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EARLY FOULING BIOFILM FORMATION IN A TURBULENT FLOW SYSTEM: OVERALL KINETICS

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Abstract—An empirical expression is presented which describes the rate of fouling biofilm development from clean surface conditions to the onset of fluid frictional resistance increase. Experiments were conducted in a CSTR with internal recycle; a system which provided control of biological activity in the bulk fluid while simulating turbulent flow conditions. Primary biofilm accumulation rate is described by a first order expression in which the rate coefficient is a function of suspended biomass concentration, Reynolds number, and suspended biomass growth rate.

THE PROBLEM

Fouling biofilms develop, within turbulent flow systems, in a sigmoidal fashion as illustrated in Fig. 1. Three phases are evident:

1. initial biofilm formation;
2. exponential accumulation;
3. steady-state or plateau phase.

The initial biofilm formation phase terminates when frictional and heat transfer resistances begin to increase; also shown in Fig. 1. The effect of biofilms on both frictional and heat transfer resistance becomes severe in the latter two stages of development. Reviews of case histories cite frictional resistance increases of 200% over clean tube conditions due to the presence of thin biofilms (Zelver, 1979; Norrman, 1976; Characklis, 1973). Fouling of electric power surface-condensers costs the U.S. power industry an estimated $400 million a year for additional fuel (Ritter & Suitor, 1975).

The majority of biofouling engineering research has focused on the latter two stages of biofilm development (Zelver, 1979; Nimmons, 1979; Trulear & Characklis, 1979). Recent work has investigated fouling biofilm development and destruction in laboratory systems with environmental control (Characklis, 1979a, b; Trulear & Characklis, 1979; Zelver, 1979). Experiments were initially conducted as batch reactors to quickly establish a biofilm and only then were continuous growth conditions started. Using this technique, the often lengthy initial formation period was circumvented.

However, research suggests that the physical, chemical, and biological properties of biofilms are determined in the early formation stage (Hartman, 1967; Heukelekian, 1956). Good research regarding initial biofilm adhesion has been accomplished (Zobell, 1939, 1943; Marshall, 1972; Corpe, 1970, 1972; Fletcher & Floodgate, 1973), but has generally ignored the effects of parameters of engineering concern. Consequently, there still remains a lack of knowledge about the kinetics of fouling biofilm early formation.

Objectives of this research were as follows:

1. Develop a biofilm detection method sensitive to early stages of biofilm formation.
2. Develop an expression for net biofilm accumulation rate as a function of microbial activity and hydrodynamic parameters during the early stages of formation.

EXPERIMENTAL PROTOCOL

Biofilm formation rates are considered dependent upon two factors:

1. The frequency of microorganism contact with the surface.
2. The overall growth activity of the suspended microorganisms.

Frequency of microbial contact with an inert surface is assumed directly dependent on the concentration of suspended organisms, properties of the microbial particles, and turbulent intensity; measured, respectively, as the suspended biomass concentration (X) and Reynolds number

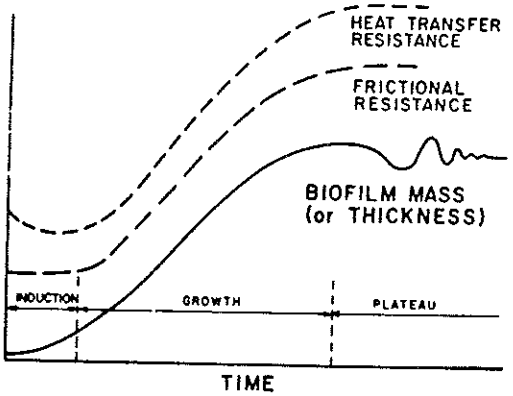

Fig. 1. Biofilm progression in turbulent flow.

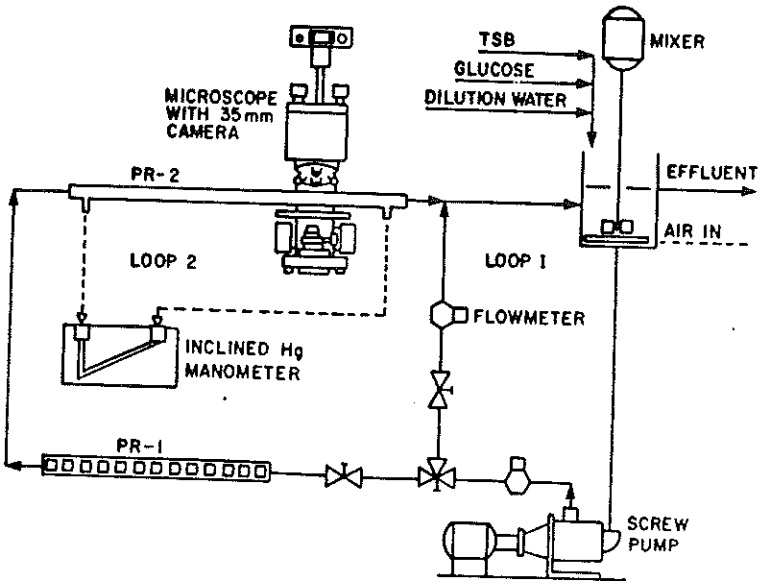

Fig. 2. Biofouling reactor system.

