Oxidation and Destruction of Microbial Films

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CHAPTER 32

OXIDATION AND DESTRUCTION OF MICROBIAL FILMS

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INTRODUCTION

The term "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, accelerating corrosion and impairing heat transfer. Biological fouling, or biofouling, results from the attachment and growth of microbial (microfouling) or macrobial (macrofouling) organisms. This chapter is directed at microfouling.

THE PROBLEM

The most common method for control of fouling biofilm is periodic chlorination. Recent concern over residual toxicity from hypochlorous acid and its reaction products has resulted in federal regulations that limit the allowable chlorine concentrations in cooling water discharges. This investigation stems from the apparent need for a better basic understanding of fouling biofilm

destruction so that the impact of these new regulations on power plant operations can be evaluated.

Description of Chlorine/Biofilm Reaction Process

The destruction of attached microbial film by chlorine can be characterized by the generalized equation:

$$\text{chlorine} + \text{attached biomass} \rightarrow \text{suspended biomass} + \text{soluble reaction products}$$

Because two phases are required (solid and liquid), the chlorine/biofilm reaction is considered a heterogeneous reaction process. To discuss a heterogeneous reaction, in addition to considering the reaction proper, attention must also be given to the physical transport of reactants and products in each phase because transport limitations can significantly affect the stoichiometry (as described by equation above) and kinetics of the overall process. Based on the experimental observations in this research, the reaction and transport processes contributing to the chlorine/biofilm reaction are as follows:

1. diffusion of chlorine from the bulk fluid into the biofilm matrix;
2. biochemical reaction (chlorine removal, biofilm destruction and/or dissolution); and
3. biofilm removal by fluid shear.

Although the results presented in this chapter are not conclusive, they form a basis for continued research in biofilm destruction by chemical oxidants. The methods are directed toward elucidation of process rates at the biofilm-liquid interface and within the biofilm; hence, the results may be equally relevant to other microbial aggregates such as those found in water and wastewater treatment effluents.

EXPERIMENTAL SYSTEM

An annular fouling reactor (AFR) system was used for this research. System components include the AFR, substrate, dilution water and chlorine feed apparatus, air supply, and temperature control. Figure 1 is a schematic diagram of the AFR system.

The AFR was constructed of acrylic plastic and consists of two concentric cylinders, a stationary outer cylinder and a rotating inner cylinder (Figure 2). Rotational velocity was electronically controlled and continuously displayed.

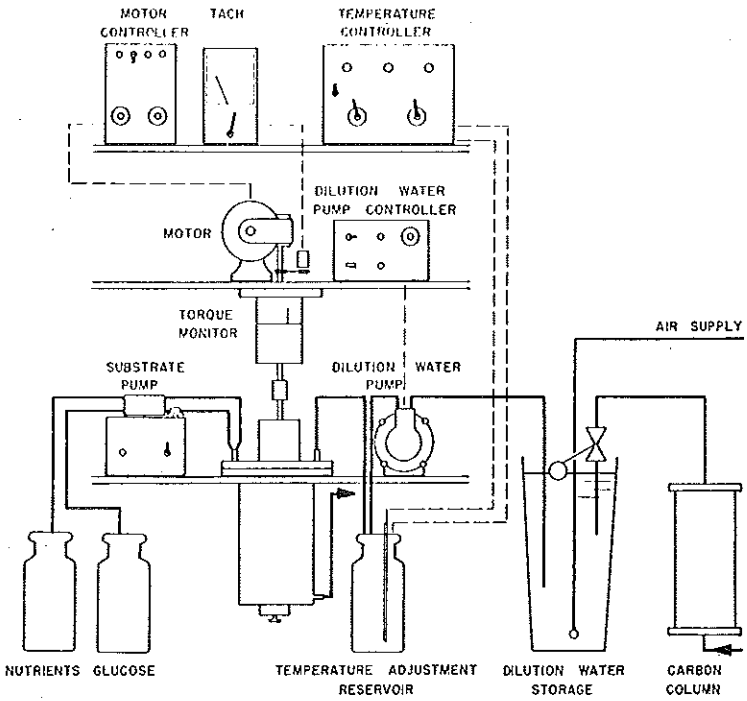

Figure 1. Schematic diagram of annular reactor system.

A torque transducer mounted on the shaft between the rotating cylinder and the motor drive continuously monitored and displayed changes in fluid frictional resistance caused by biofilm accumulation (growth phase) and biofilm removal (chlorination phase). A thin, removable slide, which formed an integral fit with the inside wall of the outer cylinder, was used to determine biofilm thickness and film mass. The AFR was completely mixed by the pumping action of four draft tubes and the impeller assembly incorporated in the inner cylinder.

Substrate and treated dilution water were fed continuously into the AFR by peristaltic pumps during the growth phase of each experiment. Dilution water treatment consisted of in-line filtration followed by carbon adsorption, primarily for the removal of residual chlorine. The effluent from the carbon column was diverted to a polyethylene storage container for aeration and subsequent use. The substrate solution consisted of a 1:4 mass ratio of glucose and trypticase soy broth. During the chlorination phase, chlorine solution was either continuously fed by the dilution water pump or manually pulsed into the AFR. Before entering the AFR, dilution water and chlorine solution (contin-

