstream portions of the coupon.

probe calibration was not affected by the presence of viable cells when measured at the end of the experiment.

Bioluminescence of 5RL Biofilms

Bioluminescence was consistent within the individual flow cells monitored and among the 4 replicate cells tested (Table 2). Both the within and among group readings were very consistent. Background light leakage was insignificant, with typical readings of 0·01–0·02 namps obtained during flow cell measurements. While the contribution of bulk phase bioluminescent bacteria to the light readings obtained was minimal, colonization of the glass viewing windows imparted a significant effect. Following replacement of the colonized viewing windows with clean glass coverslips, the light measured decreased by a factor of 2.

DISCUSSION

A multipurpose, laminar-flow adhesion cell has been designed, constructed and validated using qualitative and quantitative measures of fluid dynamics and biofilm development. The flow cell design incorporated provisions for on-line measurements of bacterial colonization and bulk-phase environmental conditions. Colonized substrata could be easily removed for off-line, non-destructive and destructive biofilm analysis. Cleaning, sterilization, use in extended flow studies, and disinfection did not appear to adversely affect the operation or appearance of the cells.

The trapping of gas bubbles between the coupons and glass viewing windows can account for a potential source of analytical error. Apparently, changes in surface tension at the biofilm substratum are partly responsible for this phenomenon (R Baier, personal communication). Trapping of gas bubbles by sterile coupons was not observed. Berg and Block (1984) have recommended autoclaving or otherwise degassing the flowing medium prior to introduction. However, this would render the bulk phase relatively anoxic; reintroduction of an oxygen/inert gas could resolve this problem. Since large volumes of medium are required for these types of flow experiments, an alternative solution to this problem seems advisable. Inclination of the downstream ends decreased the residence time of trapped bubbles. It is likely that increasing the flow channel depth would also diminish bubble trapping.

Significant differences in OCP were noted between the colonized and sterile SS substrata. The OCP is a net potential, describing the sum of cathodic and anodic reactions. The predominate cathodic reaction is described by

$$O_2 + 2H_2O + 4e^- \rightarrow\rightarrow 4OH^- \quad (3)$$

The OCP is primarily influenced by two parameters contained within the Nernst equation, *i.e.* pH and oxygen. The observed perturbations in OCP suggest that biofilms influence some aspect of pH, oxygen or, perhaps, reaction kinetics at the surface. Work is ongoing to develop a mechanistic explanation for the observed changes in surface potential as a function of biofilm formation.

Biofilm biomass measured by 4 different quantitative analyses was consistent among the individual cells tested. The reproducibility of the data was surprising given the difficulties inherent to the recovery of bacterial cells and biomass from surfaces. Unfortunately, the literature does not reveal comparable validation data for other adhesion cell designs. With the exception of the previously described radial flow cells, the majority of designs contain provisions for only one type of biofilm measurement; this is typically direct microscopic observation of colonization. While microscopical methods provide invaluable information on the kinetics, spatial distribution , and numbers of colonizing bacteria, they generally cannot yield more detailed information on the biomass constituents, community structure, metabolic activity, or physicochemical interactions between the biofilm and substratum.

The flow cells have potential for application to a number of biofilm-related studies. Studies of microbially influenced corrosion would be facilitated by these flow cells, since electrochemical parameters could be monitored continuously along with biofilm formation. Modification of the cells to include counter electrodes (*e.g.* Ti) would enable AC impedance spectroscopy and small amplitude cyclic voltammetry.

Determinations of biocide efficacy against sessile bacteria would be facilitated, particularly if bioluminescence can be utilized as an endpoint for adhesion and or metabolic activity determinations. The use of bioluminescent organisms in toxicity assays has been previously described (Dutka *et al.*, 1988). Perhaps sublethal changes in metabolic activity in biofilms could be measured in much the same manner using an adaptation of the techniques described herein. Colonization effectiveness could also be monitored continuously with the appropriate video hardware and image analysis system. Marshall (1986) describes various microscopic techniques which would be applicable to the adhesion cells.

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