(*Re*). Microbial activity is characterized by the suspended biomass growth rate (μ).

Reactor system

A tubular reactor system, illustrated in Fig. 2, was used in this study. The reactor system contained a substrate feed system, two tubular recycle loops in series, two fouling test sections, and a 3-l. mixing vessel.

Recycle loops were constructed of 1.27 cm i.d. schedule 80 poly(vinyl chloride) pipe. Flow through the recycle circuit was generated by a rotary helical screw pump; flow rates were set with globe valves and monitored using two cumulative-flow water meters.

The reactor was operated as a continuous, stirred tank reactor (CSTR) with an internal recycle circuit; suspended biomass was generated within the mixing vessel and recycle circuit.

Two fouling test sections were used to monitor biofilm accumulation. PR-1 consisted of fifteen 1.27 cm i.d. × 5.13 cm long cylindrical Pyrex glass tubes within a stainless-steel outer sleeve (Fig. 3). Glass tubes were periodically withdrawn throughout an experiment to determine biofilm accumulation. During sampling, PR-1 was isolated from the recycle flow by means of a bypass line. The PR-1 unit was then removed from the recycle circuit via two quick-disconnect unions. An exposed tube was removed by inserting a clean replacement. PR-1 was then reinstated in the recycle loop and flow reinitiated. Total sampling time was less than two minutes.

Reactor PR-2 was a 1.27 cm i.d. × 91.4 cm long Pyrex glass cylindrical tube with pressure taps 2.50 cm from each end (Fig. 4). Pressure drop across the reactor was measured with an inclined mercury manometer (range 0–15.2 cm Hg). Biofilm development on the inner surface of reactor PR-2 was also monitored microscopically.

Start-up procedure

Portions of the reactor system, the mixing vessel and Loop One (see Fig. 2) were operated as a continuous cell propagator until suspended biomass and effluent substrate concentrations reached a steady-state. The fouling test sections, situated in Loop Two, were then exposed to the desired fluid flow rate which defined zero time for the experiment. Experiments were terminated at the onset of any frictional resistance increase.

Substrate feed system

The substrate feed system is depicted in Fig. 5. A 1:1 weight ratio of glucose and trypticase soy broth (TSB) was used as limiting substrate. An inlet concentration of 200 mg l^{-1}, for example, was 100 mg l^{-1} glucose and 100 mg l^{-1} TSB.

Separate stock solutions of glucose and TSB were prepared daily with deionized water. Stock TSB was autoclaved at 20 psi and 50°C for 30 min. Glucose solutions and dilution water were not sterilized. Glucose and TSB were pumped separately into the mixing vessel with a multichannel peristaltic pump. Dilution water was pumped through a carbon adsorption column to the mixing vessel with a second peristaltic pump.

Microbial inoculum preparation

A standard inoculum was prepared to minimize effects of population variations between experiments. Twenty liters of mixed liquor from an activated sludge basin of a domestic wastewater treatment plant was settled and the sludge concentrate mixed with glycerol to 25% v/v. Ten milliliters

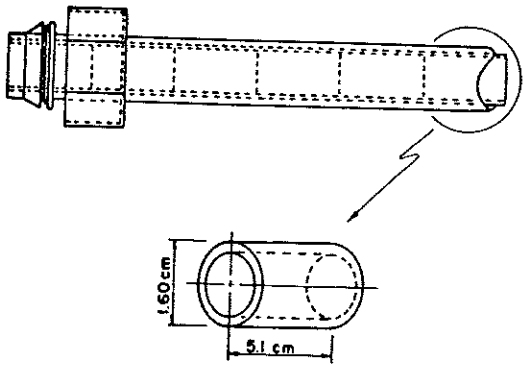

Fig. 3. Biofouling test unit, PR-1.

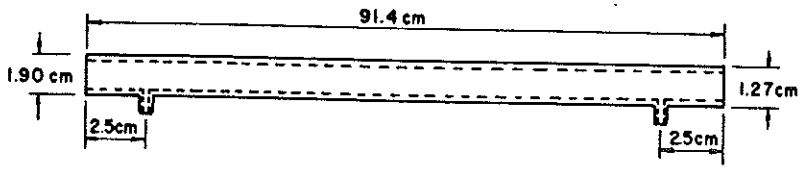

Fig. 4. Biofouling test unit, PR-2.

of the above suspension were transferred to glass ampules where they were "quck frozen" in liquid nitrogen, then stored at −20°C.

