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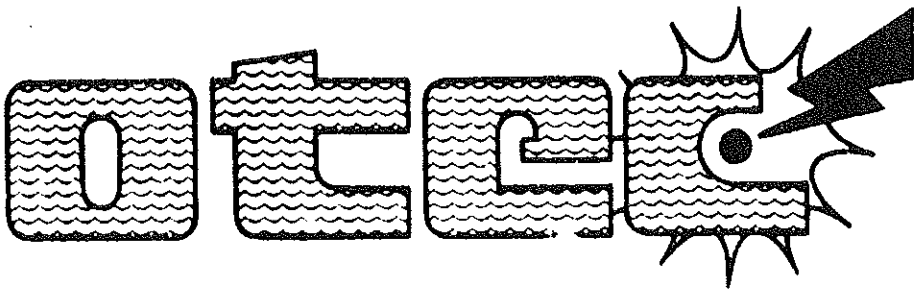
Proceedings of the OceanThermal Energy Conversion (OTEC) Biofouling and Corrosion Symposium



Robert H. Gray, Editor

October 10-12, 1977
Seattle, Washington

August, 1978

Sponsored by:
The United States Department of Energy and
Pacific Northwest Laboratory

MEASUREMENT OF THE FORMATION AND DESTRUCTION OF PRIMARY BIOFOULING FILMS

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ABSTRACT

Microbial fouling causes increased frictional resistance in pipelines, reduces heat exchanger performance and is thus responsible for large energy losses. Biofouling control to minimize these losses is generally by chlorine addition. However, this approach creates potential toxicity problems in receiving waters and a better understanding of fouling film development and destruction is needed to maintain satisfactory effluent water quality.

Several methods for direct measurement of biofilm development are presented. Indirect measurements of film development that indicate deleterious effects of fouling are introduced. Laboratory systems for determination of rates and extent of fouling as a function of wall shear stress, water quality and bulk and surface temperature are outlined. As these methods and laboratory systems have been developed for the study of primary biofilms they are directly applicable to investigations of primary biofouling development and control in OTEC.

Results indicate that substrate concentration significantly affects biofilm growth rate. However, maximum accumulation is controlled primarily by hydrodynamic wall shear stress in turbulent flow. Chlorine oxidizes biopolymers responsible for film structure and causes partial removal with aid of hydrodynamic wall shear forces. If removal is not complete, regrowth is more rapid than that observed on clean surfaces.

INTRODUCTION

Fouling or deposition of materials on surfaces is a major cause of energy loss in fluid transport and heat exchange systems. The term fouling includes precipitation or crystallization (scaling), deposition of suspended solids, corrosion, and/or biological (organic) growth. This paper concerns microbial fouling and experimental methods for observing its development, effects and destruction.

Microbial fouling consists of development of thin microbial viscoelastic biofilms and may be followed by a succession of higher life forms. The presence of films on the conduit walls of water supply lines causes large increases in fluid frictional resistance.

Characklis (1973a, 1973b) and Norrman (1976) have reviewed the literature concerning the effect of biofilm growth on frictional resistance.

Films also develop in heat exchanger tubes increasing thermal resistance and reducing efficiency (Ritter and Suitor, 1976). Fouling is primarily due to microbial growths and their extracellular polymers which accelerate absorption of fine suspended particles to the heated surfaces.

Films also accelerate corrosion processes in the metal tubes and influence deterioration in wooden cooling towers. Microbial fouling is a major barrier to economic utilization of Ocean Thermal Energy Conversion (OTEC) and membrane desalination processes.

Control of biofouling is frequently by chlorine addition. Application frequency and dose are determined arbitrarily because methods for determining the effectiveness of chlorine are unavailable. Economic considerations, energy conservation demands and increasingly stringent regulations on potentially toxic chlorine residuals and their reaction products require a systematic understanding of factors influencing microbial fouling and its control. Such understanding is particularly important in OTEC since the feasibility of the entire project hinges critically on the ability to monitor and control extremely thin microbial films.

