71-001

Effect of Hypochlorite on Microbial Slimes

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INTRODUCTION

Deterioration of pipeline capacity has been attributed, in many cases, to growth of thin, attached microbial films. Striking examples of conduits incurring frictional losses of this type frequently appear in the technical literature (1,2,3,4). Mechanical cleaning of pipelines using inflatable "pigs" has been used as a remedial measure but it requires emptying the pipeline which becomes impractical in long pipelines or lines of varying cross-section. Anti-fouling paints or coatings have been used, but their maximum effective lifetime is 2-3 years (5). Because of the ineffectiveness of these and other methods, chemical additives are generally used as a means of controlling microbial films in long pipelines.

Chemical additives, or slimicides, are conceptually presumed to disinfect the pipeline, i.e., the slime is "killed" by the chemical, resulting in a decrease in frictional resistance in the pipeline. Previous investigators have suggested however, that the hypothesis of a "dead" slime may not be tenable (3,6,7,8). The Bethlehem Steel Company in Baltimore, Maryland spends approximately $150,000 per year for chlorine to maintain the capacity of a 96 inch pipeline carrying Baltimore City's treated sewage to its Sparrow's Point plant for use as process water. Such costs justify fundamental studies of the mechanism by which slimicides effect a reduction of frictional resistance in conduits.

The effectiveness of a slimicide is usually determined by observing reduction in cell numbers with different concentrations of additive and different reaction times. Determinations of this type are frequently carried out in well-mixed, batch reactors on dispersed bacterial cells. This procedure seems inadequate for the pipeline problem, considering the flow conditions and the nature of the microbial growth in a pipeline.

The research described in the first part of this paper was designed to account for the effect of controllable factors influencing the slimicidal action of chlorine on a thin slime layer at the boundary of a flow field. Investigations were conducted to determine 1) physical, chemical and biological effects of hypochlorite on the slime, 2) effects of high shear forces on the slime, and 3) whether nutrient concentration affected the response of the slime to hypochlorite treatment. A factorial experiment design was employed to test the variables using residual glucose concentration, soluble carbon concentration and percent light transmittance as dependent variables.

In the second part of the research, a system for measuring frictional resistance was utilized as a means for evaluating changes in drag force caused by the attachment of slime organisms and their response to chemical control agents. Results indicate an increase in frictional resistance due to slime growth and subsequent decrease upon hypochlorite addition presumably due to release of polymer material from the microbial film.

STIRRED REACTOR STUDIES

The stirred reactor studies were conducted to determine the effect of certain controllable variables on the slimicidal action of hypochlorite. The investigation determined 1) some of the physical, chemical and biological effects of hypochlorite on slime, 2) effects of relatively high shear forces, and 3) the effect of nutrient concentration on the response of slime to hypochlorite. The variables and testing levels used in the factorial experiment are listed in Table I.

TABLE I

VARIABLES AND TESTING LEVELS FOR
THE 2^4 FACTORIAL DESIGN

	Low Level	High Level
A. Glucose Feed Concentration	25 mg/l	50 mg/l
B. Carbon to Nitrogen Feed Ratio	100	50
C. Chlorine Treatment	0	25 mg/l
D. Scouring Treatment	No	Yes

Analytical and Experimental Techniques

The experimental system is schematically shown in Figure 1. Two such systems were operated simultaneously. A more detailed description of the experimental system and methods can be found elsewhere (6). The reactors were constructed from 2-inch Phyrex pipe with an operating capacity of 350 ml when the lower overflow was used (Figure 2). Mixing was accomplished by a 1.75 inch magnetic stirring bar and was constant for all experiments except during scouring treatment. Temperature varied from 22.5 to 26.0 C.