Analytical methods

Chemical oxygen demand. COD was used to indirectly measure the amount of oxidizable carbon in the substrate feed, reactor effluent, and attached biomass. A modified procedure of the standard COD (APHA, 1975) method was used to detect the anticipated low levels of carbon present. Basically, dichromate and ferrous ammonium sulfate reagents used in the COD method were diluted by a factor of ten.

Total suspended biomass. Total suspended biomass in the reactor bulk fluid was determined by a membrane filtration–gravimetric procedure. Periodically, two 100 ml samples of reactor fluid were filtered through two tared Nuclepore filters (pore size = 0.45 μm). Filters were dried at 60°C for exactly 3 h and stored in sealed petri dishes within a dessicator until weighed.

Temperature, pH, and dissolved oxygen were periodically measured.

Attached biofilm: sampling and chemical analysis. Biofilm accumulation on glass test sections of PR-1 was determined by using the modified COD method. To maximize the material recovered per test section, the following procedure was employed:

1. Two glass sections were removed at prescribed intervals and rinsed with distilled water to remove any loosely adherent biomass.
2. Each glass section was then submerged in 0.022 l. of double distilled water in a pre-cleaned 2.25 cm i.d. × 20.0 cm glass culture tube.
3. Each culture tube then received a 1 min ultrasonic treatment to disrupt and disperse attached material uniformly throughout the solution.
4. Five milliliters of concentrated sulfuric acid containing 0.54% wt Ag_2SO_4 was then added to each tube.
5. Contents of each culture tube, including both the acidic solution and glass section, were transferred to precleaned distillation flasks for a modified COD analysis to determine the amount of attached chemical oxygen demand.

Attached biofilm: photomicrographic surveillance. Biofilm development per area of reactor PR-2 was recorded using a Bausch and Lomb compound microscope with a Pentax SLR 35 mm camera adaptation. Maximum magnification of the camera modification was 500× (10× eyepiece·2× zoom factor·2.5× camera factor·10× ocular). Resolving power was 2.0 μm (wavelength = 0.5 μm, numerical aperture = 0.25).

Due to the similarity in refractive index of biofilms and the bulk fluid, some artificial means of enhancing their contrast for photographs was desired. Chemical cytological staining was disregarded due to possible detrimental effects upon adherent organisms. Use of a high contrast film (Kodak SO-11 Technical Pan Film) coupled with an optical staining technique proved effective. Optical staining consisted of simultaneous application of two filtered light sources. Direct light of the microscope was filtered with a Kodak No. 25 red filter while a second light, filtered through a Kodak Wratten No. 45 blue filter, was applied at an oblique angle.

The oblique blue light was reflected from any attached biomass but was not as discernible to the red-light sensitive film as the red-filtered primary source. Results were an underexposed background with dark overexposed biofilm fibers.

RESULTS

Experiments are arranged and discussed as three groups according to the variable being evaluated (X, Re, or μ). Detailed experimental conditions are provided in Table 1.

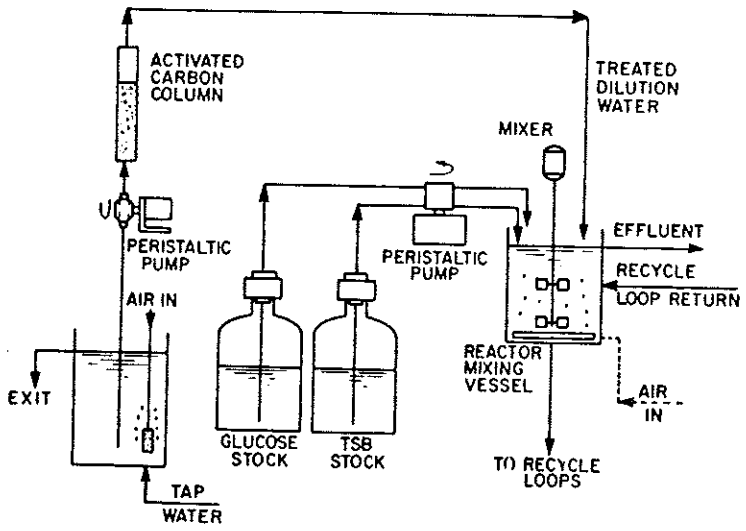

Fig. 5. Schematic of substrate feed system.