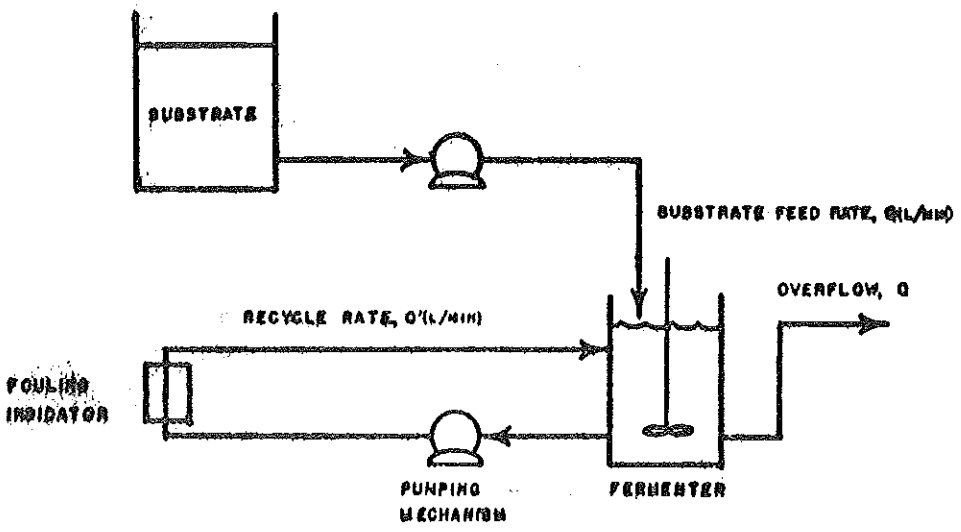
EXPERIMENTAL SYSTEMS

Rationale for Design

The objective of our experimental program is to study development, effects and destruction of biofouling films. Factors that affect attachment, growth and ultimate deposition (Table 1) must be controlled or easily determined. Figure 1 represents the salient features of our system. Advantages of the system are as follows:

TABLE 1. Factors Affecting the Biofouling Process

	Initial Attachment	Growth Rate	Ultimate Deposit
Shear Stress	•	•	•
Substrate Type		•	
Substrate Concentration		•	
Ionic Ion Concentration	•	•	•
Solid Surface	•		
Bulk Temperature	•	•	•
Surface Temperature	•	•	•
Suspended Solids	•	•	•



NOTE: Q'>>Q AND V/Q IS SMALL

FIGURE 1. Schematic of Laboratory Fouling Reactor Systems

- Since the recycle volumetric flow rate Q' is much larger than the substrate feed volumetric flow rate Q, the reactor contents are completely mixed and there are no concentration gradients. Consequently, mathematical description and sampling are simplified, temperature and pH are easily controlled, dissolved oxygen is easily monitored with one probe and biofilms in the recycle section are uniform.

- Retention time is made sufficiently short so that biomass production in the bulk fluid is eliminated. Thus, microbial activity is limited to reactor surfaces.

- Wall shear stress is independent of hydraulic retention time.

Description of Experimental Systems

Two different geometries have been used to serve as fouling indicators. A tubular fouling reactor (TFR) simulates the most common geometry encountered in practice. Additionally, turbulent flow characteristics in the system are well understood and documented. The reactor system is shown in Figures 2 and 3. Flow rate is kept constant by an electronic feedback system that controls the positive displacement screw pump. Pressure drop (Δp) along the tubular test section and temperature differential along the inner tube of a counter-current heat exchanger are the mechanical and