Experiments were initiated by growing a primary film in a batch operation for 24 hours using a sewage inoculation after which continuous feeding was maintained until the experiment ended. Mean detention time in the reactor was one hour, a sufficient time to virtually eliminate suspended organisms at experimental substrate feed levels. Treatment time was approximately 48 hours after inoculation with close monitoring of the dependent variables commencing 4.5 hours before chemical addition (i.e., treatment). The progress of a typical experiment is shown in Figures 3 and 4. The procedure in the mercuric chloride experiments was the same except that hypochlorite was added 20 hours after the initial treatment with mercuric chloride.

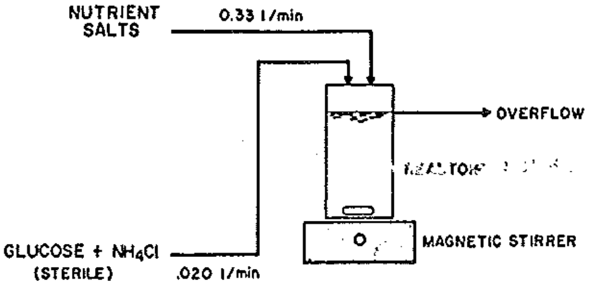

Figure 1 - Schematic diagram of experimental apparatus.

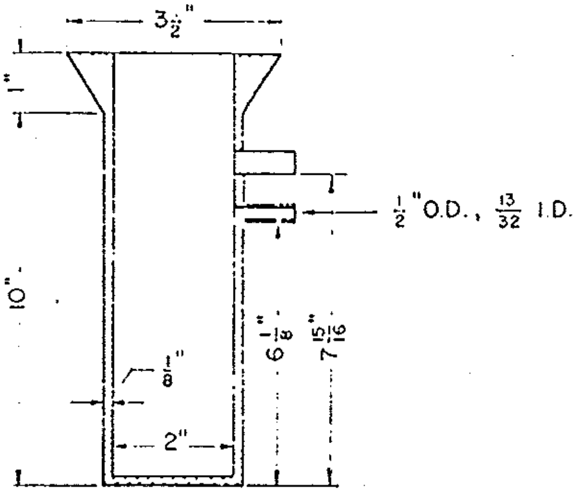

Figure 2 - Scale drawing of the experimental reactor.

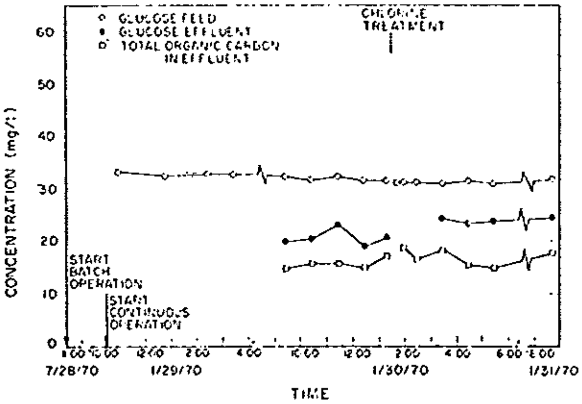

Figure 3 - Glucose and TOC concentrations during a typical experiment.

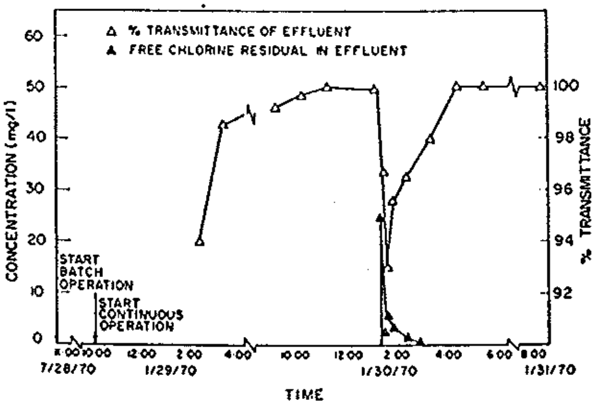

Figure 4 - Percent transmittance and free chlorine residual concentration during a typical experiment.

Chemical analyses were conducted using routine methods described elsewhere (9). Accurate glucose measurement was not possible while free chlorine or mercuric ion was present in the reactor due to the interference with the enzymatic reagent used for the analysis. Consequently glucose monitoring was suspended during those periods.