Table 1. Experimental conditions

Experiment Number	Group	X (mg-TSS l^{-1})	μ (h^{-1})	Re
1	Biomass	4.4	0.28	17,200
2		12.0	0.28	17,200
3		2.8	0.28	17,200
4		13.0	0.28	17,200
5		23.0	0.28	17,200
6		4.0	0.28	17,200
7		10.1	0.28	17,200
8		2.5	0.28	17,200
9	Reynolds number	12.0	0.28	17,200
10		12.0	0.28	10,600
11		12.0	0.28	19,300
12		12.0	0.28	23,900
13		12.0	0.28	28,800
14	Suspended growth rate	18.0	1.0	17,200
15		18.0	0.28	17,200
16		18.0	0.16	17,200
17		18.0	0.13	17,200
18		18.0	0.13	17,200

Effect of suspended biomass concentration

Results of early biofilm accumulation for three biomass concentrations (X) are illustrated in Fig. 6; Reynolds number and suspended biomass growth rate were constant at 17,200 and 0.28 h^{-1}, respectively.

An increase in pressure drop was observed at elapsed times of 70–80 hours in experiments carried out at inlet substrate and reactor suspended biomass concentrations of 200 mg l^{-1} and 50 mg-TSS l^{-1}, respectively. Biofilm was visible to the eye in the latter stages of these experiments.

All other experiments in this group were arbitrarily terminated at 70 h elapsed time, prior to any increase in system pressure drop. Microscopically, biofilms appear as discrete fibers, oscillating tangentially to the glass surface. Figure 7 provides photographs of biofilm at various elapsed times of Experiment 9. No apparent differences in attached fiber lengths or surface coverage were observed in biofilms grown at different suspended biomass concentrations.

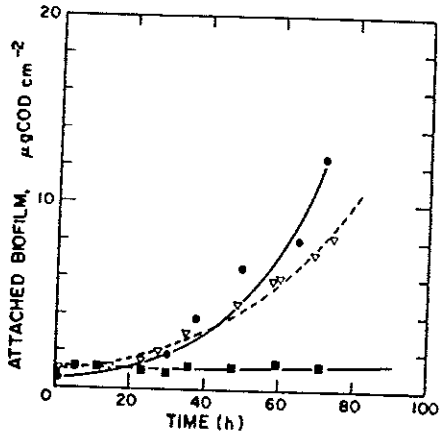

Fig. 6. Biofilm accumulation as a function of suspended biomass concentration. Re = 17,200 and μ = 0.28 h^{-1}. Suspended biomass concentration (X) = 23.0 (●), 12.0 (▽), and 2.4 (■) mgTSS l^{-1}. Curve is based on equation (12).

Effects of Reynolds number

Results of initial biofilm COD accumulation at different Reynolds number (Re) are shown in Fig. 8, for a constant suspended biomass concentration and growth rate of 12 mg-TSS l^{-1} and 0.28 h^{-1}, respectively. Experiments in this group were terminated at elapsed times of between 70–100 h, prior to any increase in system frictional resistance.

Biofilms were visibly evident only during latter stages of the low Re experiments, i.e. 10,000–13,000. Microscopically, attached biofilm fibers grown at the low Re conditions varied in length (c. 1.5–1500 μm) and were randomly distributed on the surface. Attached fibers grown at the high Re conditions were more uniform in length (c. 1.5–3.0 μm) and surface coverage. This effect of Re on fiber length is illustrated by the photographs in Fig. 9.

Effects of suspended biomass growth rate

Initial biofilm COD accumulation at various suspended organism growth rates is illustrated in Fig. 10 at a constant biomass concentration and Reynolds number of 18.0 mg-TSS l^{-1} and 17,200, respectively.

Experiments at the growth rate of 1.0 h^{-1} were terminated at 20–30 h when increases in frictional resistance were observed. Biofilm was so extensive that *in situ* biofilm thickness measurements were obtained using the microscope micrometer (Sanders, 1966; Trulear & Characklis, 1979). Thickness results for Experiment 14 are shown in Fig. 11.

Little evidence of attached fibers were microscopically detected at the low biomass growth rate of 0.13 h^{-1}. Frictional resistance increase was not detected in 220 h and experiments were terminated after 2 weeks.

DISCUSSION

Rate of initial biofilm accumulation was determined from material balances on the reactor system. Material balances on suspended biomass and limiting substrate can be written as follows:

Rate of suspended biomass accumulation = Rate of net biomass inflow + Rate of suspended biological growth + Rate of biofilm detachment

$$V dX/dt = F(X_i - X) + \mu XV + R_d A \qquad (1)$$

where

X = suspended biomass concentration, (ML^{-3})
X_i = inlet suspended biomass concentration, (ML^{-3})
F = volumetric flow rate, (L^3t^{-1})
V = reactor volume, (L^3)
μ = suspended biomass growth rate, (t^{-1})

