Water Res. Vol. 16, pp. 1207 to 1216, 1982
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0043-1354/82/071207-10$03.00/0
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DYNAMICS OF BIOFILM PROCESSES: METHODS

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(*Received November* 1981)

Abstract—Methods available for measuring biofilm accumulation are presented. The methods are conveniently classified as either direct or indirect measurement techniques. The direct techniques involve measurement of biofilm thickness or biofilm mass. The indirect measurement techniques include (1) methods for measuring specific biofilm constituents (e.g. polysaccharide) and (2) methods for determining microbial activity within the biofilm. These methods are discussed in relation to their use in both laboratory studies and in the field for continuous monitoring of biofilm processes.

INTRODUCTION

Bacteria stick firmly, and often with specificity, to almost any surface submerged in an aqueous environment. The bacteria attach by means of a matrix of polymers, primarily polysaccharide, that extend from the cell surface and form a mass of tangled fibers, termed a "glycocalyx" (Costerton *et al.*, 1978). The adhesion mediated by the glycocalyx determines particular locations of bacteria in many aquatic environments. The cells grow and reproduce at the surface increasing the biomass and associated material. The entire deposit is termed the *biofilm*.

Biofilm processes may be beneficial as exemplified by fixed-film waste-water treatment processes (e.g. trickling filters and rotating biological contactors). In addition, biofilms frequently play a major role in stream purification processes. In fact, microbial activity in natural waters has been found predominantly at interfaces (Marshall, 1976; Costerton *et al.*, 1978). However, biofilms can be quite troublesome in certain engineering systems. For example, biofilms in water conduits can cause energy losses resulting from increased fluid frictional resistance and increased heat transfer resistance. Table 1 lists the effects, relevance and concerns related to biofilm processes.

The majority of the methods described in this paper were developed for research concerned primarily with biofilm development in relation to fouling. Fouling refers to the formation of inorganic and/or organic deposits on surfaces. In cooling systems, these deposits form on condenser tube walls increasing fluid frictional resistance and heat transfer resistance. Fouling biofilm development, referred to as biofouling, results from the attachment and growth of microorganisms on surfaces.

The most common method for controlling fouling biofilm development and maintaining heat exchange performance is periodic chlorination. Chlorine, added to the cooling water, serves either to kill the microorganisms or to hydrolyze the extracellular polymers which hold the biofilm together. The chlorine dose and application schedule are typically determined by (1) observation of condenser performance as indicated by plant steam back-pressure, or (2) operator experience.

Recently, concern over residual toxicity from hypochlorous acid or its reaction products has resulted in federal regulations which limit the allowable concentrations of free available chlorine in cooling water discharges. At the present time, there is no sound basis for assessing the impact of the regulations. Our investigations stemmed from the apparent need for a better basic understanding of fouling biofilm development and fouling biofilm destruction so that the impact of these new regulations on power plant operations could be evaluated.

The nature of the problem and our investigative method led us to more fundamental concerns regarding biofilm processes in an attempt to establish a systematic framework for analysis of biofilm processes irrespective of the specific environments where they are found or their particular engineering application. This paper, the first of a series, reviews methods for measuring biofilm development processes in the laboratory and in the field. Other papers in the series will consider the dynamics of biofilm development, energy losses associated with biofilm development and biofilm control.

EXPERIMENTAL SYSTEMS

Two reactor geometries were used in this research to study biofilm development and destruction:

1. The *circular tube* was used because it is the prevalent geometry in heat exchangers.
2. The rotating *annular reactor* was tested because of its compactness and ease of operation.

Nutrients, glucose, and in some cases, a synthetic growth media were added to experimental reactors to provide the necessary mineral, energy and carbon requirements for mi-

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Table 1. Effect and relevance of biofilms on various rate processes

Effects	Specific process and result	Concerns
Heat transfer reduction	Biofilm formation on condenser tubes and cooling tower fill material. *ENERGY LOSSES*	Power industry Chemical process industry U.S. Navy Solar energy systems
Increase in fluid frictional resistance	Biofilm formation in water and waste water conduits as well as condenser and heat exchange tubes. Causes increased power consumption for pumped systems or reduced capacity in gravity systems. *ENERGY LOSSES*	Municipal utilities Power industry Chemical process industry Solar energy systems
	Biofilm formation on ship hulls causing increased fuel consumption. *ENERGY LOSSES*	U.S. Navy Shipping industry
Mass transfer and chemical transformations	Accelerated corrosion due to processes in the lower layers of the biofilm. Results in *MATERIAL DETERIORATION* in metal condenser tubes, sewage conduits, and cooling tower fill	Power industry U.S. Navy Municipal utilities Chemical process industry
	Biofilm formation on remote sensors, submarine periscopes, sight glasses, etc. causing *REDUCED EFFECTIVENESS*	U.S. Navy Water quality data collection
	Detachment of microorganisms from biofilms in cooling towers. Releases *PATHOGENIC ORGANISMS* (e.g. *Legionella* in aerosols)	Public health
	Biofilm formation and detachment in drinking water distribution systems. Changes *WATER QUALITY* in distribution system	Municipal utilities Public health
	Biofilm formation on teeth. Causes *DENTAL PLAQUE AND CARIES*	Dental health
	Attachment of microbial cells to animal tissue. Causes *DISEASE* of lungs, intestinal tract, and urinary tract	Human health
	Extraction and oxidation of organic and inorganic compounds from water and wastewater (e.g. rotating biological contactors, biologically-aided carbon adsorption and benthal stream activity). *REDUCED POLLUTANT LOAD*	Wastewater treatment Water treatment Stream analysis
	Biofilm formation in industrial production processes *REDUCES PRODUCT QUALITY*	Pulp and paper industry
	Immobilized organisms or community of organisms for conducting *SPECIFIC CHEMICAL TRANSFORMATIONS*	Chemical process industry
	Fouling biofilm accumulation *REDUCES EFFECTIVENESS* of ion exchange and membrane processes used for high quality water treatment	Desalination Industrial water treatment