Treatment Procedures

Chemical treatment consisted of instantaneous addition of a small volume of concentrated solution (Table II). Scouring treatment consisted of increasing the stirring in the reactor to twice the normal operating speed (measured in rpm) for a 5 minute period.

TABLE II

CONCENTRATION AND VOLUMES OF CHEMICAL ADDITIVES

	Concentration of Stock Solution (gm/l)	Volume Added (ml)	Concentration in Reactor (mg/l)
Sodium hypochlorite	1.75	5	25
Sodium hypochlorite (conc.)	50	1.75	250
Sodium carbonate	31.1	2	178
Mercuric chloride	25.0	7	500

Results and Discussion

Hypochlorite Treatment. The results of the statistical analysis showed that only hypochlorite treatment had any effect on the dependent variables. Addition of sodium hypochlorite caused a significant decrease in the precent light transmittance (T) and increase in soluble organic carbon (SOC) of the effluent indicating that material was removed from the growth surface (Figures 5 and 6).

Previous research from the starch industry suggested that sodium hypochlorite was reacting with polysaccharide material in the slime causing transfer of polymer material to the bulk fluid. Research (7,10,11,12,13) has disclosed that the reaction of alkaline hypohalite with polysaccharide causes cleavage of polymers resulting in dissolution of previously bound molecules or fragments of molecules (causing increases in SOC). In the experimental system described above, changes in surface properties resulting from hypochlorite addition probably caused further detachment of slime material due to shear forces at the slime-liquid interface. In fact, subsequent analysis immediately after hypochlorite treatment showed total organic carbon concentration (TOC) to be twice the SOC, indicating that much of the material in the bulk fluid after treatment was not dissolved (i.e., did not pass a 0.45 μ filter) and was simply detached from the surface.

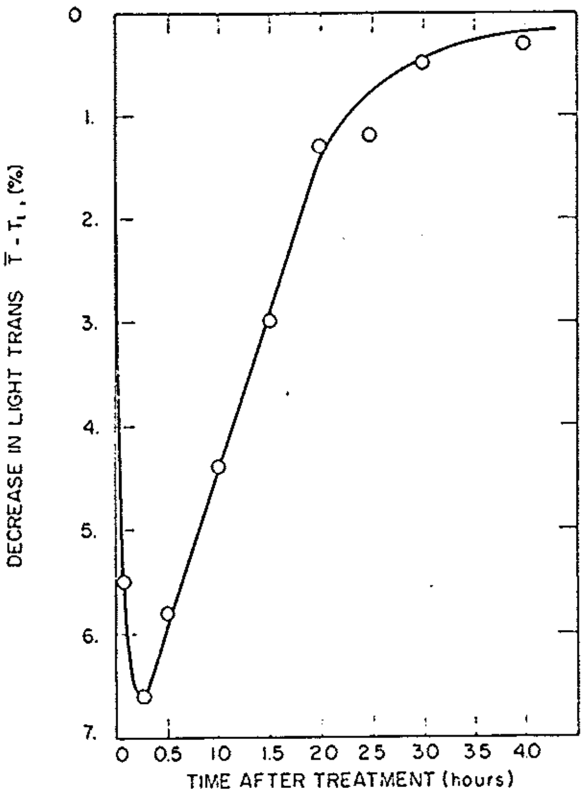

Figure 5 - Average change in percent light transmittance after hypochlorite treatment in 8 experiments.