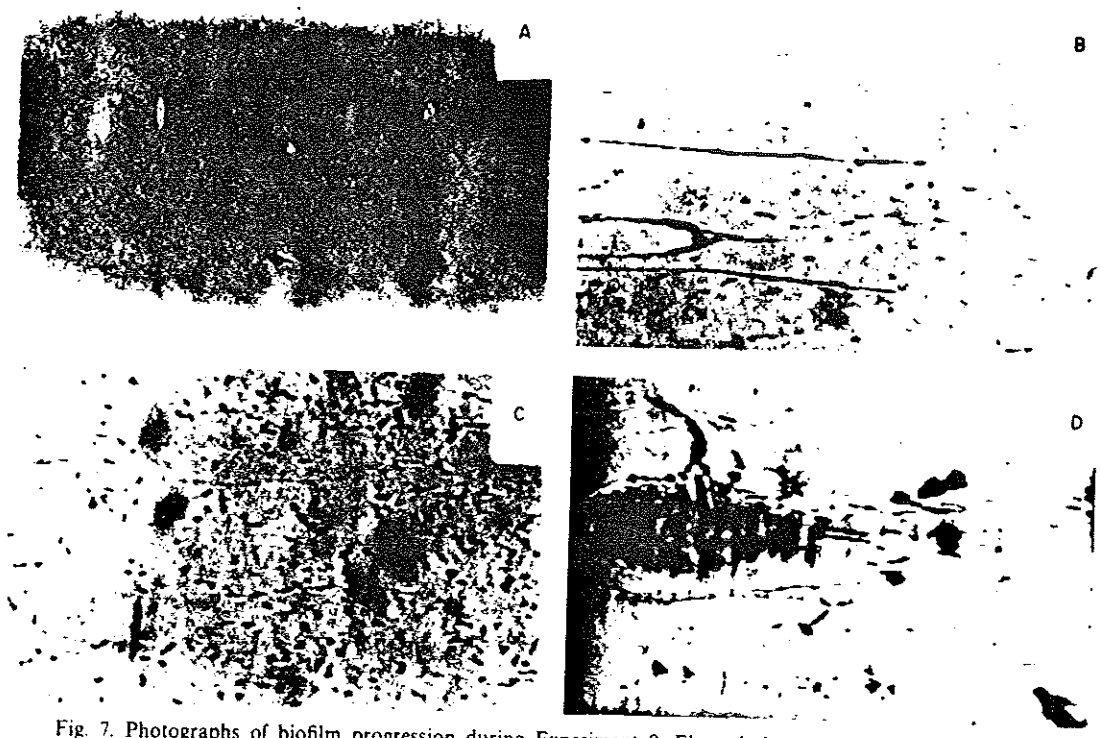

Fig. 7. Photographs of biofilm progression during Experiment 9. Elapsed time = (A) 0 h; (B) 10 h; (C) 35 h; (D) 55 h. Scale in all frames: 1 cm = 145 μm. Flow is from left-to-right. Magnification = 100×.

A = reactor surface area, (L^2)
R_s = biofilm detachment rate, ($ML^{-2}t^{-1}$)

and

Rate of limiting substrate accumulation = Rate of net substrate inflow − Rate of substrate depletion by suspended organisms − Rate of substrate depletion by attached organisms

$$V dS/dt = F(S_i - S) - \mu XV/Y - R_g A/Y \quad (2)$$

where

S = limiting substrate concentration, (ML^{-3})
Y = biomass yield, ($M M^{-1}$)
R_g = biofilm growth rate, ($ML^{-2}t^{-1}$).

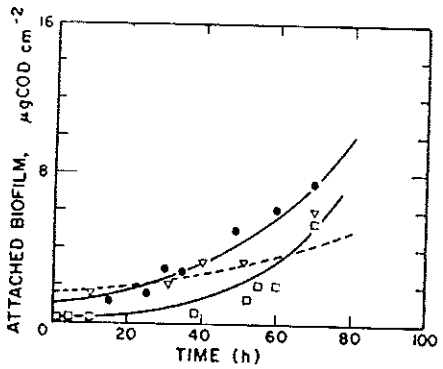

Fig. 8. Biofilm accumulation as a function of Reynolds number. X = 12.0 mgTSS l^{-1} and μ = 0.28 h^{-1}. Reynolds number (Re) = 10,000 (□), 17,200 (●), and 23,900 (▽). Curve is based on equation (12).

Equations (1) and (2) require an additional equation describing biofilm accumulation:

Rate of biofilm accumulation = Rate of microorganism deposition and attachment + Rate of biofilm production − Rate of biofilm detachment

$$A dB/dt = R_d A + R_g A - R_s A \quad (3)$$

where

B = biofilm amount, (ML^{-2})
R_d = biomass deposition rate, ($ML^{-2}t^{-1}$).

Equations (1)–(3) can be simplified with the following assumptions:

1. Effluent biomass and substrate concentration are at a steady state.
2. Inlet biomass concentration is zero.
3. Substrate depletion by biofilms is negligible compared to that of the suspended organisms.
4. Biofilm production rate, $R_g A$ XV, is small compared to suspended biomass growth rate.