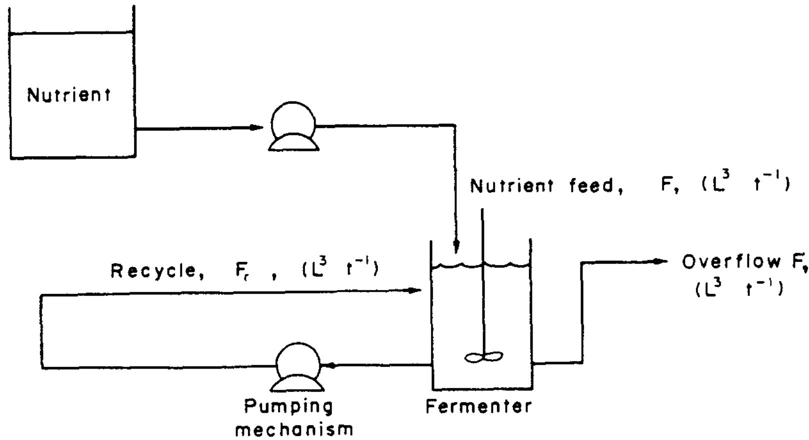

Fig. 1. Schematic diagram of the tubular reactor with recycle.

crobial growth. The feed water to the reactors was tap water which had been treated to remove residual chlorine and suspended solids.

Tubular reactor

The tubular reactors are CSTR's (continuous stirred tank reactors) with internal recycle as indicated in Fig. 1. Advantages of this configuration for laboratory experimentation include the following:

1. At the high recycle rates employed ($F_r \gg F$), the reactor contents are completely mixed and no concentration gradients exist. This simplifies mathematical descriptions and sampling. It also provides a relatively uniform biofilm in the recycle section while allowing simple control of pH and temperature. From a practical standpoint, this system minimizes the consumption of water and microbial nutrients.
2. A short mean residence time can be maintained which minimizes biomass activity in the bulk fluid and restricts microbial activity in the system to the reactor surfaces.
3. Fluid shear stress at the wall in the recycle loop is independent of mean residence time in the reactor system.

Each tubular reactor system incorporated 2 and sometimes 3 test sections (Fig. 2):

1. A test section (glass or stainless-steel) in which fluid frictional resistance was monitored by a manometer or pressure transducer during biofilm development (A). In some cases, biofilm development was observed microscopically in this test section.
2. A test heat exchanger section, in which changes in heat transfer resistance were monitored as a function of biofilm development (B).
3. A test section or sleeve which contained 12–16 removable sample tubes (glass or stainless-steel) each 5.0 cm in length (C). Sample tubes were removed periodically during biofilm development for determination of biofilm thickness, biofilm mass, or chemical analysis.

Annular reactor

The annular reactor is a potential method for monitoring biofilm development because of its sensitivity, particularly to changes in fluid frictional resistance. Furthermore, changes can be monitored continuously and non-destructively.

The annular reactor consists of two concentric cylinders, a stationary outer cylinder and a rotating inner cylinder (Fig. 3). A torque transducer, mounted on the shaft between the cylinder and the motor drive, monitors drag force. Rotational velocity is controlled electronically and displayed continuously. A removable slide, which forms an integral fit with the inside wall of the outer cylinder, is used to determine biofilm thickness, biofilm mass and provide samples for determining chemical composition. The reactor is completely mixed by virtue of the pumping action of four draft tubes and an impeller mounted at the bottom of the inner cylinder (Kornegay & Andrews, 1967). The shear force at the inner wall can be varied independently of mean residence time.

Reactor feed

The reactor feed is a combination of three constituents: dilution water, nutirent solution and microbial inoculum.