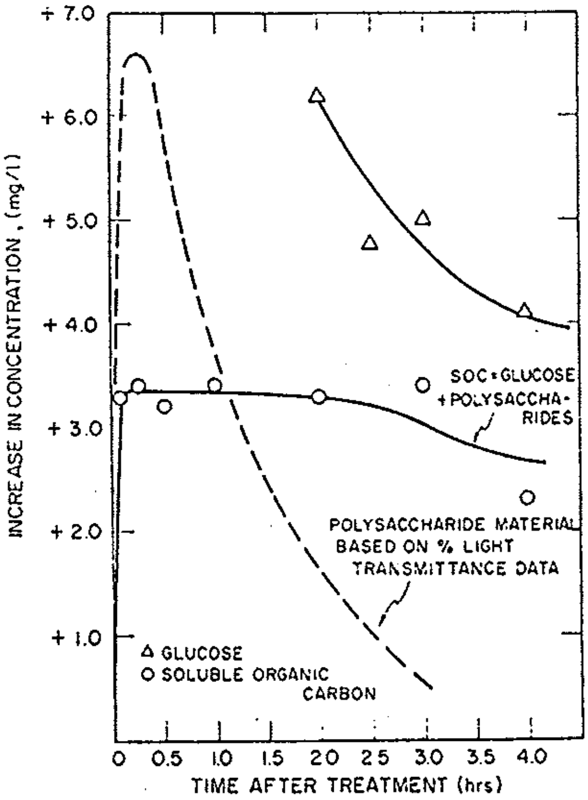

Figure 6 - Average change in glucose and soluble organic carbon in the effluent after hypochlorite treatment in 8 experiments.

Electron photomicrographs by Jones, et al (14) show the slime layer to consist of relatively few cells imbedded in a polymer matrix. Considering the rapid reaction of hypochlorite with the polymer material as evidenced by light transmission measurements, it was hypothesized that the change in activity of the microbial cells was not a major factor in the observed "sloughing" phenomenon. The hypothesis was tested by a series of experiments using mercuric chloride as a chemical additive.

Mercuric Chloride Treatment. To observe the possible change in the effect of hypochlorite in live and dead slimes, another bactericide had to be chosen. Ideally, the bactericide would stop cullular respiration (glucose uptake = 0) without affecting the slime physically (no change in effluent light transmittance and no change in effluent SOC except that due to decreased glucose uptake). Mercuric chloride (500 mg/l) served this purpose.

Ionizable mercury compounds poison biological systems by release of mercuric ion which reacts with sulfhydryl groups (15). The mercuric ion does not react appreciably with other materials in the slime. Glucose uptake started decreasing after Hg^{++} addition and was essentially zero 24 hours later.

Addition of hypochlorite at this point (24 hours after Hg^{++} addition) caused the same responses in the inactive slime. The percent light transmittance of the effluent decreased significantly in conjunction with increases in the SOC and TOC. Because of the hypochlorite addition, no further glucose measurements could be made.

Effects of Na^+ and pH. In order to establish a possible effect of Na^+, experiments employing NA_2CO_3 and NaOCl as treatment were compared. The Na^+ concentrations were equal (250 mg/l NaOCl and 178 mg/l Na_2CO_3) and 10 times normal experimental levels to exaggerate any possible effects. Maximum pH levels for the reactors after this treatment were pH 8.5 for NaOCl and pH 10.2 for Na_2CO_3, significantly higher than normal experimental conditions. The maximum pH change during the previous experiments was 0.5 pH units and the pH never exceeded 7.0 even after the addition of NaOCl (25 mg/l).

The high concentration of NaOCl induced complete removal of slime from the reactor surface. The observed removal was a process of dissolution occurring over a 3 hour period rather than a massive, instantaneous sloughing. Light transmittance and SOC measurements displayed the expected response to treatment based on previous experiments (Figure 7). Sodium carbonate treatment caused the same initial qualitative response although to a lesser degree. In contract to NaOCl treatment, the system treated with Na_2CO_3 recovered within 3-4 hours (Figure 7).

ANNULAR REACTOR STUDIES

The annular reactor apparatus was used to measure changes in drag force caused by the attachment of slime organisms and their response to hypochlorite addition.