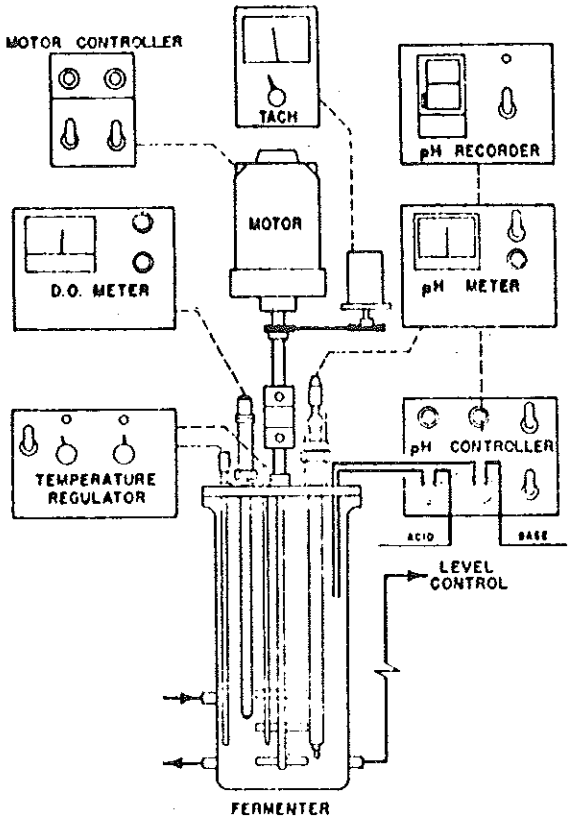


FIGURE 3. Controls and Monitors for Tubular Fouling Reactor Experimental System

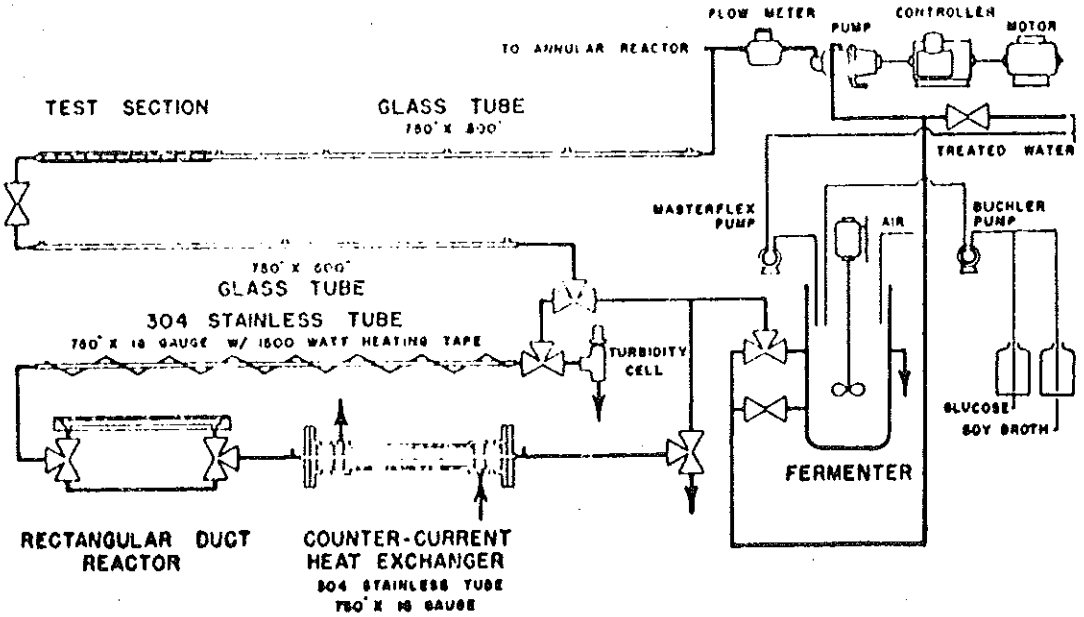


FIGURE 2. Experimental System for Evaluating Effect of Velocity, Water Quality, Bulk Temperature and Surface Temperature of Biofouling Rate in a Tubular Fouling Reactor (TFR). Also Used for Determining Kinetics of Fouling Control via Chemical Treatment (e.g., chlorine, ozone, etc.).

thermal measures of fouling, respectively Overall hydraulic retention time is maintained at about 10 min. An in-line rectangular duct section contains removable metal discs which serve as platens in a rheogoniometer. Thus, dynamic rheological properties of in situ biofilms can be determined.

An annular fouling reactor (AFR) consists of two concentric cylinders (Figure 4) with the inner cylinder rotating. Shear stress, which depends on the speed of rotation, is independent of hydraulic retention time. Inside, the reactor is completely mixed by virtue of the pumping action of four draft tubes and an impeller at the bottom of the inner cylinder (Kornegay and Andrews, 1968). Fouling is indicated by a change in the torque required to maintain constant rpm.

MEASUREMENT TECHNIQUES FOR BIOFOULING FILMS

Measurement techniques can be divided in direct determinations of film thickness and indirect detection of film through measurement of factors sensitive to presence of film.

Direct Measures

Volumetric

The volumetric method consists of measuring the displacement of a water-surfactant solution surface due to firm accumulation in a 5 cm x 1.27 cm (I.D.) test section. The difference between displacement by the fouled test section and displacement by the same test section after cleaning and drying is the film volume. Dividing film volume by the surface of the test section (20.0 cm^2 within ± 0.6%) gives wet thickness.

Areal Mass Density

The above test sections are integral parts of the tubular reactor wall. At regular intervals, a test section is removed, dried and weighed, then cleaned, dried and weighed again. The difference is the dry film weight. Dividing by the surface of the test section yields areal mass density. Dividing by the film volume yields wet mass density.

Microscopic

The microscipic technique was adapted from Sanders (1964) and requires film growth on a transparent surface. In our experiments, microbial film develops on a thin acrylic plastic slide which is an integral part of the AFR reactor wall. The slide is withdrawn from the reactor and placed on a microscope stage. The 43x objective is lowered until the film surface is in focus and the fine adjustment setting is recorded. The objective is lowered further until the plastic surface is in focus. The difference in fine adjustment settings is compared to a calibration curve and the thickness obtained.

Light Transmittance

Determination of film accumulation by light transmittance is similar to comparison spectrophotometry. Selenium photoelectric cells on opposite sides of a rectangular duct section monitor incident and transmitted light intensities normal to the direction of flow. Percent reduction in light transmitted (%RLT) is proportional to film accumulation. A valving arrangement allows flushing with clean water to eliminate turbidity effects. Measurements are consistent and repeatable. Independent determinations of film thickness are needed to provide calibration curves for this method. Once this is accomplished the method can be used for in situ non-destructive measurement of film thickness development.