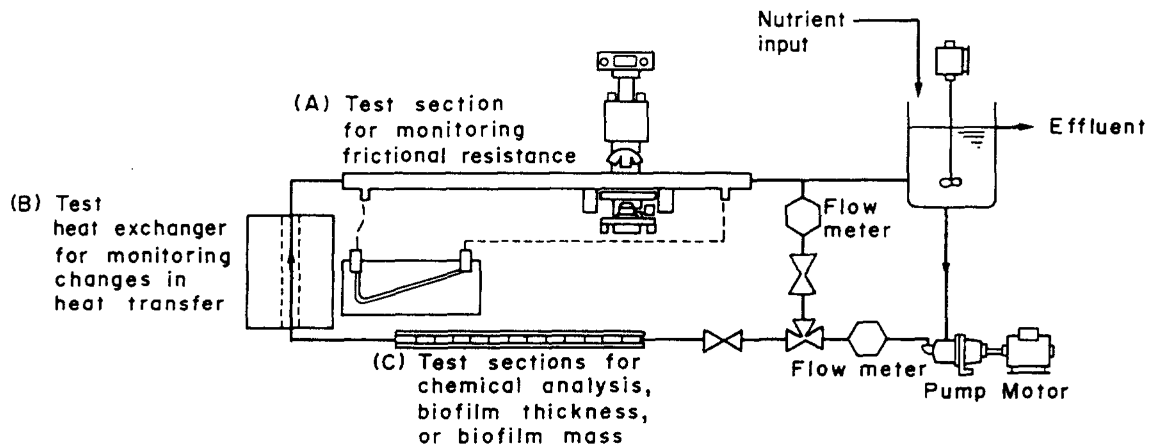

Fig. 2. Composite diagram of a tubular reactor incorporating the important features of three tubular reactors used in this research.

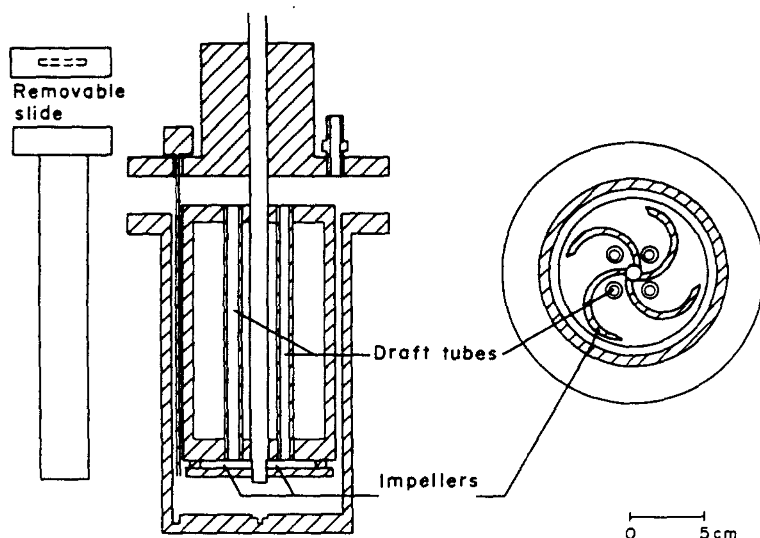

Fig. 3. Scale drawing of the annular reactor. The scale bar represents 5 cm.

Dilution water. Rice University (Houston, TX) tap water was the source of dilution water for all laboratory experiments. The tap water was treated prior to entering the experimental systems in the following way:

1. Flow through an in-line filter to remove particulates.
2. Flow through a downflow carbon adsorption column for removal of residual chlorine.
3. Storage and aeration in a 200 l. epoxy-lined tank.

A chemical analysis of the tap water is presented in Table 2.

Microbial inoculum. A standard inoculum was prepared in order to minimize the effects of population distribution differences in the laboratory experiments. Twenty liters of mixed liquor from a domestic wastewater treatment plant was settled and the concentrated sludge mixed with glycerol to approximately 25% v/v glycerol. Ten milliliter aliquots of the resulting suspension were transferred to glass ampules (10 cm^3) which were "quick frozen" in liquid nitrogen and then stored at $-20°C$. Growth rate tests on the standard inocula were conducted periodically by inoculating 250 ml Trypticase Soy Broth (12 g) with an ampule and observing increase in culture turbidity indirectly by light transmittance measurements. Results using this method indicate no significant changes in growth rate over a period of one year. However, significant differences in activity were apparent when another standard inoculum was prepared.

ANALYTICAL METHODS

Direct measurement of biofilm quantity

The only two direct measures of biofilm quantity are biofilm thickness (Th) or biofilm mass (B) (Table 3). The two quantities are related by the biofilm density (ρ_{Th}).

Biofilm thickness. Biofilm thickness has been determined in the tubular reactor system by Zelver (1979). Small sample tubes (1.27 cm i.d. and 5 cm in length) are inserted as an integral part of the tubular reactors. The sample tubes are inserted end-to-end in an acrylic plastic test section (1.9 cm i.d. and 76 cm in length) as shown in Fig. 2(c). The test section is connected to the recycle loop with pipe unions to provide easy access to the sample tubes. At designated intervals, a sample tube is removed from the reactor and a clean sample tube inserted in its place. The fouled sample tube is then drained to reduce excess water. Drainage time is generally 10 min but, in some cases, is reduced to 2.5 min when the biofilm appears to be drying around the end of the sample tube.