Experimental System and Techniques

The annular reactor with a mounted torque monitor is shown in Figure 8. A more detailed description of the experimental system can be found elsewhere (9). The reactor was constructed of acrylic plastic with an operating capacity of 1750 ml

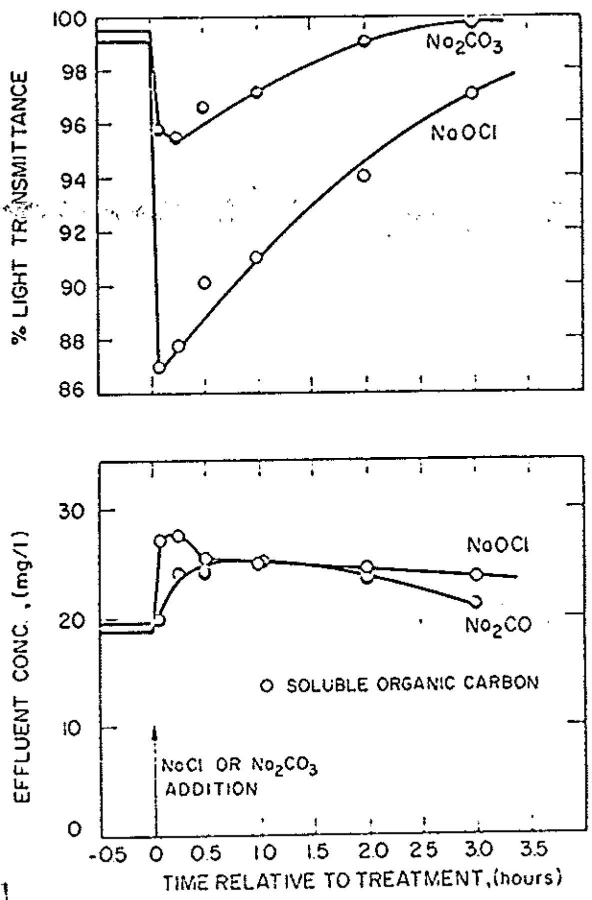

Figure 7 - *The effects of the addition of NaOCl (250 mg/l) and Na_2CO_3 (178 mg/l) on soluble organic carbon concentration and percent light transmittance of effluent.*