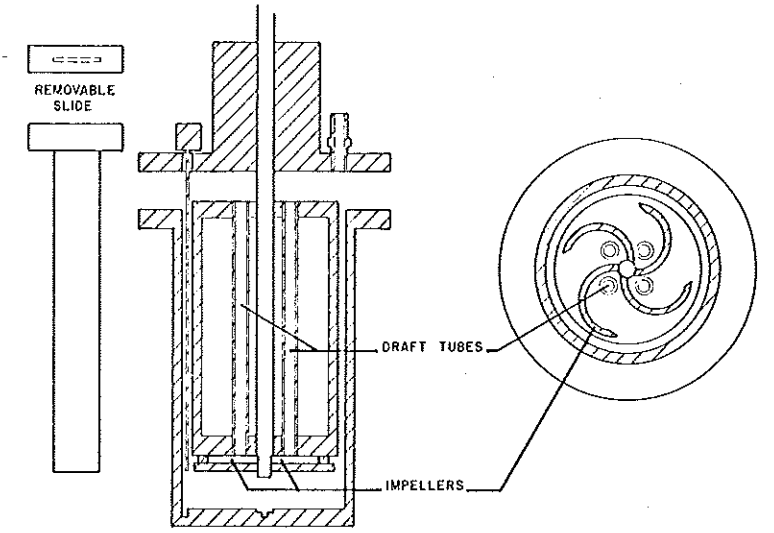

Figure 2. Schematic diagram of annular reactor.

uous chlorination experiments) were passed through a temperature adjustment reservoir which maintained the reactor solution at 30 ± 0.5°C.

EXPERIMENTAL PROCEDURE

Batch Operation–Phase 1

The initiation of biofilm attachment was made in batch operation of the reactor for 5-8 hr. The initial medium contained 20 mg/l glucose and 80 mg/l trypticase soy broth. The inocula were prepared from activated sludge from a local wastewater treatment plant. The sludge was concentrated by settling and mixing with glycerol to 25% (v/v). Glass ampules filled with 10 ml of this suspension were quick-frozen in liquid nitrogen and stored in a freezer at -20°C. One ampule of these inocula was used as standard inoculum for each experimental run.

Table I. Summary of AFR Results Pertaining to Process Stoichiometry[a]

Input Chlorine Concentration (mg/l)	Reactor Residence Time (min)	Chlorine Loading Rate (mg/m^2-min)	Rotational Speed (rpm)	pH	Biomass Removed in 30 min (mg)	Chlorine Reacted in 30 min (mg)	Biomass[b] Removed in 30 min (mg)	Biomass[b] Removed per Chlorine Reacted (mg/mg)
5	5	2.8	175	7.8	40 ± 18(4)	17 ± 1(5)	15	0.9
5	1	14.2	175	7.8	221 ± 121(5)	55 ± 18(5)	196	3.6
5	2.5	5.7	175	7.8	45 ± 24(5)	27 ± 5(5)	20	0.7
12.5	2.5	14.2	175	7.8	99 ± 2(5)	40 ± 10(5)	74	1.9
5	2.5	5.7	175	7.0	22	30	0	0
5	2.5	5.7	175	6.0	24.2	29	0	0
5	2.5	5.7	175	9.0	49	28	24	0.9
5	2.5	5.7	175	8.0	46	27	21	0.8
0	2.5	0	175	7.8	25			
5	2.5	5.7	200	7.8	53	29	28	1.0
5	2.5	5.7	225	7.8	57	28	32	1.1
5	2.5	5.7	175	7.8	117	22	92	4.2
					175[c]	31[c]	150	4.8

[a]Reactor surface area = 0.2 m^2; reactor volume = 570 cm^3; temperature = 30°C.
[b]Corrected for removal when no Cl_2 present.
[c]60 min period.

Continuous Operation–Phase 2

During the growth phase, glucose and sterile trypticase soy broth were fed into the reactor by a peristaltic metering pump at a constant inflow concentration of 4 mg/l for glucose and 16 mg/l for trypticase soy broth and a mean residence time of 10 min. Normally, two days were required for the growth phase to be completed.

Chlorination–Phase 3

At the beginning of the chlorination phase, the reactor was drained and filled with deionized water. The effect of deionized water on the biofilm was significant, as indicated by the results of the ninth experiment in Table I. The chlorine solution was then fed into the reactor. At the end of this phase, the reactor was again replaced with deionized water and continuous operation (i.e., Phase 2) was resumed.

The pH of the chlorine solution was 7.8 except for four experiments in which the chlorine solution was buffered to maintain the pH at 6, 7, 8 and 9.

Laboratory-grade sodium hypochlorite (4-6%) was used to prepare the chlorine solution. The chlorination phase was 30 min except for the experiments in which rotational speed was varied (60 min).

Effluent chlorine and suspended solids (SS) concentrations were determined at the beginning and during the chlorination phase. Biofilm thickness in the reactor was measured at the beginning and end of the chlorination phase.

Concentrations of stock chlorine solutions were determined by iodometric methods described in *Standard Methods*.[1] Free and total chlorine in samples were measured by a colorimetric method developed by Black and Whittle.[2] The SS were measured by the gravimetric method using polycarbonate filters (0.4 μm). Total and fixed SS determinations employed glass-fiber filters. For the total solids, the residue and tared filter were dried at 60°C for 3 hr. For fixed solids, the filter and residue were ignited in a muffle furnace at 550°C.

The thickness of microbial film was determined with the stage micrometer of a microscope in a method adapted from that of Sanders.[3] The removable slide was withdrawn from the reactor, placed in a vertical position for 1 min to allow excess water to drain and placed on the microscope stage. The 10X objective was lowered until the biofilm surface was in focus, and the fine adjustment dial setting of the stage micrometer was recorded. The objective was then lowered further until the inert plastic surface was in focus. The difference in fine adjustment settings was compared with a calibration curve and the thickness determined. The reported biofilm thickness was the mean from four or five measurements along the slide from top to bottom.