The volumetric displacement of the biofilm is determined using the apparatus pictured in Fig. 4. The displacement cell is filled with a water-surfactant solution (0.3% v/v Turgitol). Initial liquid level (i.e. without the sample tube immersed) is measured by lowering the conductive probe by means of a micromanipulator until contact is made with the water surface. Contact is indicated by deflection of an ammeter connected in series with the cell and a 1.5 V power source or by visual observation. A fouled sample tube is them immersed in the cell and the new liquid level (and hence, displacement) due to the fouled sample tube is determined. The sample tube is cleaned and its volumetric displacement is determined. The difference between the displacements of the fouled and clean sample tubes is the biofilm volume. The biofilm thickness is determined by dividing biofilm volume by the inner surface area of the sample tube. Overall sample standard deviation for this method of determining biofilm thickness is approx. $\pm 10\ \mu m$ based on five (5) replicate measurements on 73 samples.

Table 2. Chemical analysis of Rice University tap water

Constituent	Concentration ($mg\ l^{-1}$)
Dissolved residue at 350°C	311
Total dissolved solids	452
Silica	14 (as SiO_2)
Calcium	13 (as Ca)
Magnesium	4 (as Mg)
Sodium (diff) Na + K	111 (as Na)
Carbonate	0 (as CO_3)
Bicarbonate	278 (HCO_3)
Sulfate	7 (as SO_4)
Chloride	38 (as Cl)
Total iron	0.15 (as Fe)
Total hardness	48 (as $CaCO_3$)
Total alkalinity	228 (as $CaCO_3$)
Free carbon dioxide	0.3 (as CO_2)
	Units as indicated
Conductance (as 25°C)	553 μmhos cm^{-1}
Color	2 color units
Turbidity	1 JTU
pH	8.12

Table 3. Measurement of biofilm accumulation

Classification	Analytical method	References
(A) Direct measurement of biofilm quantity	Biofilm thickness	Hoehn & Ray (1973): Little & Lavoie (1979); Norrman *et al.* (1977); Sanders (1966); Trulear (1980); Trulear & Characklis (1982)
	Biofilm mass	Trulear (1980); Trulear & Characklis (1982); Zelver (1979); Picologlou *et al.* (1980)
(B) Indirect measurement of biofilm quantity: specific biofilm constituent	Polysaccharide	Bryers (1980); Dubois *et al.* (1956)
	Total organic carbon	Little & Lavoie (1979); Trulear (1981)
	Chemical oxygen demand	Bryers (1980); Bryers & Characklis (1980)
	Protein	McCoy (1979)
(C) Indirect measurement of biofilm quantity: microbial activity within the biofilm	Viable cell count	Corpe (1973); Costerton & Colwell (1979); Gerchakov *et al.* (1977)
	Epifluorencence microscopy	Geesey *et al.* (1978); Trulear (1981)
	ATP	Bobbie *et al.* (1979); LaMotta (1974); Little & Lavoie (1979)
	Lipopolysaccharide	Dexter *et al.* (1975); Watson *et al.* (1977)
	Substrate removal rate	Costerton & Colwell (1979); Trulear (1980); Trulear & Characklis (1982)
(D) Indirect measurement of biofilm quantity: effects of biofilm on transport properties	Frictional resistance	Norrman *et al.* (1977); Zelver (1979); Trulear (1980); Picologlou *et al.* (1980); Trulear & Characklis (1982)
	Heat transfer resistance	Fetkovich *et al.* (1977); Knudsen (1980); Nimmons (1979); Characklis *et al.* (1981)

Biofilm thickness has also been determined using various methods which locate the biofilm–fluid interface and the biofilm–inert surface interface. Trulear (1980) used an optical microscope method to determine biofilm thickness in the annular reactor. The technique was adapted from Sanders (1966) and requires biofilm growth on a thin acrylic plastic slide which forms an integral part of the annular reactor wall. The slide is withdrawn from the reactor and placed on a microscope stage. The 10 × objective (100 × magnification) is lowered until the biofilm surface is in focus and the fine adjustment dial setting is recorded. The objective is then lowered further until the inert plastic growth surface is in focus (Fig. 5). The difference in fine adjustment settings is compared with a calibration curve and the thickness obtained. Sample standard deviation of the measurement was approx. 10–12 μm. The variation includes any irregularities in the biofilm surface. The accuracy of the method may be influenced by the refractive index of the wet biofilm.

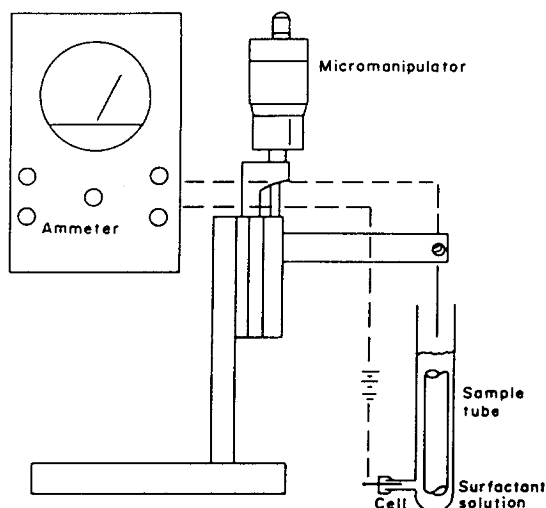

Fig. 4. Apparatus for measuring wet biofilm volume (Zelver, 1979).