Conductivity

The conductivity technique was adapted from Hoehn and Ray (1973) and utilizes an apparatus consisting of a steel needle mounted on a micromanipulator. Film thickness is measured in a removable circular test section made of acrylic plastic tubing. The test section has measurement points distributed over a length of 15 cm (two at the top, two at the bottom, and one on each side). Each measurement point consists of a stainless steel rod (3 mm dia) mounted flush with the inside tube wall. Opposite each rod is a threaded hole in the tube wall which can be sealed with a screw and O-ring. Biofilm thickness is measured on the stainless steel surface.

The test section is removed from the reactor, the screws are withdrawn and the section is drained for 2 min. The steel needle and stainless steel rod at one measurement point are connected to an electrical circuit with an electrometer. The steel needle is lowered into the section through the threaded hole. When the needle contacts a biofilm, current (approximately 10^{-8} amp) is registered and depth noted. The needle is lowered further until it contacts the steel surface, further deflection (approximately 3×10^{-5} amp.) occurs and depth is noted. The difference in depths is the film thickness. Precision is about ± 6% and accuracy, compared with Vernier micrometer measurements on thin metal foil, is within 5%.

Damped Oscillation

The damped oscillation method exploits the viscous properties of biofilms. The apparatus consists of a thin wire, with attached ferromagnetic material, suspended from a ceramic phonograph cartridge through a solenoid coil. An alternating current applied to the solenoid causes the wire to vibrate through an extremely small amplitude, monitored with an oscilloscope. The assembly is lowered by micromanipulator until the wire contacts the film and damping of the amplitude occurs. Depth is recorded and the wire is lowered further until it contacts the solid surface where complete damping occurs. The difference in depths is the film thickness. Response in agar films (0.25-5.0% by weight) was good. Although evident, response has not been consistent in biofilms. Potential advantages of this in situ measurement have stimulated continuing research and development of the method.

Indirect Measures

Frictional Resistance

Biofilms on wetted surfaces increase fluid frictional resistance. Thus, friction factor increase is a good indicator of microbial fouling and biofilm development. In the TFR, friction factor f is defined as:

$$f = \frac{2D}{L} \frac{\Delta p}{\rho v_m^2} \qquad (1)$$

where D = tube diameter (L)
L = tube length (L)
ρ = fluid density (M/L^3)
v_m = mean fluid velocity (L/t)
Δp = pressure drop across length L ($M/L\text{-}t^2$)

Pressure drop is measured with a pressure transducer (Validyne DP-7), valved to 6 ports in the TFR system. The transducer is monitored by a modulator-demodulator (Validyne CD-101) and strip chart recorder and operates on a 0-5 psig range with 0-1 psig option. A mercury manometer is used for calibration, zeroing and backup.

In the AFR, friction factor is defined as:

$$f = \frac{T_q}{\rho \cdot R_i^3 (R_i + R_o) \Omega^2 H} \qquad (2)$$

where R_i = radius of inner cylinder (L)
R_o = radius of outer cylinder (L)
Ω = rotational velocity (t^{-1})
H = height of cylinders (L)
Tq = torque (M^2L/t^2)

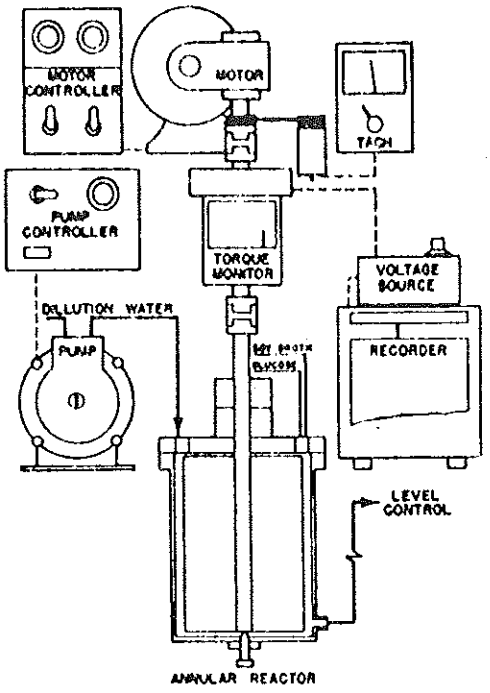


FIGURE 4. Experimental System for Evaluating Effect of Velocity, Water Quality and Bulk Temperature on Biofouling Rate in an Annular Fouling Reactor. Also used for determining kinetics of fouling control via chemical treatment (e.g., chlorine, ozone, etc.).

Torque is measured by a torque transducer (General Thermodynamics, Inc.) mounted to the shaft which drives t e inner cylinder (Figure 4). Torque is monitored with a strip chart recorder and has a range of 0-360 cm-g.