These assumptions were experimentally verified by Bryers (1980). Equations (1)–(3) reduce to the following:

$$F/V = \mu \quad (4)$$

$$Y/(S_i - S) = X \quad (5)$$

$$dP/dt = k f_s(B) \quad (6)$$

where

k = biofilm net accumulation rate constant, (t^{-1})

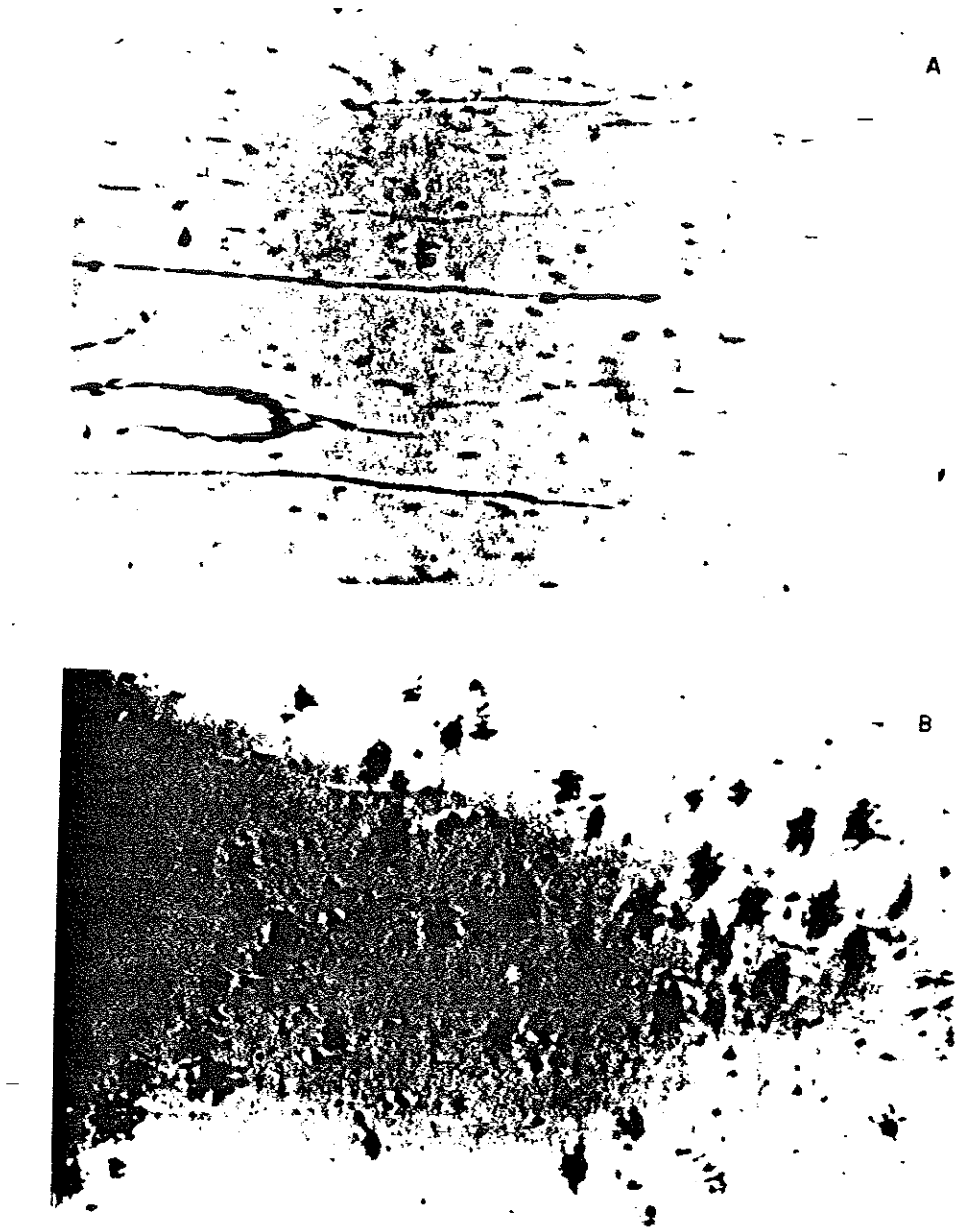

Fig. 9. Photograph of Reynolds number effects on biofilm filament length and surface coverage. Frame A at Re = 17,200 and X = 12.0 mgTSS l^{-1}. Scale in frame A: 1 cm = 145 μm. Frame B at Re = 28,800 and X = 12.0 mgTSS l^{-1}. Scale for frame B: 1 cm = 5 μm. Elapsed time in both frames = 10 h.

$f_N(B)$ = biofilm net accumulation as a function of biofilm amount, (ML^{-2}).

Equations (4) and (5) are expressions describing the steady state chemostat. Equation (6) describes the overall rate of biofilm accumulation. The observed increase of biofilm amount with time suggested a first order rate expression of the form:

$$dB/dt = kB. \tag{7}$$

The numerical value of the rate constant, k, was determined from statistical regression of B vs time according to the integrated form of equation (7). These values of k are illustrated for each experimental group in Figs 12–14.