Norrman *et al.* (1977) located the biofilm–fluid and biofilm–inert surface interfaces by means of electrical conductance using a technique adapted from Hoehn & Ray (1973) which utilizes an apparatus consiting of a steel needle mounted on a micromanipulator. Figure 6 shows details of the test section in which biofilm is grown and thickness measured. The test section consists of 6 measurement points, each one being a stainless-steel rod (3 mm o.d.) mounted flush with the inside tube wall. Opposite each rod is a threaded hole which can be sealed with a screw and O-ring. The thickness of the biofilm on the stainless-steel rod surface is measured using the electrical conducting probe depicted in Fig. 7. The probe and one of the stainless-steel rods are connected to an electrical circuit completed by an electrometer. To obtain a measurement, the test section is removed from the reactor, the screws are withdrawn and the test section drained for two minutes. The probe is then lowered into the test section through the threaded hole until contact is made with the biofilm surface: a current (approx. 10^{-8} A) is registered and the depth noted on the micromanipulator (Fig. 7A). Next, the probe is lowered until contact is made with the stainless-steel rod surface, a greater current flow is registered (approx. 3×10^{-5} A), and the depth is again noted (Fig. 7B). The difference in depth is the biofilm thickness. The procedure is repeated at the other 5 locations. Precision is about $\pm 6\%$ and accuracy, compared to Vernier micrometer measurements, is within 5%.

Biofilm mass. Glass sample tubes (tubular reactors) or acrylic slides (annular reactor) containing biofilm are dried to constant weight at an elevated temperature (≤ 105°C). The surface is then cleaned, dried and weighed again. The difference in the two measurements is the dry biofilm mass. The surface area available for growth is generally known so an areal mass density can be determined. If biofilm thickness or biofilm volume has been measured, volumetric biofilm density (ρ_{Th}) can be determined. The volumetric biofilm density has units of dry mass per wet volume. The areal film density has units of dry mass per unit area of growth surface.

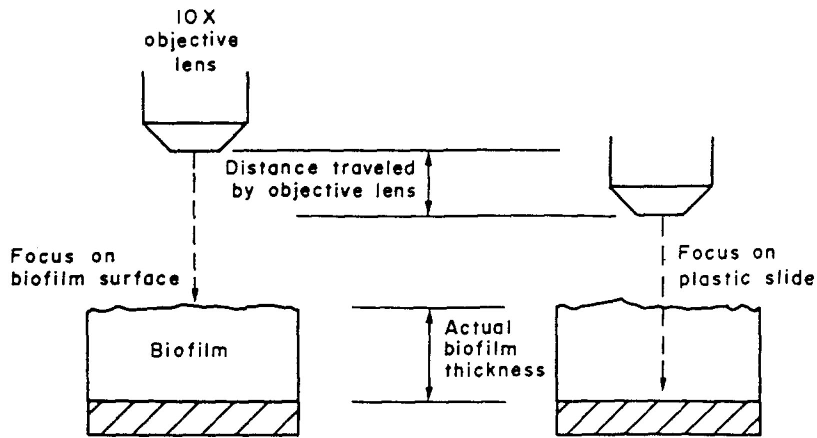

Fig. 5. Method for measuring wet biofilm thickness with an optical microscope (Trulear, 1980).

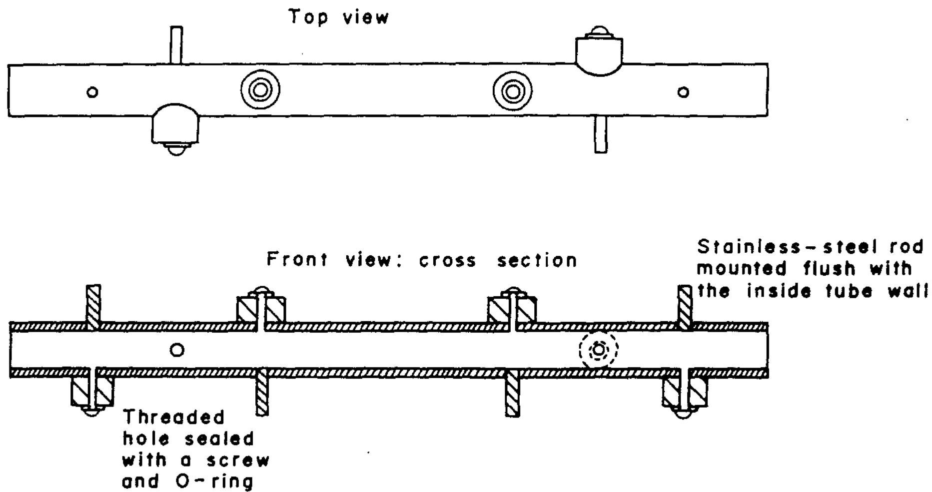

Fig. 6. Tubular test section used in measurement of wet biofilm thickness by electrical conductance (Norrman *et al.*, 1977).