RESULTS

Batch Reactor Tests: Chlorine Demand of Biomass

Batch reaction experiments were conducted with a pseudohomogeneous suspension of biofilm. The primary purpose of these tests was to determine stoichiometric coefficients for the reaction:

$$\text{chlorine} + \text{biofilm} \rightarrow \text{soluble reaction products}$$

An approximate value for the reaction rate constants was also obtained. The tests were conducted with biofilm and chlorine. The biofilm was developed on the surfaces of the annular reactor under conditions described in the preceding section. The biofilm was scraped from the surface and blended. Serial dilutions of the biomass suspensions were prepared and chlorine solution added. The reactors were mixed well and maintained at 37°C and pH 7.8.

Table II presents the result of the batch reaction tests. Figure 3 graphically depicts the progress of one of the tests. The rate of chlorine reaction can be determined by fitting the data to an exponential decay curve as follows:

$$C = C_0 \exp(-kt)$$

where C = free-chlorine concentration, M/L^3
C_0 = initial free-chlorine concentration, M/L^3
k = rate constant, t^{-1}
t = reaction time, t

The calculated rate constants are presented in Table II.

Stoichiometry

The stoichiometric ratio, or chlorine demand, was calculated to be 0.9 mg free chlorine reacted per milligram biomass destroyed. The basis for the calculation is presented in Figure 4. The data from the two highest initial biomass concentrations were not used because all of the chlorine was consumed before the final biomass concentration was determined; biomass decay, even without chlorine present, would cause further decrease in biomass (Table II, column 1).

Table II. Results of Batch Tests for the Reaction between Biofilm and Chlorine at 37°C

	Free Chlorine Concentration (mg/l)						
Initial Biomass Concentration, mg/l	66.5	0.0	22.2	44.3	66.5	88.6	110.8
Reaction Time, min							
0	0.0	80.9	80.3	80.5	81.3	82.3	80.8
3	0.0	80.8	78.0	76.8	76.5	76.0	75.0
7	0.0	80.8	74.3	73.3	72.3	71.5	71.0
15	0.0	80.5	72.8	72.5	69.0	67.5	66.0
30	0.0	80.3	72.3	63.5	60.3	59.5	57.8
60	0.0	79.5	71.8	59.0	53.5	53.5	54.0
240	0.0	75.0	49.3	43.0	32.8	19.8	6.8
1470	0.0	68.3	41.3	25.3	9.0	0.0	0.0
Final Biomass Concentration, mg/l	38.9	0.0	0.0	2.1	3.8	5.3	7.5
Percent Volatile				21.0	18.0	18.0	15.0
Rate Constant $k_1 \times 10^3$, min^{-1}		0.3	1.9	2.4	3.5	5.6	10.1

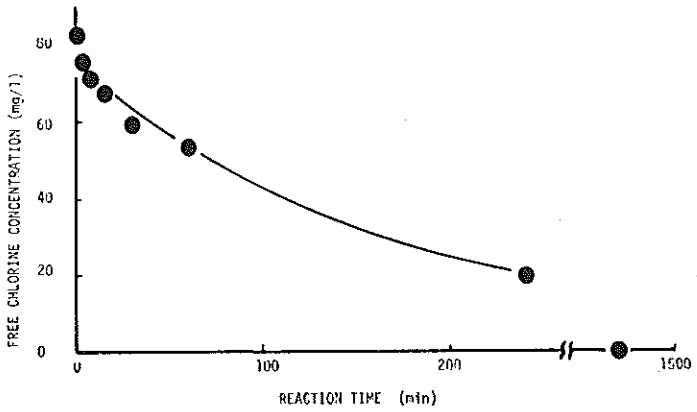

Figure 3. Decrease in chlorine concentration with time caused by reaction with dispersed biofilm in a batch reactor at 37°C.

Rate

The rate constant k is about 10^{-3} min^{-1} and is a function of initial biomass concentration, as indicated by Figure 5. Fundamentally, k is a function of instantaneous biomass concentration x. Then

$$C = f(C_o, k', x)$$

where x = instantaneous biomass concentration, M/L^3
k' = rate constant (units dependent on rate expression)

Insufficient data for x preclude further analysis of this expression. However, the decay constant for biomass based on limited data is approximately 10^{-3} min^{-1}, assuming a first-order rate expression. This result could also be determined from the stoichiometric ratio reported in the previous section and knowledge of the chlorine decay rate.

Continuous AFR Reactor Tests: Biofilm Removal

Continuous reactor tests were conducted at a variety of operating conditions in an attempt to clarify process stoichiometry and kinetics and to determine the influence of some relevant process variables such as pH and fluid shear stress.

Process Stoichiometry

Chlorine apparently induces two fundamental changes in the AFR: (1) biofilm is solubilized, and (2) biofilm detaches from the wall. In these experiments, biofilm solubilization was not measured. Table I summarizes the results of the continuous experiments pertaining to process stoichiometry.