Heat Transfer Resistance

The growth of biological films on wetted surfaces usually increases heat transfer resistance in a counter current flow tube and shell heat exchanger. A fouling factor may be determined using:

$$\frac{1}{U_f} = \frac{1}{U_d} - \frac{1}{U_{cl}} \qquad (3)$$

where U_f = overall heat transfer coefficient fouling factor (M/t^3T)
U_d = overall heat transfer coefficient of fouled tube (M/t^3T)
U_{cl} = overall heat transfer coefficient of clean tube (M/t^3T)

The overall heat transfer coefficient U, provided it remains constant along the entire length of the tube is given by:

$$U = \frac{Q}{\Delta T_m \cdot A} \qquad (4)$$

where Q = heat transfer rate ($M L^2/t^3$)
A = heat transfer area (L^2)
ΔT_m = log-mean temperature difference (T)

Overall heat transfer coefficient under clean and fouled conditions is computed from equation (4) after determining Q and ΔT_m. Inlet and outlet temperatures of heat exchanging fluids are measured by thermistors (YSI-406) and volumetric flow-rates are measured by a water meter (Carlon) and rotometer (Dwyer).

RESULTS

Properties of Biofilm

Measurements of biofilm thickness, density and water content are interrelated and are "system specific." For example, thickness is generally measured when the film is wet, but solids content is determined by drying at a specified temperature and time. Thus, dry mass per unit wet volume are the units of film density. Comparisons are difficult because measurement of film thickness and drying procedures vary among investigators. Growth conditions, including substrate and its concentration, temperature and shear stress at the growth surface also affect physical properties of films.

Table 2 shows physical, chemical and biological properties of films grown under laminar and turbulent conditions. Biological properties of films are limited. Viable cell concentrations in films were remarkably consistent in our laboratory system. Organisms listed were those most frequently observed in attached films in natural, cooling and waste waters.

Chemical properties of films were reviewed by Characklis (1973a, 1973b). Films developed in our laboratories in a 1:1 weight ratio of glucose and trypticase soy broth were analyzed for carbon, nitrogen and phosphorous (Table 3).

The inorganic composition of films varies considerably and, undoubtedly, affects film properties. Based on bioflocculation and filtration literature calcium, magnesium and iron are implicated in film bonding strength. Aluminum, silica and aluminum silicates, could be important in stabilizing film structure.

TABLE 2. Some Properties of Biofilms

	Flow under which film was grown	
PHYSICAL	Laminar	Turbulent
Maximum thickness (μm)[a]	1300	300
Volumetric density (mg/cm³)	30-100	5-50
Areal density (mg/cm²)	0.5-13.0	0.05-1.5
Solids content (%)	0.5-5.0	0.5-5.0
Rheological character	viscoelastic	viscoelastic
Sloughing characteristics	intermittent	continuous
BIOLOGICAL		
Bacterial cell density in our laboratory system (cells/cm³)		10^9-10^{10} (grown under turbulent flow conditions)
Organisms frequently observed in natural, cooling and waste water		Sphaerotilus Crenothrix Gallionella Pseudomonas Flavobacteria Achromobacter Aerobacter

CHEMICAL (Data obtained from case histories of closed conduits experiencing excessive slime frictional losses)

Water (%)	85.6 - 95.4
Volatile solids (%)	1.9 - 3.2
Fixed solids (%)	1.4 - 11.7
Si as % of fixed solids	7.0 - 12.5
Fe as % of fixed solids	1.4 - 18.5
Al as % of fixed solids	3.9 - 7.5
Ca as % of fixed solids	1.0 - 5.6
Mg as % of fixed solids	2.5 - 3.2
Mn as % of fixed solids	4.9 - 59.5

TABLE 3. Biofilm and Media Concentration of Organic Carbon, Kjeldahl Nitrogen and Total Phosphorus

	Biofilm	Media	Biofilm[a]
Organic carbon (%)	19.0	47.0	6.4 - 13.8
Kjeldahl nitrogen (%)	9.2	7.73	0.51 - 3.0
Total phosphorus (%)	1.8	9.24	

a) Anderson et al., 1975

DELETERIOUS EFFECTS OF BIOFOULING

The presence of microbial films causes undesirable increases in resistance to fluid flow and heat transfer.

Fluid Frictional Resistance

The increase in frictional resistance from development of thin, microbial films is larger than expected due to the decrease in available cross-sectional area. Exceedingly high energy losses have been documented (Characklis, 1973a, 1973b). Explanation for the pronounced increase in resistance may be found in the viscoelastic nature of biofilms and/or their morphology. Film viscoelasticity may allow resonating vibration of the film causing losses within the biofilm (Schuster, 1971) and irregular film surface morphology may give rise to increased roughness.

Norrman et al. (1977) investigated macroscopic effects of biofouling in 1.27 cm I.D. glass circular tubes. Figure 5 shows biofilm development has little effect on frictional resistance, as indicated by pressure drop, until some critical thickness is attained. Then, Δp increases rapidly with increasing thickness. For v_m = 150 cm/sec (Q = 11.4 l/min) and v_m = 100 cm/sec (Q = 7.6 l/min), the critical thickness is between 50-100 μm. In turbulent flow past a rigid rough surface, the rough surface acts as a smooth surface unless the roughness elements extend through the viscous sublayer. In Norrman's system, the viscous sublayer was between 45 and 70 μm thick which corresponded with the onset of experimentally determined high energy losses. This suggests the increase in roughness is responsible for the increased pressure drop. Further experiments to corroborate this effect are underway.