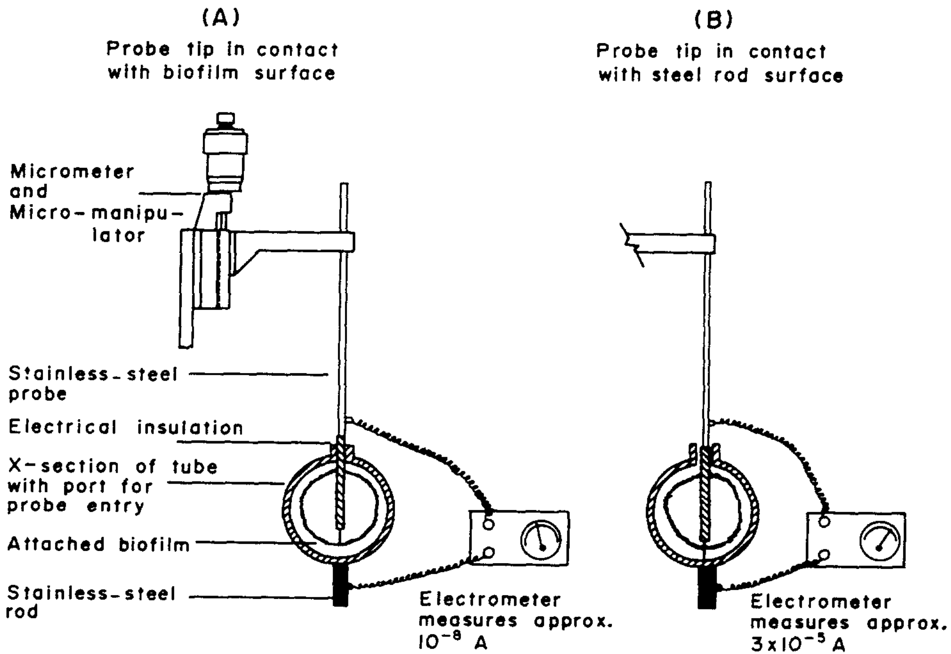

Fig. 7. Apparatus for measuring wet biofilm thickness by electrical conductance (Norrman *et al.*, 1977).

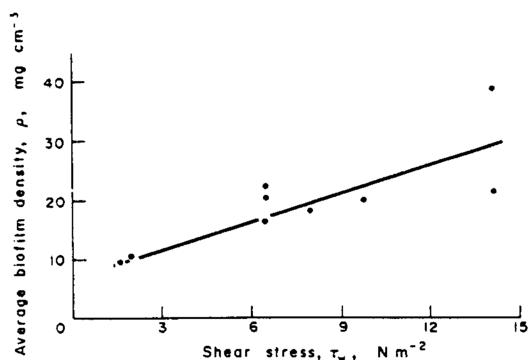

Fig. 8. Influence of fluid shear stress on biofilm density (Zelver, 1979).

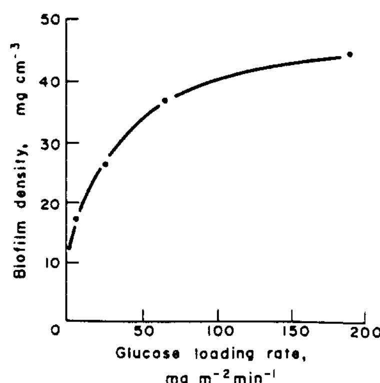

Fig. 9. Influence of glucose loading rate on biofilm density (Trulear, 1980).

Laboratory experiments indicate no significant change in biofilm density *during* a given experiment. However, biofilm density increases with increasing fluid shear stress and nutrient loading *between* experiments as indicated in Figs 8 and 9. Biofilm thickness and biofilm mass must be determined simultaneously in a given experimental system so a consistent relationship between the two can be determined.

Indirect measurement of biofilm quantity: specific biofilm constituent

Biofilm quantity can be determined indirectly by measuring a biofilm constituent indicative of the total biofilm amount. Various biofilm constituents have been used to monitor biofilm development as indicated by Table 3. A calibration between the specific constituent and biofilm mass is necessary to apply material balance concepts to the biofilm system unless some conservative quantity (e.g. carbon) is used for measuring for biomass, substrate and product. Consequently, mass should be measured if possible. Note, however, that mass measurements may not be as sensitive as some analytical techniques. Table 4 presents the limits to detection and standard error for various analyses used in our laboratory to determine biofilm quantity.

Two chemical detection methods have been used to indirectly measure initial biofilm formation in our research:

1. Total attached polysaccharide.
2. Biofilm organic carbon or chemical oxygen demand.

Total attached polysaccharide. Biofilms contain relatively large quantities of polysaccharide (Jones *et al.*, 1969; Costerton *et al.*, 1978). One quantitative method for polysaccharide concentration is based on a reaction of the carbohydrate "reducing" end—either a ketone or an aldehyde. A strong non-oxidizing acid will react with the reducing end to yield hydroxymethyl furfural plus other by-products. Condensation between these activated aldehydes and phenolic compounds (e.g. resorcinol, napthol, anthrone, or phenol) results in formation of colored compounds proportional in intensity to the polysaccharide concentration.

The phenol-H_2SO_4 method of Dubois *et al.* (1956) has been used for indirectly monitoring biofilm, during the early stages of development. Figure 10 demonstrates the increase in attached polysaccharide using the tubular reactor (Bryers, 1980).