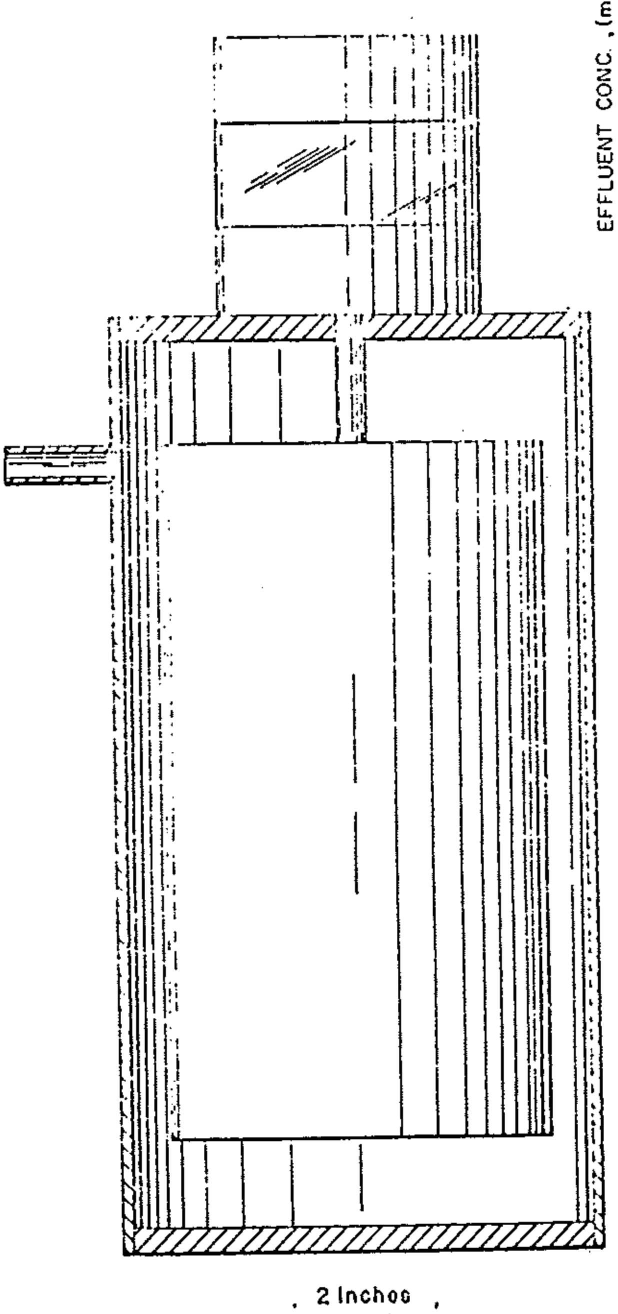

Figure 8 - *Schematic diagram of annular reactor with mounted torque monitor.*

and mixing was accomplished by a rotating inner cylinder.

The torque monitor (Model TDS-DN-TMI, General Thermodynamics Corporation, Wilmington, Mass.) with range of 0-5 inch-ounces measured the drag force at the rotating cylinder surface. Temperature in the reactor was maintained at 25.0 C. ± 0.5 C.

Initial attachment of the slime was accomplished in a batch operation for 24 hours using a sewage inoculation after which continuous feeding of glucose (50 mg/l) and nutrient salts was maintained. The hydraulic detention time was one hour.

Results and Discussion

Experiment WC-6 indicates the increase in torque exerted on the inner cylinder due to the growth of slime over a 6 day period. On the 7th day hypochlorite addition resulted in a substantial decrease in frictional resistance (Figure 9).

The system response to hypochlorite addition was similar to that observed in the stirred reactor experiments with regard to changes in percent light transmittance, SOC and TOC in the effluent. The torque values following hypochlorite treatment were sometimes below those observed in sterile systems (i.e. no slime or suspended microorganisms) which suggested that polymers released from the treated slime were effecting a drag reduction.

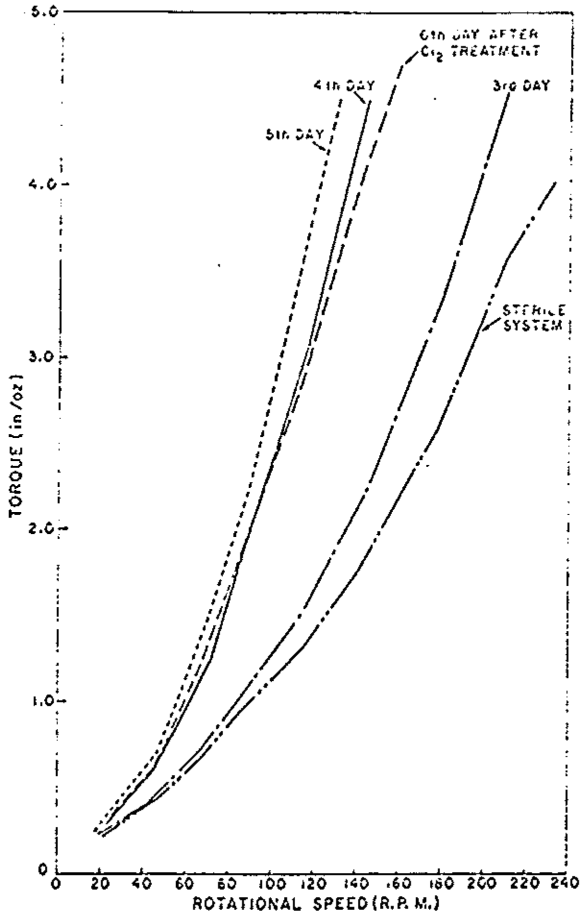

Figure 9 - Results of experiment WC-6.