Experiments where substrate feed concentrations ranged from 5-500 mg/l (temperature 28°-30°C) indicated the extent of microbial fouling depended strongly on wall shear stress and not substrate concentration, if concentration exceeded some minimum value. However, both shear stress and feed substrate concentration (C_{so}) affect the rate of microbial fouling (Figure 6). Data indicated the effect of C_{so} on biofouling rates, based on change of friction factor, could be described by saturation kinetics. Experiments lasted 1 to 14 days until a logarithmic development rate and plateau were observed. For wall shear stress of τ_o = 32.6 g/cm-sec^2 growth rate as a function of C_{so} is described by the equation:

$$R_f = \frac{R_f^* C_{so}}{R_s + C_{so}} = \frac{0.86\ C_{so}}{30 + C_{so}}$$

where R_f = fouling rate (days^{-1})

R_f^* = maximum fouling rate (days^{-1})

R_s = saturation constant (mg/l)

C_{so} = feed substrate concentration (mg/l)

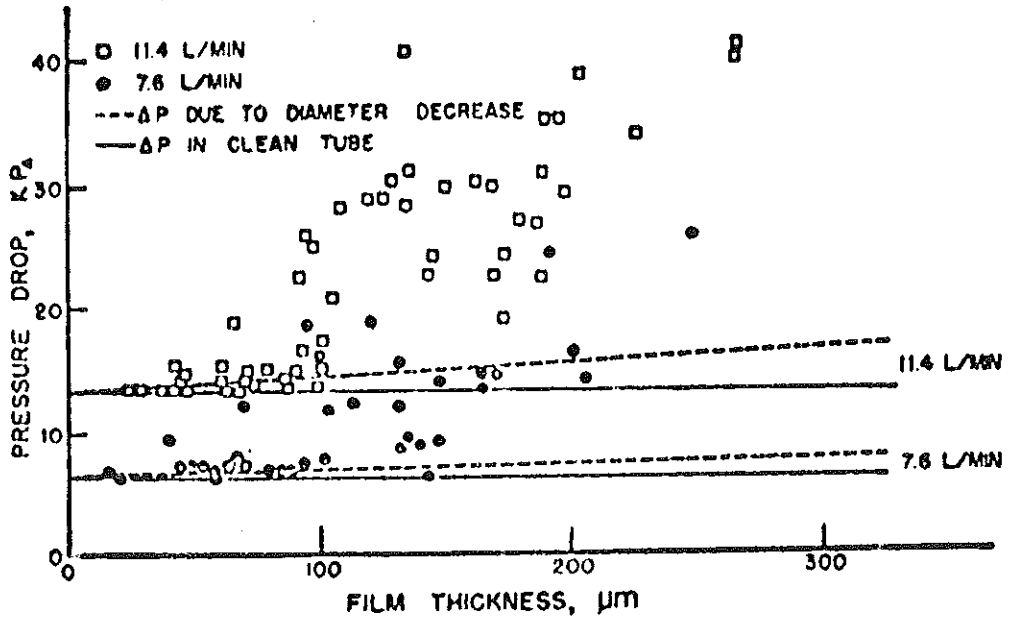


FIGURE 5. Effect of Film Thickness on Pressure Drop in a 1.27 cm Circular Tube

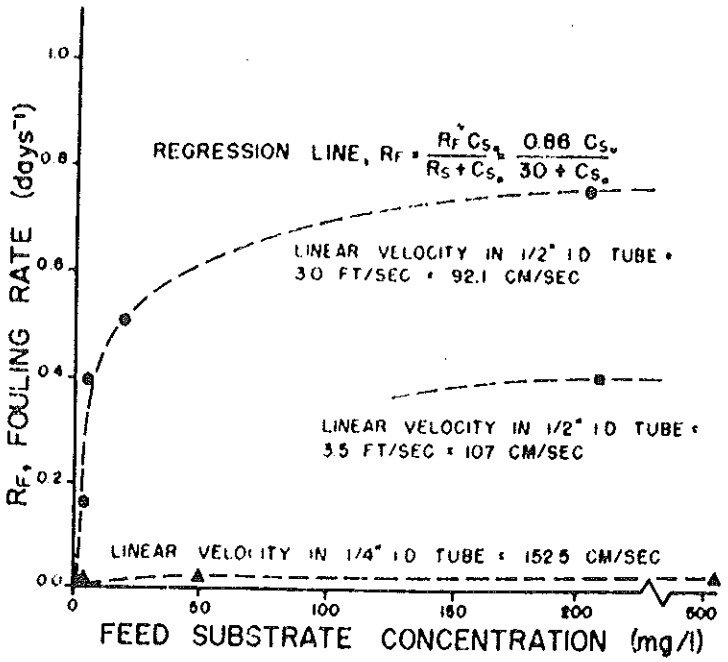


FIGURE 6. Biofouling Rate as a Function of Feed Substrate Concentration and Fluid Velocity in Tubular Reactors (at 30°C)