Biofilm organic carbon or chemical oxygen demand. Indirect quantification of biofilm can be accomplished by measuring the oxidizable organic material (organic carbon) attached. Bryers (1980) reports good sensitivity (6 μg COD cm^{-2}) and precision ($\pm$0.10 μg COD cm^{-2}) with a modified chemical oxygen demand (COD) analysis in the tubular reactor system. Modifications in the *Standard Methods* (APHA, 1976) COD procedure consisted of diluting the dichromate oxidant and ferrous ammonium sulfate titrant.

Trulear (1981) has indirectly monitored accumulation in the annular reactor using total organic carbon (TOC). Sensitivity and precision of the method are less than 2.0 and $\pm$0.45 μg TOC cm^{-2}, respectively.

Indirect measurement of biofilm quantity: microbial activity within the biofilm

Table 5 enumerates several analytical methods—current or proposed—for the indirect monitoring of biofilm using metabolic activity as an indicator.

Table 4. Sensitivity and precision of various biofilm accumulation measurement techniques

Method	Sensitivity: Measured quantity	Sensitivity: Equivalent COD*	Precision (±): Measured quantity	Precision (±): Equivalent COD*	References
Biofilm thickness	9 μm	25.6 mg cm^{-2}	9 μm	26.6 mg cm^{-2}	Zelver (1979)
	10	28.5	10	28.5	LaMotta (1974)
	10	28.5	9	25.6	Trulear (1980)
Biofilm mass	0.11 mg cm^{-2}	0.13 mg cm^{-2}	0.01 mg cm^{-2}	0.01 mg cm^{-2}	Zelver (1979)
Biofilm COD	0.006 mg cm^{-2}	—	0.0001 mg cm^{-2}	—	Bryers (1980) Bryers & Characklis (1980)
Biofilm TOC	0.002 mg cm^{-2}	0.0057	0.00045 mg cm^{-2}	0.00128 mg cm^{-2}	Trulear (1981)

*Calculated from biofilm thickness values assuming biofilm density = 25 mg cm^{-3}, COD = 1.14 mg COD mg^{-1} and biofilm carbon content = 0.4 mg carbon per mg biofilm (Trulear, 1980; Bryers, 1980; Trulear, 1981).

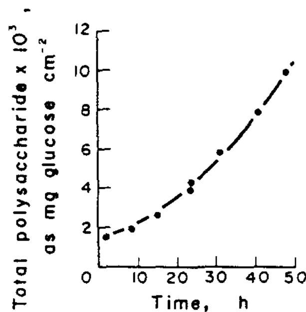

Fig. 10. Increase in biofilm polysaccharide content during a typical experiment in a tubular reactor (Bryers, 1980).

Direct enumeration. Corpe (1973) measured biofilm development on glass slides immersed in seawater using *total cell count* and *viable cell count* attached per unit area. Results indicated an increasing total count attached while viable cells fluctuated. Total biofilm quantity (i.e. mass or thickness) was not determined.

Gerchakov *et al.* (1977) used *viable cell counts* to monitor biofilm development on a variety of metal surfaces exposed to tropical seawater. Results indicate an exponential increase in viable cell counts with exposure time for all material. Stainless-steel showed the highest rate of development followed by brass, glass and copper–nickel. No direct measure of biofilm quantity was made.

Epifluorescence techniques for *total cell counts* involve the disruption of large aggregates of attached biofilm, recovery of bacterial cells by filtration, staining cells with acridine orange (a fluorescent stain) and direct counting by epifluorescence microscopy (Zimmerman & Meyer-Riel, 1974). Trulear (1981), using direct counts by epifluorescence microscopy, has counted up to 5.6×10^7 cells cm^{-2} in the annular reactor. Geesey *et al.* (1978), using direct counts by epifluorescence, demonstrated the importance of attached bacteria in alpine streams. Costerton (1980) reports rapid bacterial attachment (1.3×10^7 cells cm^{-2} day^{-1}) to a heat exchanger surface as determined by epifluorescence measurements.

Cellular components. Measurement of the adenosine triphosphate (ATP) content of biofilms has been the most widely used chemical measure of biofilm activity. ATP exists only in living organisms with no residual ATP detectable after cell death (Hamilton *et al.*, 1967). Activity appears limited to the upper layers of the biofilm. La Motta (1974) observed an increase in ATP content with biofilm thickness up to approx. 320 μm in an annular reactor. However, the ATP content remained constant as biofilm thickness measured beyond 320 μm suggesting an "active" layer of biofilm.

This may also suggest some analytical limitation in the ATP extraction technique. Attached ATP per unit area has been measured by Little & Lavoie (1979) and Bobbie *et al.* (1979) in two separate field heat exchange units.

Lipopolysaccharides (LPS) are unique to gram negative bacterial cell walls. Dexter and co-workers, (1975, 1976) reports that LPS attached is proportional to total attached cells as determined by scanning electron microscopy, however the precision is poor. LPS from Gram negative bacteria is not confined to cell walls but may "leach" into surrounding fluids (Janda & Work, 1971). Costerton (1980) suggests that the presence of biofilms can be detected by measuring the net increase in soluble LPS across a power plant condenser. However, the method is costly and yields inconsistent results.

