Water Research Vol. 10, pp. 515 to 522. Pergamon Press 1976. Printed in Great Britain.

THE INFLUENCE OF CARBON–NITROGEN RATIO ON THE CHLORINATION OF MICROBIAL AGGREGATES

W. G. CHARACKLIS and S. T. DYDEK*

Environmental Science and Engineering Department, Rice University, Houston, Texas 77001, U.S.A.

(*Received* 8 *January* 1975; *in revised form* 9 *July* 1975)

Abstract—Experiments were conducted with attached microbial films in a continuous flow reactor to determine the response of the film to hypochlorite treatment as a function of influent substrate concentration, influent carbon–nitrogen ratio (C/N) and shear force at the slime-water interface. Experiments were also conducted in batch systems with suspended organisms grown at varying C/N.

Results were obtained regarding the relationship of bactericidal action to slime destruction. Comparison of experiments with HOCl and Hg^{2+} indicate that bactericidal efficiency does not influence slime destruction. Destructive effects of hypochlorite oxidation were noted by measuring decrease in solids concentration of bacterial cultures.

INTRODUCTION

Organic material in sterile, dilute aqueous solution frequently reorients itself in order to attain a minimum energy state. This is accomplished by concentration of the matter at the air–water and/or solid–water interfaces (Renn, 1956). Work is done through decrease in the interfacial tensions existing at these points. In biologically active systems of this type, microorganisms accumulate at these interfaces, frequently with beneficial effects as in trickling filter operation, for organic waste water treatment and benthal removal of organics from natural streams.

More often, however, microbial attachment is undesirable as evidenced by problems of marine fouling (Lee, 1962; Horbund and Freiberger, 1970). Fouling of heat exchangers and cooling towers by microbial growths is a continuing maintenance problem for industry. In water and waste water treatment, attached growths reduce the operating effectiveness of carbon adsorption, filtration, ion exchange and membrane processes. Surface attachment also causes difficulties in scale-up of laboratory biological reactors (Hamer, 1972; Howell, 1972).

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 (Arnold, 1936; Derby, 1947; Minkun, 1954; Pollard and House, 1959). In one instance (Seifert *et al.*, 1950), the maximum capacity of a 24 in. (nominal diameter), 50 mile long water supply line was reduced to about 55% of its original value within a few years. The loss was due to a thin, slimy layer that consisted largely of organic material and was not caused by a substantial decrease in effective internal diameter (average height 0.025 in.). Further discussion of frictional resistance due to attached microbial growths can be found in a review paper by Characklis (1973b).

Mechanical cleaning of pipelines using inflatable "pigs" has been used but it requires emptying the line 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 yr (Jennings *et al.*, 1967). Because of the ineffectiveness of these and other methods, chemical additives are generally used as a means of controlling microbial films in long pipelines.

The effectiveness of a slimicide is usually determined by observing the reduction in cell number with different concentrations of additive and different reaction times (Mueller, 1968; Stundl, 1963). Determinations of this type are frequently carried out in well-mixed batch reactors and frequently omit adsorption of the chemical on solids and reaction with other chemicals on the system. Consequently, estimates pertaining to the amount of chemical required are frequently low (Curtis, 1969; Freedman, 1967; Stundl, 1963). Cell counts are usually made on non-specific culture media and all cells growing on the medium are counted. This procedure is based on several questionable assumptions.

(1) All cells growing on the medium are slime-forming organisms.

(2) Slime-producing organisms react with the chemical additive in the same way as other organisms.

(3) Organisms react to the additive in the same way in attached and suspended form.

(4) "Killing" the cells eliminates their attachment to surfaces.

Although chlorine has been used extensively under process conditions, it has frequently been ineffective

* Presently with Region VI, Environmental Protection Agency, Dallas, Texas, U.S.A.

in alleviating slime problems (Chambers, 1966; Curtis, 1969; Freedman, 1967). Because of its reactivity, it is reasonable to assume that chlorine is dissipated by the combined processes of diffusion and chemical reaction in the slime layer. It has been suggested that the gelatinous covering of the slime bacteria protects the cell from the lethal effects of the chlorine molecule (Curtis, 1969; Lamanna, 1965; Sanborn, 1944; Stundl, 1963), and consideration of hypochlorite–polysaccharide reactions indicates possible reasons.

This paper reports on experiments conducted with suspended microbial aggregates to evaluate "overall" kinetics and stoichiometry for hypochlorite oxidation as a function of carbon-to-nitrogen ratio (C/N) in the growth media.