For these preliminary data, R_f^* is affected by τ_o and good correlation is obtained with the expression:

$$R_f^* = a \exp b \cdot \tau_o = 9.0 \exp (-0.07 \cdot \tau_o)$$

Fluid flow affects growth and development of microbial films by transporting nutrients to the film surface and shearing off portions of the film. Thus, R_f^*, as a function of wall shear stress, should have a maximum. The last equation does not exhibit a maximum and its validity should be limited to cases where flow conditions are such that sufficient nutrients are transported to the film surface.

Heat Transfer Resistance

Large volumes of water are used in heat exchangers and, unless treated, result in formation of deposits of low thermal conductivity which reduce heat transfer and flow capacity. Since biofilms are at least 95% water, thermal conductivity of films is almost identical to that of water (Purkiss, 1972). Therefore, the primary effect of biofilms is to increase the stagnant liquid layer next to the tube wall. Experiments are being conducted to evaluate changes in heat exchanger performance with film growth.

Anderson et al. (1975) indicated that the upper temperature for growth and division or reproduction of cells from condenser biofilm was about 60°C. However, the experiments were conducted in the laboratory. Biofilm in the TFR accumulated preferentially on heated surfaces and "crusty" material has been removed from immersion heater surfaces with viable counts exceeding $10^6/cm^3$ of deposit. Experimentation to quantitatively establish the effect of surface temperature on microbial growth rate and ultimate deposit is underway.

CONTROL OF BIOFOULING WITH CHLORINE

Chlorination of power plant intake waters is the predominant means of controlling or preventing biofouling. Recently, concern over toxicity of chlorine residuals in natural waters led to investigations into the mechanism of control. Characklis and Dydek (1976) showed chlorine functioned primarily as a chemical oxidizer and disrupted microbial films by hydrolysis of exocellular polymers, especially polysaccharides. Polymers lend structural strength to the deposit. Chlorine, combined with fluid shear stress, caused detachment and partial solubilization of the film. Mercuric chloride (500 mg/l) had no physical effect on films although it completely inactivated cells. In the same

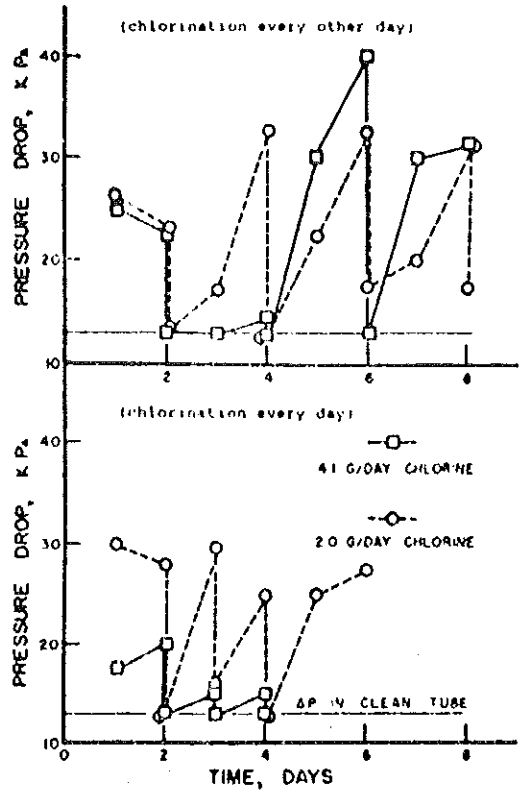


FIGURE 7. Shock Chlorination Effect on Frictional Resistance. Flow Rate = 11.4 l/min

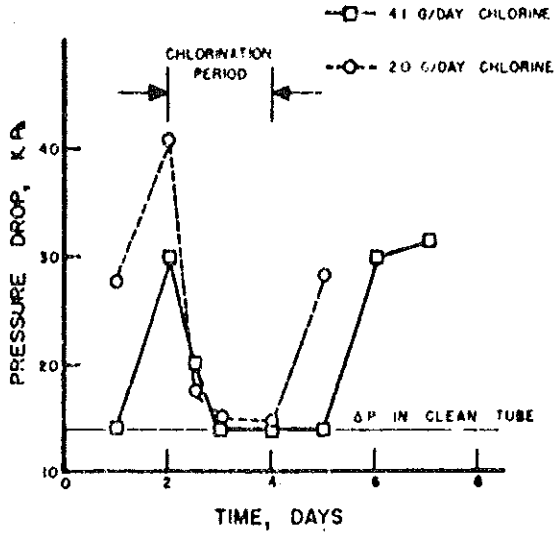


FIGURE 8. Continuous Chlorination Effect on Frictional Resistance. Flow Rate = 11.4 l/min

study material balance on soluble chlorine species showed that total chlorine and chloride output accounted for over 95% of total chlorine input. Reaction rates are expected to vary with biofilm properties which are controlled to a large degree by substrate composition (Pavoni et al., 1972).