Three experiments (HC-1, HC-2 and HC-3) were performed to test this hypothesis. HC-1 was a control with no treatment. For HC-2, the slime was treated with NaOCl after the initial torque measurement and an hour later was drained to remove any polymer materials in the reactor fluid. Subsequent measurements indicated an increase in torque which was possibly due to swelling or other surface irregularities caused by the addition of hypochlorite. For the third experiment, HC-3, the slime was treated with NaOCl after the initial torque measurements and an hour later the torque measurements were repeated without draining the reactor. Consequently, the reactor contained any polymer material that was released due to hypochlorite addition.

The results shown in Figure 10 indicate a substantial decrease in frictional resistance for HC-3. In some cases, torque measurements after NaOCl addition were less than those in a sterile system at the same rotational velocity. The results suggest that the drag reduction is being caused by polymers in the bulk reactor fluid. Drag reduction by polymer addition is well documented (14,16) and is most effective when it occurs in the boundary layer of the flow (16,17).

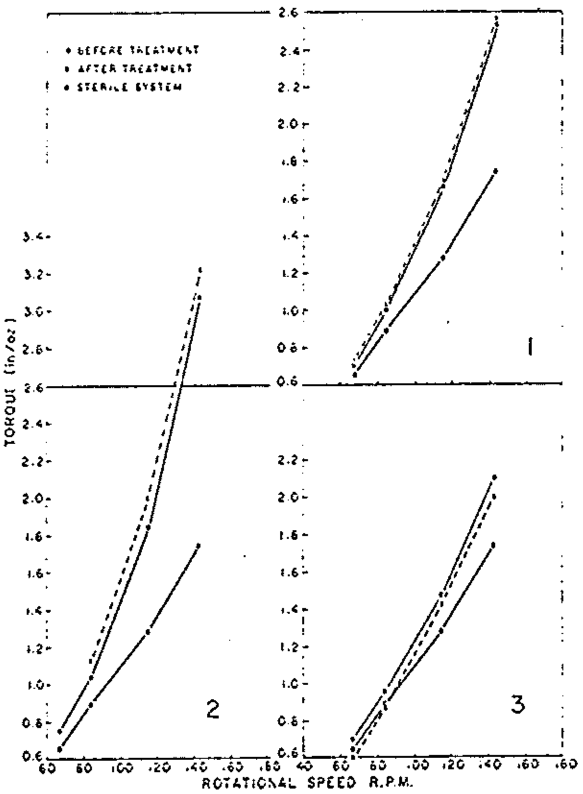

Figure 10 - Results of experiments HC-1, HC-2, and HC-3.

CONCLUSIONS

The data from this research and previous work suggest a mechanism by which hypochlorite interacts with attached microbial slimes. It should be understood that the conclusions pertain to conditions under which the experiments were conducted.

1. Hypochlorite reacts with polysaccharide material in the slime causing the release of organic polymers into solution.
2. Hypochlorite reaction with polymer material in the film causes surface changes resulting in the removal of these irregularities by fluid shear forces.

3. Simultaneously, hypochlorite inactivates exposed microbial cells which are washed out in association with detached polymer material.

Results also indicate that scouring treatment has no effect on the slime. Glucose feed concentration and C/N ratio in the feed did not affect the response of the slime to hypochlorite additions.

It is also suggested that methods for testing the effectiveness of a slimicide should be revised. Reduction in cell number as a function of additive concentration and reaction time is unsatisfactory. Conducting these experiments in a well-mixed batch reactor on suspended (as opposed to attached) organisms is also inappropriate since the effect of hypochlorite on slime appears to be largely a surface reaction.

On the basis of experiments conducted it is presumed that the release of polymers from the slime into the boundary layer of a turbulent flow regime due to the addition of hypochlorite causes the observed reduction in frictional resistance at the slime-fluid interface.

ACKNOWLEDGEMENTS

The study was supported in part by the U.S. Department of the Interior, Federal Water Quality Administration, under Contract No. 11010 DUZ (formerly WP-01262) and conducted at the research facilities of the Geography and Environmental Engineering Department, The John Hopkins University.

The author extends many thanks to Dr. Charles E. Renn and Dr. John C. Geyer for their advice and encouragement during the experimental work.

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