Further experiments were conducted with attached microbial films for the following purposes.

(1) To determine the response of attached microbial films to hypochlorite treatment as a function of influent substrate concentration, influent C/N and shear force at the slime–water interface.

(2) To determine whether bactericidal action contributes to slime destruction.

METHODS

Flocculent growth reactor

Organisms. A bacterial seed was obtained from the primary effluent of a nearby sewage treatment plant. The seed was filtered (Whatman No. 2 filter paper) and 0.1 l. was added to a four liter Erlenmyer flask containing glucose (5 g) and tryptic soy broth (5 g) in a liter of water. The mixture was stirred by a mangetic stirrer and aerated with a submerged diffuser at 37°C. Ninety per cent of the contents were wasted daily followed by the addition of 5 g glucose and 5 g tryptic soy broth made up to one liter with tap water. Inocular for subsequent experimental cultures grown at different C/N were obtained from this seed culture.

An experimental culture was prepared by adding a 5 ml inoculum to a 2 l. Erlenmyer containing glucose and mineral salts (Tables 1 and 2). Total reactor volume was 500 ml. The flask was shaken mechanically at 180 min^{-1} at 20°C for 24 h.

Table 1. Initial concentration of mineral salts medium in homogeneous reactor batch experiments

$MgSO_4 \cdot 7H_2O$	22.5 mg/l
$CaCl_2$	27.5
$FeCl_3 \cdot 6H_2O$	.025
KH_2PO_4	8.5
K_2HPO_4	21.79
$Na_2HPO_4 \cdot 7H_2O$	33.4

Table 2. Initial carbon and nitrogen concentrations for batch experiments in the homogeneous reactor

	CARBON : NITROGEN			
	3	15	50	100
NH_4Cl	2900	578	174	88
Nitrogen	667	133	40	20
	Glucose - 5000 mg/l			
	Carbon - 2000 mg/l			

Table 3. Carbohydrate content of filtrates from cultures of varying C:N after 24 h incubation

C:N	Carbohydrate (mg/l)
3	72
15	60
50	106
100	100

Table 4. The composition of the inoculating seed mixture for the film reactors

Reactor Volume (liters)	0.350
Primary Effluent (liters)	0.340
Culture Media (liters)	0.010
Glucose (mg)	35
Peptonized milk (mg)	54.2
Peptonized meat (mg)	33.4

Apparatus. The batch reaction system consisted of 400 ml beakers stirred by magnetic bars on a six-place stirring apparatus. The reactor temperature varied between 25–26°C.

Media. Table 2 indicates carbon and nitrogen concentrations initially present for the batch experiments. The initial mineral salts concentration is shown in Table 1.

Experimental Protocol. After the 24 h incubation period in the shake flask, solids were measured gravimetrically by filtering through tared 0.45 μm membrane filters. Three replicates were run for each experiment (error $\pm$ 50 mg l^{-1}). Appropriate dilutions produced the desired solids concentration and 150 ml of the resulting suspension was added to the appropriate reactor.

Sodium hypochlorite (5.25% NaOCl) was diluted to 100 mg l^{-1} free chlorine and 150 ml measured into separate flasks, one for each reactor. The chlorine concentration of each flask was determined (variations in concentration at 95% confidence level were 1% for total chlorine and 2% for free chlorine). The experiment was initiated by adding 150 ml hypochlorite solution to the reaction vessel containing the bacterial suspension (dilution decreased initial free chlorine concentration to 50 mg l^{-1}). Because of the rapid reaction, more samples were withdrawn during the first hour of each experiment. Because the reaction continued during analysis until the titration endpoint, the time of reaction was the elapsed time to the end of the titration. During the first hour, variations in the total chlorine readings were $\pm$1.0 mg l^{-1}. After the first hour, variations decreased to $\pm$0.6 mg l^{-1}.

Fixed film reactor

Organisms. The source of organisms, or seed, for an experiment was obtained from the primary effluent of a nearby sewage treatment plant. The sample was added to a culture media (Table 4) and the resulting mixture was added to the reactor. The reactor was operated batch-wise for 24 h prior to starting the experiment to allow for the initial attachment of a slime film.

Apparatus. Two identical heterogeneous reactors were used. The reactors were fabricated from 2 in. glass (Pyrex) pipe. The contents were stirred by a 1.75 in. teflon coated magnetic bar. The reactors were supplied with a continuous feed and constant volume was maintained by an overflow tube (Fig. 1).