Norrman et al. (1977) described the effects of chlorination on established biofilms. Figure 7 shows the effect of shock chlorination at rates of 2.0 and 4.1 g/day and application intervals of 24 and 48 hr. Chlorine application was by injection over a 30 min period. Figure 8 shows the effect of continuous chlorination at the same dosages/day. Evidently, a stoichiometry exists for the chlorination of biofilms. If the reaction is not "completed," the remaining biofilm will "catalyze" film redevelopment because the induction period for film growth is eliminated.

ACKNOWLEDGMENTS

We gratefully acknowledge the following: the National Science Foundation (Grant No. ENG 74-11957 and Faculty Science Fellowship to the senior author) and the Electric Power Research Institute (Project No. 902-1) for partial financial support; Maurine Lee and Audrey Yarletts for manuscript preparation; Duane Marks for graphics; and contributions from J. D. Bryers, M. Nimmons, Dr. F. L. Roe, E. Swinford, M. Trulear, and N. Zelver.

We also thank Dr. L. V. McIntire and Dr. P. B. Bedient for their review of the manuscript.

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COMMENTS AND QUESTIONS

Question: (H. A. Schlesinger, Gibbs and Hill, Inc., New York, NY)

Roughly what dosage of chlorine in milligrams per liter would those relate to at 4 and 2 grams per day?

Answer: (Dr. Picologlou)

I don't think I have the number available right now. I can look it up and let you know during lunchtime.

Question: (L. Wayne Adamson, Naval Ship Research and Development Center, Annapolis, MD)

I had a question on the shear stress curves that you showed. Basically what you were plotting there was the effect of velocity, was it not? In other words, you really were not looking at shear stress as such because shear stress is also dependent on diameter. It seems to me that you would have to look at diameter and velocity to understand whether it was a shear stress or velocity effect you're seeing.

Answer: (Dr. Picologlou)

Yes, this is certainly true, and these slides are deficient in that respect. However, the slide corresponds to a tube of two different diameters, quarter inch and half inch. The way they are shown you can deduce fairly quickly the shear stress from the velocity. As the shear stress increases, the rate decreases. I could very well make the computation and show you the corresponding shear stresses.

Question: (J. G. Knudsen, Oregon State University, Corvallis, OR)

I was quite interested in the plots you showed of the increase in pressure drop. I have a couple of questions related to it. Number 1, I don't recall you mentioning the roughness height that would correspond to that increase. Number 2, is there a possibility that there would be polymeric materials in the solution itself that may cause the increase? Normally, you would expect that to be a drag reduction effect.

Answer: (Dr. Picologlou)

I'll answer your second question first. We have been monitoring the viscosity of the solution, and it was very close to that of water. To answer the first question: I thought I mentioned the height of the viscous sub-layer which was about 50-70 microns. If the model we have about rough pipes tells if the roughness protrusion extends past the viscous sub-layer, the pipe can no longer be considered smooth. Does that answer your question?

Question: (Dr. Knudsen)

I was just wondering if you had compared it with the conventional Moody plot. That is, compared the friction factor you were getting and related it to a relative roughness in the pipe.

Answer: (Dr. Picologlou)

Yes, and that was of the same order of magnitude as the film thickness. Perhaps it's not relevant, but upon chlorination one observes quite an increase of suspended solids which indicates that the film is sloughed off.

Question: (L. C. Trimble, Lockheed Missiles and Space Company, Inc., Sunnyvale, CA)

Lyle, I think my question is for you. In the fouling rates that were projected here do you intend to try to do any cross-correlation with the biofouling experiments being conducted on the CMU device?

Answer: (L. D. Perrigo, Battelle, Pacific Northwest Laboratories, Richland, WA)

At the present time we have no particular program under development that would be specifically pointed at Rice University. We are acquiring data from several different sites. I failed to mention this morning when I put the viewgraph on the projector that the data I was showing included those from St. Croix and Keahole Point. Those data were all grouped together. We have two different sites, but we do not have two different techniques for acquiring data.

Comment: (Mr. Trimble)

I guess the reason I asked the question, Lyle, is because there may be a simpler way to get there from where we are now. If you have the fouling rates as a function of nutrient, you can simply measure the nutrient in a given locale and it might be a faster way to predict what your fouling rates would be.

Answer: (Mr. Perrigo)

I understand now, Lloyd, what your point is. I have other input that suggests nutrient rate is certainly important but not all-inclusive.