The overall rate constant, k, is considered a function of the three chosen system parameters, i.e.

$$k = k_1 X^a Re^b \mu^c \tag{8}$$

where

k = biofilm net accumulation rate constant, (t^{-1})
k_1 = specific biofilm accumulation rate constant
a,b,c = empirical constants.

Figure 12 indicates the change in the rate constant (k) as a function of suspended biomass concentration

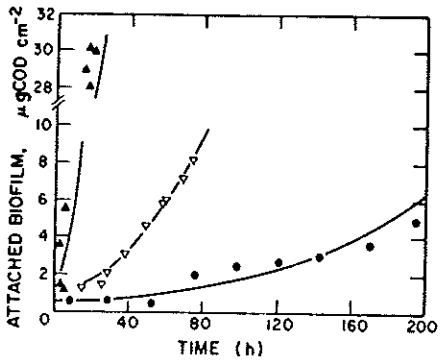

Fig. 10. Biofilm accumulation as a function of suspended biomass growth rate. $X = 18.0$ mgTSS l^{-1} and $Re = 17{,}200$. Suspended biomass growth rate (μ) = 1.0 (▲), 0.28 (▽), and 0.13 (●) h^{-1}. Curve is based on equation (12).

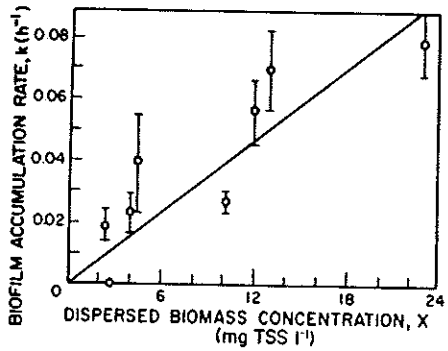

Fig. 12. Accumulation rate constant (k) as a function of biomass concentration. $Re = 17{,}200$ and $\mu = 0.28\ h^{-1}$.

(X) over the range tested. A linear regression of k to x yields the following:

$$k = k^* X^{1.0}. \tag{9}$$

Mass transport theory and particle deposition models (Bird *et al.*, 1966; Freidlander & Johnstone, 1957; Beal, 1970) postulate that the flux of particles to a surface in turbulent flow is directly proportional to the bulk average particle concentration, i.e. a doubling in bulk fluid particle concentration doubles the particle flux. Figure 12 does indicate an approximate doubling in the net accumulation rate constant with a doubling in suspended biomass concentration. In addition, Fletcher (1977) reports a linear increase in the rate of bacterial attachment to styrene with increasing suspended bacteria concentration.

Several experiments were conducted at suspended biomass concentrations ≥ 30 mg-TTS l^{-1}, but this data was not used in the above regression for the following reasons:

1. An inoculum with a lower specific growth rate was used.
2. Higher inlet substrate concentrations were necessary and oxygen may have been limiting biofilm accumulation rate.

An increase in Reynolds number, Re, results in a linear decrease in initial biofilm accumulation rate, k, as illustrated in Fig. 13. Correlation of k to Re yields

$$k = k^{**}(Re)^{-1.0}. \tag{10}$$

Only those Reynolds number experiments at X equal 12 mg-TSS l^{-1} were used in the regression (equation 10).

Particle deposition models (Lin *et al.*, 1953; Freidlander & Johnstone, 1957; Beal, 1970) predict an increasing particle flux to a surface with increasing fluid velocity. Results of Fig. 13 show the rate constant (k) to decrease with increasing Re (or fluid velocity). This suggests that particle flux due to convective transport is only one process contributing to net biofilm accumulation rate. Increasing shear forces by increasing Re may enhance biofilm detachment, or

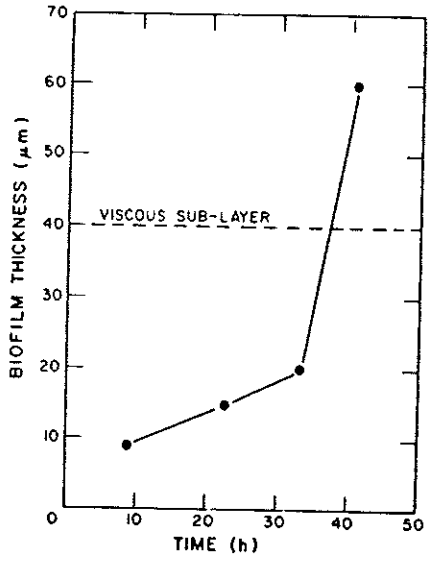

Fig. 11. Biofilm thickness *in situ* PR-2 for Experiment 14. $X = 18.0$ mgTSS l^{-1}, $Re = 17{,}200$, and $\mu = 1.0\ h^{-1}$.