Dilution water was pumped to a constant head tank from which it flowed to the reactor. Sterile substrate was

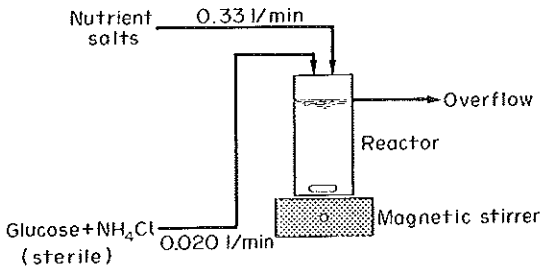

Fig. 1. Schematic diagram of experimental apparatus.

pumped directly into the reactors. Temperatures varied from 22.4–26°C.

Media. Feed substrate solution was prepared by adding the appropriate amounts of glucose and ammonium chloride to distilled water. The substrate solution was autoclaved at 120°C for 15 min. The substrate feed solution was added to a mineral salts medium just prior to entering the reactor. The resulting reactor feed concentrations are shown in Tables 5 and 6.

Experimental design. A 2^4 factorial experimental design was used to determine the effect of certain variables on the slimicidal action of hypochlorite in the heterogeneous reactor. The four independent variables were glucose feed concentration, C/N, hypochlorite treatment and scouring treatment. Table 7 describes the design. To insure valid statistical evaluation of the results, the experiments were performed in a random order.

Table 5. Concentrations of carbon and nitrogen in the film reactor feed

	C/N = 100		C/N = 50	
Glucose	50 mg/l	25 mg/l	50 mg/l	25 mg/l
Carbon	20	10	20	10
NH_4Cl	0.87	0.44	1.74	0.87
Nitrogen	0.2	0.1	0.4	0.2

Table 6. Concentration of mineral salts medium in the film reactor feed

$MgSO_4 \cdot 7H_2O$	22.5 mg/l
$CaCl_2$	27.5
$FeCl_3 \cdot 6H_2O$	0.25
KH_2PO_4	8.5
K_2HPO_4	21.79
$Na_2HPO_4 \cdot 7H_2O$	33.4

Table 8. Concentration of chemical additive in stock solutions and in the heterogeneous reactor

	Conc. of Stock Solution (mg/l)	Volume Added (ml)	Conc. in Reactor (mg/l)
Sodium hypochlorite	1,750	5.0	25
	50,000	1.75	250
Mercuric chloride	25,000	7.0	500

Table 9. Maximum fluid velocities at the wall of the heterogeneous reactor

	Linear Velocity (cm/sec)	
	Normal	Scour
Reactor 1	79	159
Reactor 2	146	324

Experimental protocol. Experiments were initiated by batch operation for 24 h using a sewage inoculum to enhance attachment. Following attachment, continuous feed was maintained at a mean detention time of 1 h, a flow rate sufficient to virtually eliminate suspended organisms at the prescribed substrate feed. Hypochlorite, mercuric chloride or scouring treatment occurred 48 h after beginning continuous operation, a sufficient time to attain steady state. In the case of mercuric ion treatment, an additional treatment consisting of hypochlorite dosing, occurred 20 h later. Samples were obtained from the reactor overflow and were immediately filtered (0.45 μm) and/or stored at 1°C for a maximum of 5 h before analysis except that analysis for hypochlorite was done immediately.

Chemical treatment consisted of an instantaneous or "pulse" addition of a small volume of concentrated solution (Table 8). Scouring treatment consisted of increasing the linear velocity at the wall to approximately twice the normal operating condition (Table 9).

Methods of analysis

Glucose was measured colorimetrically through use of a commerical enzyme preparation (Glucostat, Worthington Biochemical Corporation). Free chlorine residual was measured by a colorimetric analysis developed by Black and Whittle (1967). All light transmittance measurements were made with a Spectronic 70 (Bausch and Lomb). Total chlorine was measured by the iodometric method (Standard Methods, 1971). Total and soluble organic carbon were measured with a Beckman Model 915 TOC Analyzer. Polysaccharide was determined using the method of Dubois (1956). All other analytical techniques are described in Standard Methods (1971).