Several other methods, previously used in quantifying free floating bacterial populations, have been proposed to indirectly detect biofilm development. Mason *et al.* (1978) report an accurate, practical, and rapid method for the estimation of immobilized whole cell biomass based on the chemiluminescent oxidation of luminol in the presence of cellular haematin catalysts. Sensitivity of less than 1 mg biomass was reported.

Table 5. Measures of microbial activity within the biofilm

Direct enumeration	Total and viable cell count Total cell count by epifluorescence microscopy
Cellular component chemical analysis	Adenosine triphosphate (ATP) Lipopolysaccharide (LPS) Total proteins Alkaline phosphates HAEM catalyzed luminescence Muramic acids Poly-β-hydroxybutyrate
Substrate removal	Direct measurement Heterotrophic potential

White *et al.* (1979) proposed several measures of biomass activity: (1) alkaline phosphatase, a bacterial cell wall component; (2) muramic acid, an unique procaryotic storage material; (3) poly-β-hydroxybutyrate; and (4) phospholipids. At this time, there is little data available for determining accuracy or precision of these methods when applied to biofilms.

Substrate removal rate. Microbial activity within the biofilm can be determined by measuring the removal rate of a particular substrate, nutrient or electron acceptor. Using the annular reactor, Trulear & Characklis (1982) observed an increase in glucose removal rate with increasing biofilm accumulation up to some critical biofilm thickness termed the *active thickness*. Similar observations have been reported by many others (Atkinson & Daoud, 1970; Harremoes, 1977; LaMotta, 1976; Kornegay & Andrews, 1967). The active thickness is the effective depth of penetration of substrate, nutrient or electron acceptor before it is exhausted.

Heterotrophic potential is a similar test developed to measure microbial activity in natural waters (Costerton & Colwell, 1979). The test essentially measures glutamic acid removal due to microbial activity in a sample.

For biofilm samples, results can be reported as substrate mass removed per unit biofilm area, per unit biofilm volume, or per unit biofilm mass. The three quantities are related through the biofilm thickness and biofilm density.

Indirect measurement of biofilm quantity: effects on biofilm transport properties

The development of a biofilm in a flowing fluid system affects fluid frictional resistance as described by Picologlou *et al.* (1980), and Zelver (1979) in the tubular reactor and Trulear (1980) in the annular reactor. Biofilm accumulation also influences heat transfer resistance as described by Characklis *et al.* (1981) and Nimmons (1979) in the tubular reactor. The use of these measurements for monitoring biofilm development will be discussed in detail in a future paper.

SUMMARY

Methods available for monitoring the progress of biofilm processes can be conveniently classified as follows:

Direct measurement of biofilm quantity.

Indirect measurement of biofilm quantity: specific biofilm constituent.

Indirect measurement of biofilm quantity: microbial activity within the biofilm.

Indirect measurement of biofilm quantity: effects on biofilm transport properties.

This paper has discussed methods for accomplishing measurements in the first three classifications.

Direct measurement of biofilm quantity is essential in many instances including the following:

(1) Calibration of indirect measurement techniques for determining biofilm quantity.

(2) Use of mass conservation equations for determining process rates and stoichiometry.

(3) Relating biofilm development to fluid frictional resistance and heat transfer resistance in a rational manner.

Indirect measurement of biofilm quantity using a specific biofilm constituent has certain advantages including the following:

(1) Some of the indirect methods are more sensitive.

(2) Indirect measures provide information regarding biofilm composition and, hence, stoichiometry of biofilm processes.

Indirect measurement of biofilm quantity through microbial activity within the biofilm is useful in certain instances including the following:

(1) Determining the physiological state of the organisms in the biofilm.

(2) Some measurements of biofilm activity are extremely sensitive.

(3) Substrate removal rates are necessary when employing mass conservation equations.

Activity measures are very useful in biofilm studies relating to natural waters and wastewater treatment systems employing biofilm reactor systems (e.g. trickling filters or rotating biological contactors). In these instances, the activity of the biofilm in terms of substrate removal is most critical.

Measures of biofilm quantity, however, are critical when fouling processes are being considered. For example, biocides used for ameliorating the detrimental effects of biofilms on fluid frictional resistance and heat transfer resistance must *remove* attached biomass to be effective. Merely "killing" or inactivating the microbial cells within the biofilm will not be sufficient since the "hydraulic roughness" and thermal conductive resistance caused by the biofilm will be unaffected. Consequently, measures of activity are not very useful for such a task.

Biofilm quantity and biofilm activity may not necessarily be directly related. As the biofilm develops, diffusion and reaction within the biofilm will ultimately deplete oxygen or some essential nutrient in the lower layers. Any further increase in biofilm quantity may not increase biofilm activity, i.e. an *active thickness* is reached. This concept has been discussed by others in detail.

The choice of analytical method for monitoring biofilm processes will be dependent on the questions which prompt the investigation.

Acknowledgements—Much of the work described was accomplished at Rice University, Houston, TX. The authors gratefully acknowledge partial financial support from the National Science Foundation (ENG 77-26934), Electric Power Research Institute (RP 902-1), Office of Naval Research (N00014-80-C-0475), Calgon Corporation and Amoco Foundation. Ms Sharlene Vehnekamp prepared the manuscript.

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