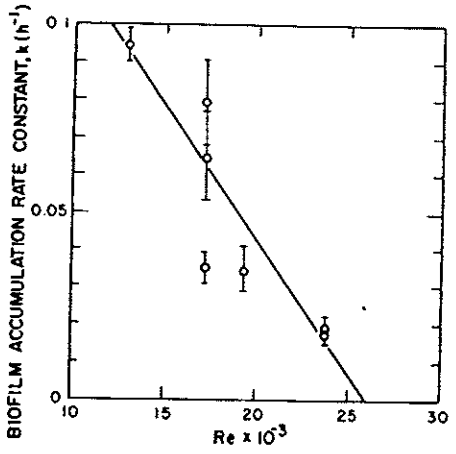

Fig. 13. Accumulation rate constant (k) as a function of Reynolds number. $X = 12.0$ mgTSS l^{-1} and $\mu = 0.28\ h^{-1}$.

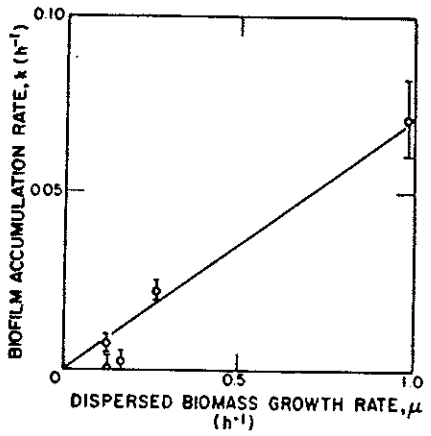

Fig. 14. Accumulation rate constant (k) as a function of suspended biomass growth rate. X = 18.0 mgTSS l^{-1} and Re = 17,200.

other processes near the wall, to a greater extent than deposition.

Figure 14 indicates that the initial biofouling accumulation rate, k, is approximately a linear function of the suspended organism growth rate. Statistical regression yields a linear expression of the form:

$$k = k^{+}\mu^{1.0} \tag{11}$$

Suspended biomass growth rate in a chemostat is directly related to the residence time of the reactor as shown in equation (4). Decreasing residence time, at constant substrate and biomass concentration, increases the growth rate of the microbial culture. In a mixed-species culture, decreasing residence time generally selects for those microorganisms with higher growth rates (Veldkamp & Jannasch, 1972; Bailey & Ollis, 1977), with the slower growing organism being diluted out. Biofilms cultivated in a chemostat would reflect an organism population (and metabolic activity) resembling the steady-state suspended population. Consequently, a higher biofilm accumulation rate is expected at higher suspended biomass growth rates.

Once the empirical constants a,b,c, are known, the specific rate constant, k_1, can be calculated from any known set of experimental conditions.

Using equations (9)–(11), equation (7) can be written as follows:

$$dB/dt = k_1(X\mu/Re)B \tag{12}$$

where

$k_1 = 125.0 \pm 25.0$ mgTSS l^{-1}.

The integrated form of equation (12)

$$B(t) = B_0 \exp((k_1 X\mu/Re)t) \tag{13}$$

where

B_0 = attached COD per area at time zero, (ML^{-2})

is evaluated for the selected experiments and superimposed in Figs 12–14 for comparison to actual data.

It must be noted that equations (12) and (13) are empirical rate expressions which describe the overall rate of accumulation of biofilm within the initial stages of development in a specific experimental system. The above analysis does provide a simple empirical expression for biofilm accumulation in the early stages of formation in turbulent flow for the range of variables cited. An in-depth consideration of the more fundamental rate processes that contribute to early fouling biofilm formation is presented elsewhere by Bryers (1980).

SUMMARY

The influence of three parameters—suspended biomass concentration (X), Reynolds number (Re), and suspended biomass growth rate (μ)—on initial biofilm formation has been described in this paper. Effects of these chosen parameters on more fundamental processes involved in biofilm accumulation are presented elsewhere (Bryers, 1980).

Biofouling experiments were conducted in a CSTR with internal recycle at constant biological activity and turbulent intensity. Results provided the following information:

1. Chemical analysis (i.e. COD attached) provided a sensitive measure of biofilm accumulation prior to the observation of any fluid frictional resistance changes.
2. The rate of initial biofilm COD accumulation was described mathematically using a first-order rate expression. The resultant first order rate constant k was a linear function of X, Re, and μ.
3. Overall biofilm accumulation rate increased linearly with increasing suspended biomass concentration.
4. Biofilm accumulation rate increased with increasing suspended organism growth rate.
5. Mass transport consideration indicates particle flux from the bulk fluid to the wall increases with increasing Reynolds number, Re. Experimental results indicate a decrease in initial biofilm accumulation with increasing Re, suggesting that particle flux from the bulk fluid is but one of many rate processes contributing to the net biofilm COD accumulation.

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