As much as 5 mg of biomass exited the reactor per milligram chlorine reacted. Note that this is a minimum estimate of the biomass detached because it is calculated from filterable solids exiting the reactor. Biomass that detaches and dissolves (i.e., passes a 0.4-μm filter) is not included in the measurement. Figure 6 indicates that increasing mean residence time decreases the filterable biomass produced per unit chlorine reacted. This result may be attributed

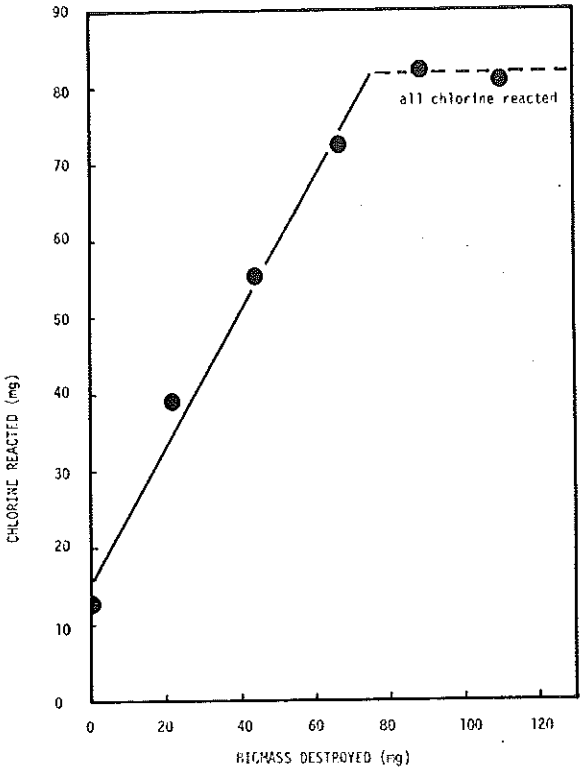

Figure 4. The relationship between chlorine reacted and dispersed biofilm destroyed in a batch reaction test.

partially to increased biomass dissolution in the bulk fluid at higher mean residence times. The data suggest that as much as 4-5 mg of biomass can be removed per mg chlorine reacted when residence time is reduced further.

Figure 7 indicates the effect of inlet chlorine concentration on detachment stoichiometry for the same mean residence time. The increased concentration may result in "deeper biofilm penetration" by the chlorine and, therefore, better removal per unit chlorine reacted.

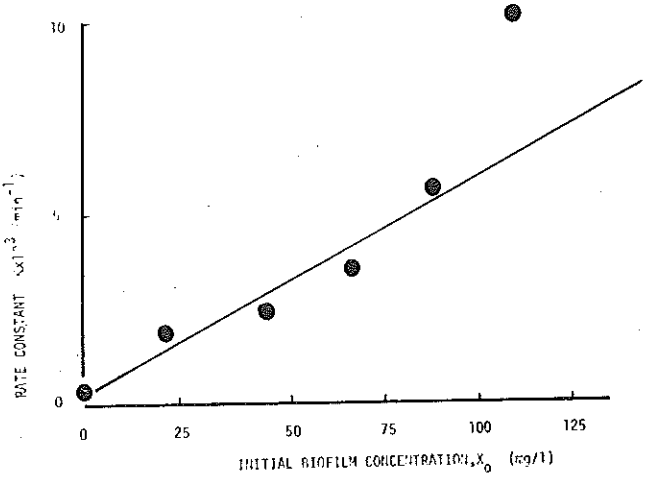

Figure 5. Influence of initial biomass concentration on rate constant k.

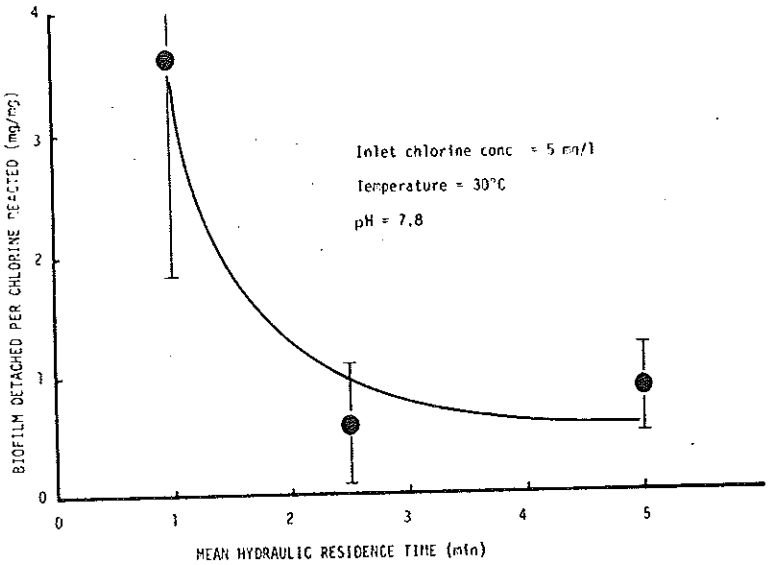

Figure 6. Effect of reactor mean residence time on biofilm detached per chlorine reacted.

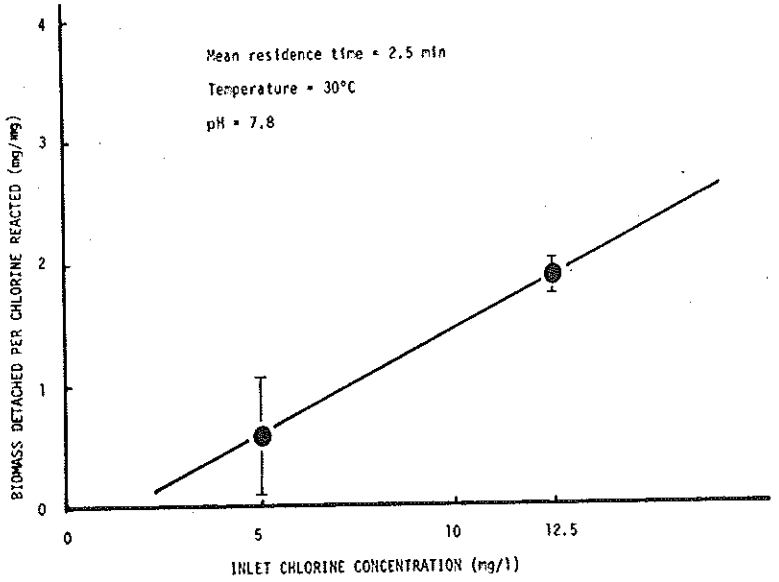

Figure 7. The influence of inlet chlorine concentration on biofilm detached per chlorine reacted.

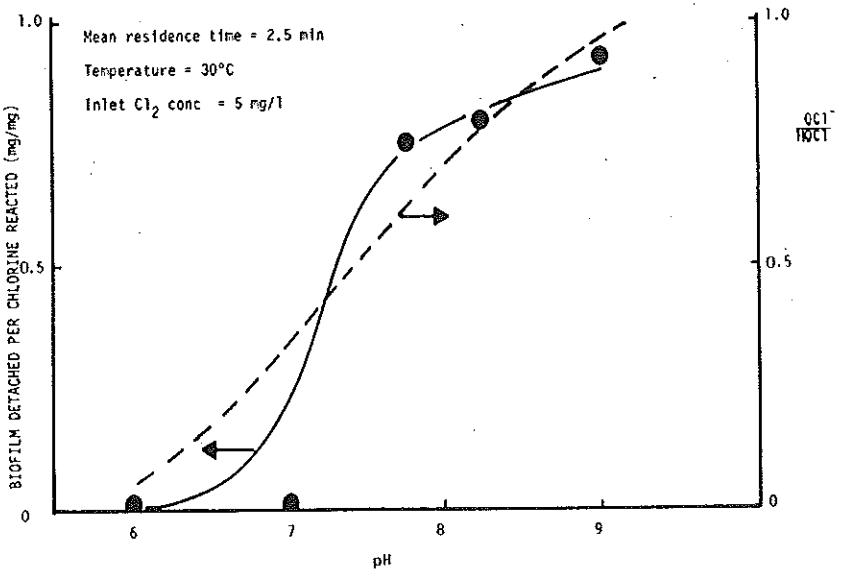

Figure 8. The influence of pH on biofilm detached per chlorine reacted.

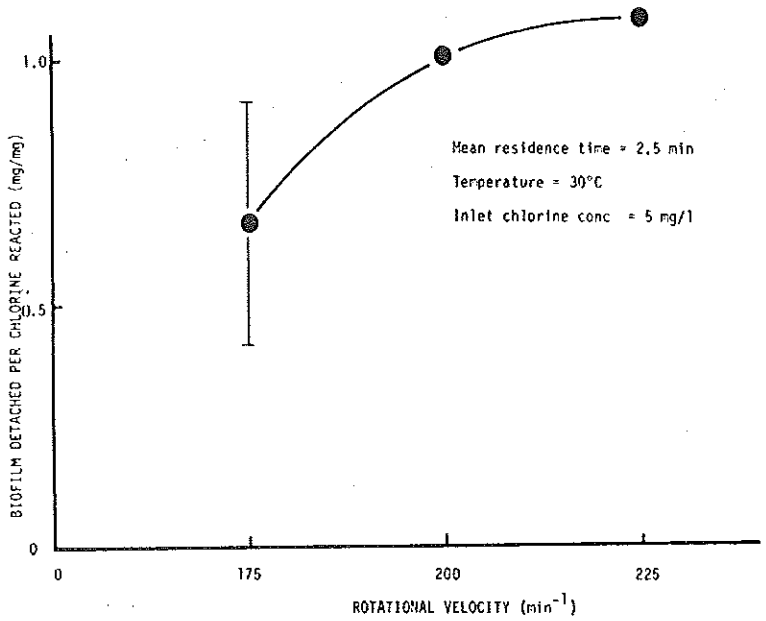

Figure 9. The influence of rotational velocity on biofilm detachment per chlorine reacted.

Data on the effect of pH on biofilm detachment (Figure 8) suggest that biofilm detachment occurs to a greater extent at higher pH were OCl^- is the predominant species. This presumes, without any basis, that HOCl does not dissolve biomass more rapidly than OCl^-. Consequently, biofilm detachment is favored by high pH as opposed to disinfection processes that are more efficient at lower pH. The effect of the OCl^-/HOCl ratio represents an interesting trend; however, the results do not imply a fundamental mechanism for biofilm removal.

In the range tested, rotational velocity has little effect on biofilm detachment, as indicated by Figure 9. Duplicate tests are necessary to establish significant differences.

Process Kinetics

The rate processes contributing to overall chlorine removal rates, torque decay rates and biomass exiting the reactor are complex. The measured rates are undoubtedly the net result of several rate processes. For example, chlorine removal rate could well be the net result of diffusion/reaction in the biofilm as well as reaction with the detached biomass in suspension. A summary of the rate data is presented in Table III.

Table III. Summary of AFR Results Pertaining to Process Kinetics[a]

Input Chlorine Concentration (mg/l)	Reactor Residence Time (min)	Chlorine Loading Rate (mg/m^2-min)	Rotational Speed (rpm)	pH	Torque Decay Rate Constant (min^{-1})	Decrease in Biofilm Thickness (μm)
5	5	2.8	175	7.8	0.003 ± 0.004(5)	21 ± 9(5)
5	1	14.2	175	7.8	0.008 ± 0.010(5)	30 ± 7(5)
5	2.5	5.7	175	7.8	0.006 ± 0.004(5)	46 ± 27(5)
12.5	2.5	14.2	175	7.8	0.053 ± 0.030(5)	29 ± 8(4)
5	2.5	5.7	175	7.0	0.009	39
5	2.5	5.7	175	6.0	0.005	24
5	2.5	5.7	175	9.0	0.021	32
5	2.5	5.7	175	8.0	0.068	30
0	2.5	0	175	7.8	0	0
5	2.5	5.7	200	7.8	0	36
5	2.5	5.7	225	7.8	0.008	43
5	2.5	5.7	175	7.8	0.012 0.010[b]	12[b]

[a]Reactor surface area = 0.2 m^2; reactor volume = 570 cm^3; temperature = 30°C
[b]60-min period.

Figure 10 indicates that chlorine consumption is almost directly proportional to the chlorine loading rate. The chlorine surface-loading rate is appropriate as a parameter if all chlorine is consumed at the reactive surface (i.e., the biofilm). The amount of biomass exiting the reactor increases significantly with increasing chlorine loading rate, as indicated in Figure 11. The influence of chlorine loading rate on torque reduction is minimal for a chlorine concentration of 5 mg/l. However, increasing the chlorine concentration to 12.5 mg/l significantly increases the torque reduction, as indicated in Figure 12.

The torque decay rate decreases slightly as reactor mean residence time increases (Figure 13). Torque is reduced much faster at higher chlorine inlet concentration (Figure 12). It is probable that the increased concentration gradient allows deeper penetration into the biofilm. The effect of pH on torque reduction is not clear (Figure 14), although the rate of torque reduction appears to increase with increasing pH. Finally, the effect of rotational velocity on the rate of torque reduction was insignificant based on these tests (Figure 15), primarily because of the range of torque values that could be measured. At higher rotational velocities, the torque value was "off-scale" and decreases were not measurable until the torque dropped to a value below the maximum on the torque monitor (5 in.-oz or 3.5 N-cm).

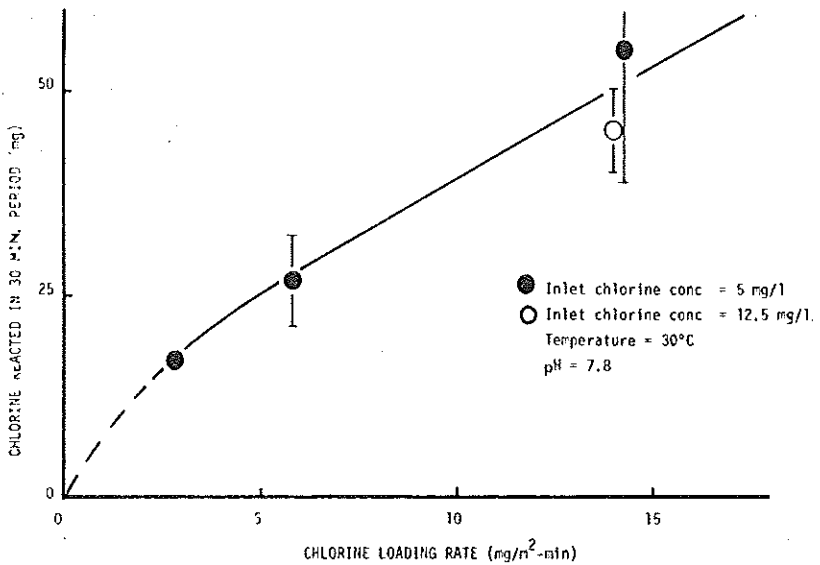

Figure 10. The influence of chlorine loading rate on chlorine reacted in biofilm.

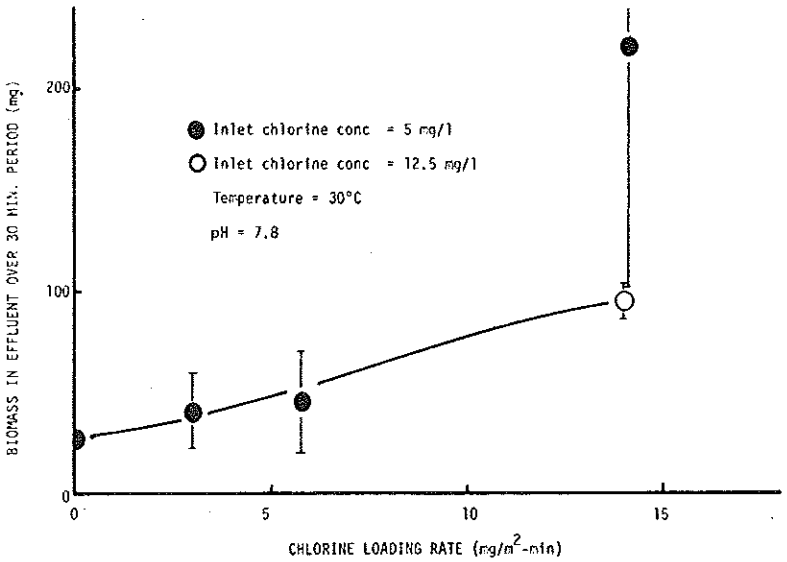

Figure 11. The influence of chlorine loading rate on biomass exiting the reactor.

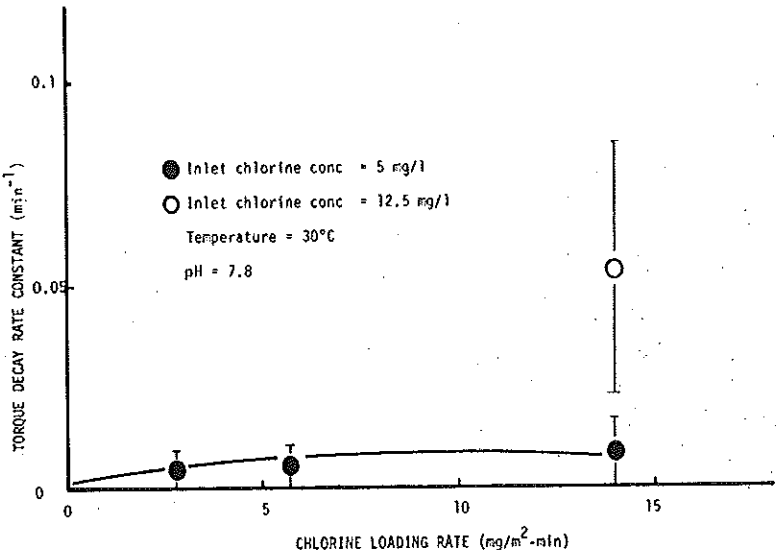

Figure 12. The influence of chlorine loading rate on torque reduction.

The influence of daily chlorine application on biofilm formation (as indicated by torque measurements) is presented in Figure 16 for two different inlet chlorine concentrations. The initial growth of biofilm is relatively slow compared to the rate of increase following chlorine applications. This may be due to any or a combination of the following:

1. Insufficient chlorine was applied for complete biofilm removal, and the partially oxidized, remaining organic deposit accelerated "regrowth."
2. A microbial population shift occurred because of chlorination, and the surviving organisms influenced torque to a higher degree (e.g., filamentous organisms).
3. The organic portion of the biofilm was oxidized but the inorganic fraction remained, providing a thin, rough surface more conducive to microbial attachment and growth.

The results suggest that continued application at the two rates would lead to increasing fouling in one case (inlet chlorine conc = 5 mg/l) and decreased fouling in the second case (inlet chlorine conc = 12.5 mg/l). Longer test periods are necessary for verification.

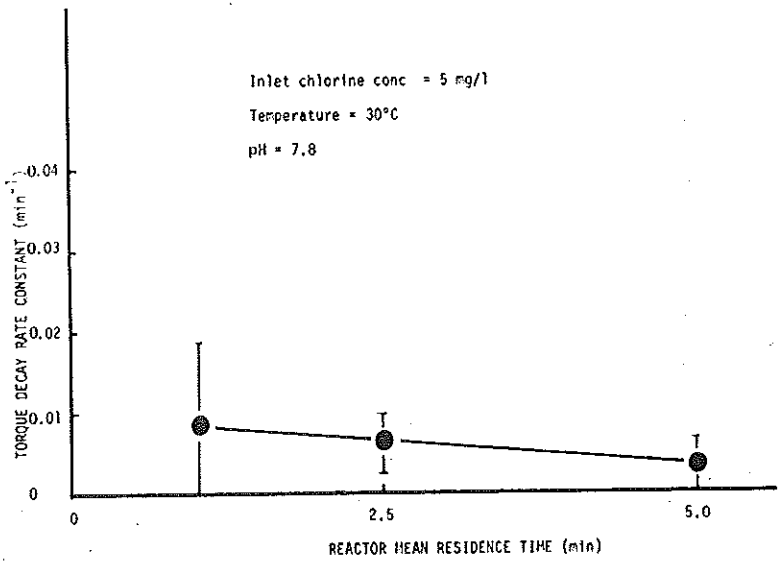

Figure 13. The influence of reactor mean residence time at constant inlet chlorine concentration on the torque decay rate constant.

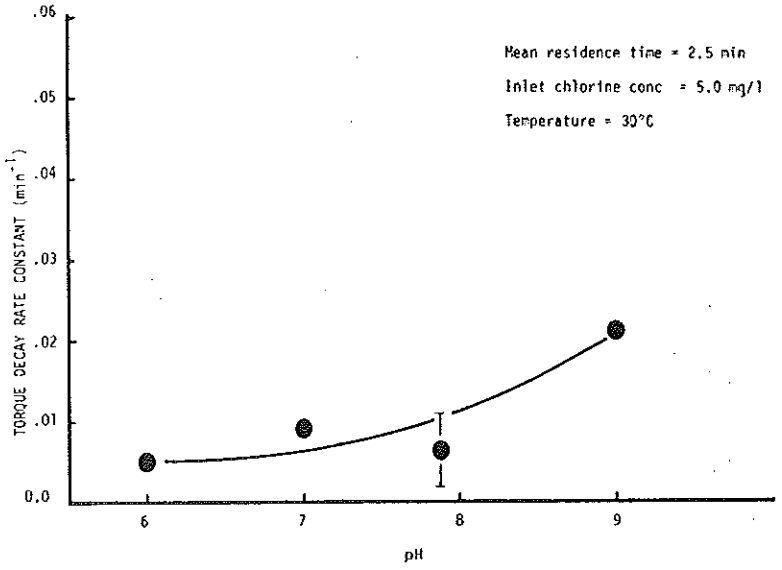

Figure 14. The influence of pH on the torque decay rate constant.

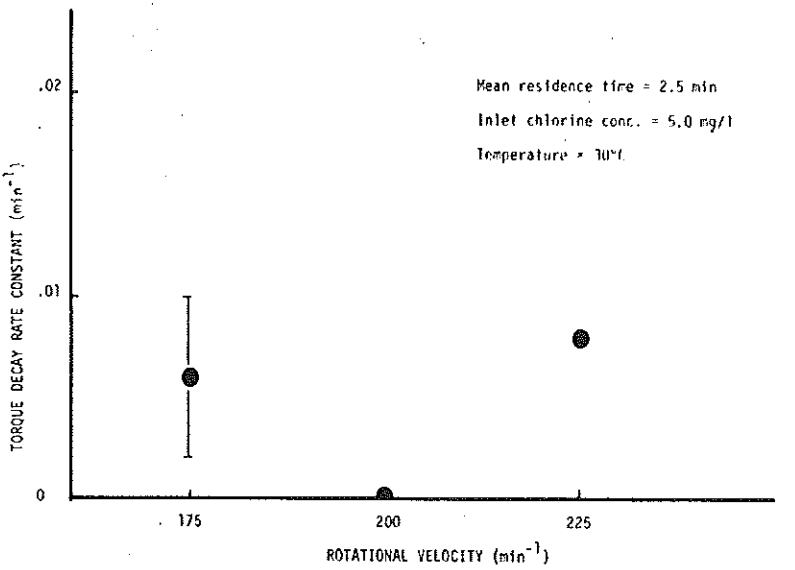

Figure 15. The influence of rotational velocity on the torque decay rate constant.

SUMMARY

Contacting attached biofilms with chlorine results in detachment of biofilm, dissolution of attached and detached biofilm and consumption of chlorine. The reported results suggest the following rate processes contribute to the overall rate of chlorine consumption.

1. Diffusion and reaction of chlorine in the attached biofilm: (1) diffusion rate into the biofilm is increased by increasing reactor chlorine concentration, as suggested by Figures 7 and 12, (2) reaction rate of chlorine is generally independent of depth as long as some biofilm remains. None of the reported experiments resulted in complete biofilm removal, (3) chlorine reacts with structural components of the biofilm (e.g., polysaccharides) inducing biofilm detachment,[4] (4) detachment of biofilm results in torque reduction, as indicated by Figures 10 through 12, although the relationship between torque reduction and biomass removal is not clear; and
2. reaction of chlorine with detached biofilm in the bulk reactor fluid (see results of batch reaction tests).

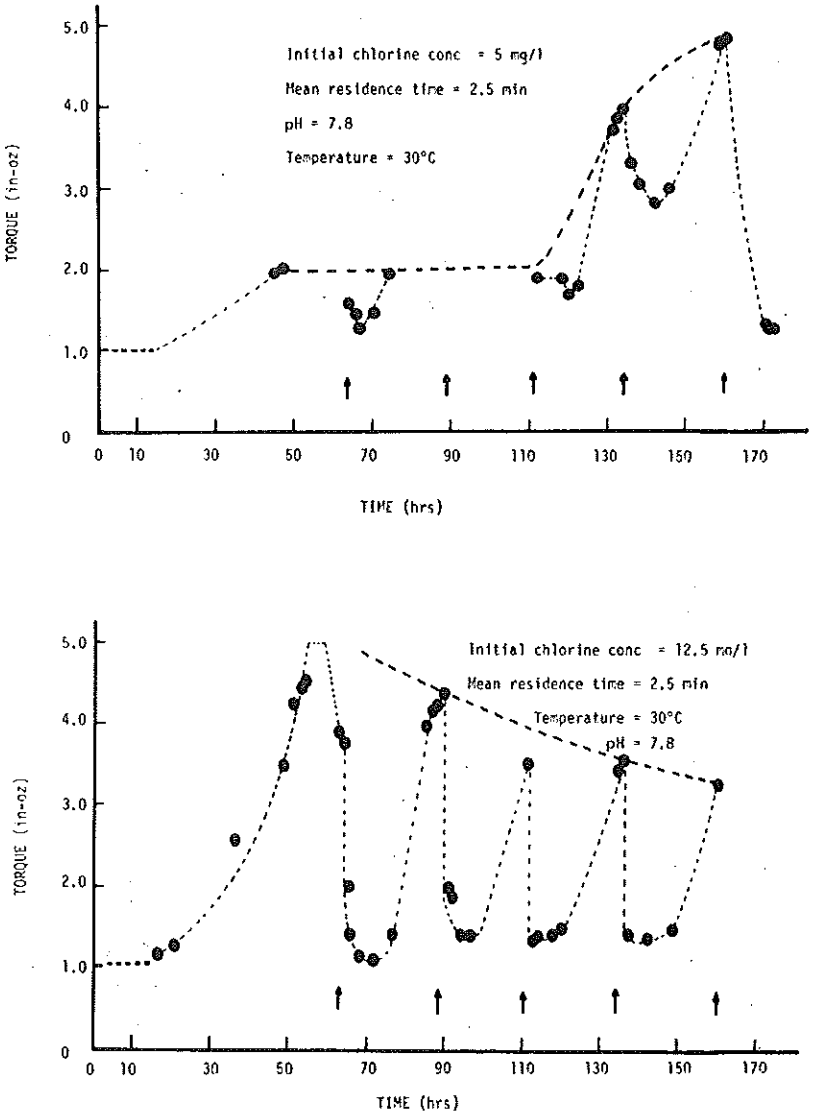

Figure 16. The influence of daily chlorine applications on biofilm formation as indicated by torque measurements.

Much more definition is needed to determine the fundamental rates in this reaction system and the factors that influence the rates. This research is being continued in our laboratories with more attention to complete oxidant and carbon material balances as well as molecular composition of the biofilm (e.g., protein, polysaccharide and nucleic acid).

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