Table 7. 2^4 Factorial experiment design to determine effects of variables on the slimicidal action of hypochlorite in the film reactor

Independent Variables	Levels Low (-)	High (+)	Variable Designation
Glucose feed concentration	25 mg/l	50 mg/l	x_1
Carbon : nitrogen in feed	100	50	x_2
Hypochlorite treatment	0	25	x_3
Scouring treatment	no	yes	x_4

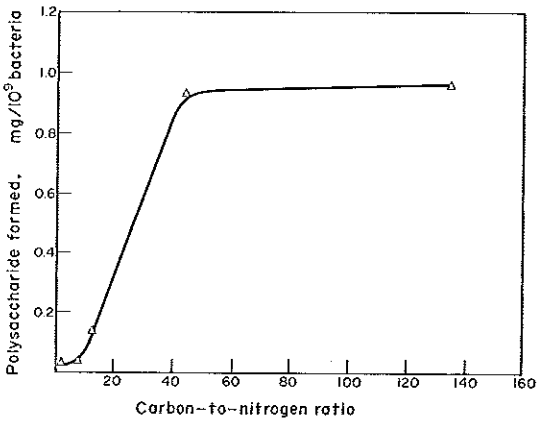

Fig. 2. Effect of carbon–nitrogen ratio on polysaccharide production by *Aerobacter aerogenes* (Duguid and Wilkinson, 1953).

Chemicals

Commercial sodium hypochlorite was used for all experiments (Clorox Co., Oakland, California). Bacteriological media used were manufactured by Baltimore Biological Laboratories. All other chemicals used were reagent grade.

RESULTS AND DISCUSSION OF HOMOGENEOUS REACTOR EXPERIMENTS

Polysaccharide production

A basic premise of this research is that bacteria grown under nitrogen-limited conditions produce larger slime capsules and thus, more polysaccharides than under balanced growth conditions. Duguid and Wilkinson (1953) found increased polysaccharide production at high C/N (Fig. 2). This was experimentally confirmed by microscopic examination and carbohydrate analyses of various culture filtrates (Table 3) using the method of Dubois *et al.* (1956).

Bacteria grown at C:N = 3 and 15 produced smaller capsules and less carbohydrate than bacteria grown at C:N = 50 and 100. A C/N greater than 7.5 is considered nitrogen-limited for similar systems (Busch, 1971). There was no significant difference in capsule size or filtrate carbohydrate concentration between C:N = 3 and C:N = 15, or between C:N = 50 and C:N = 100 (Table 3). The two cases are subsequently referred to as "high" or "low" C/N cultures.

Chlorine demand

Experiments conducted with 500 mg l^{-1} biomass growth at each of the four C/N indicated no significant difference between chlorine demand of cells grown with C:N = 3 and 15 or between demand of cells cultivated with C:N = 50 and 100 (Fig. 3). Subsequent chlorine uptake experiments were done with cultures grown at C:N = 3 and C:N = 50.

Microbial cultures grown in a high C:N environment exhibit a more rapid initial uptake and greater ultimate chlorine demand than cells grown at low C:N as indicated by data presented in Fig. 3. The difference is attributed to differences in polysaccharide content. As further evidence, chlorine uptake by bacterial culture filtrates was measured for a high and low C:N. The filtrate higher in carbohydrate content had a higher chlorine demand (Fig. 4).

Chlorine uptake was characterized by a rapid initial rate followed by a subsequent much slower rate due to the disappearance of free chlorine (Fig. 5). In the oxidation–reduction reaction, free chlorine is completely reduced to the chloride form or partially reduced to combined chlorine detectable by the total chlorine test. An overall materials balance for chlorine species yielded 90–95% recovery of starting materials (Figs. 6 and 7).

The effects of different initial chlorine concentrations were also observed. Experiments were conducted with cultures grown at C:N = 3 and C:N = 50, solids concentrations 500 mg l^{-1}, and chlorine concentrations of 25, 50 and 100 mg l^{-1} (Fig. 8). Further kinetic assessment of the data would require differentiation of reacting components (HOCl, OCl^-, etc).

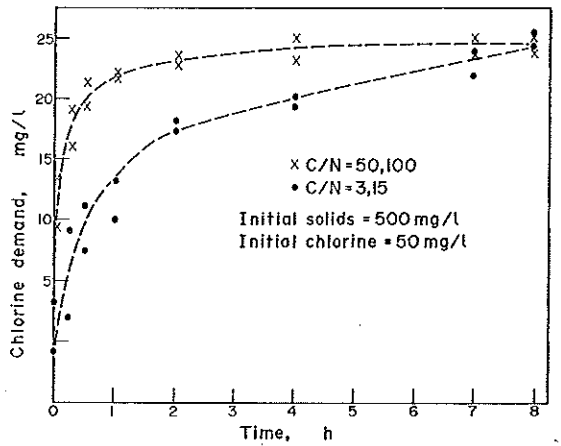

Fig. 3. Chlorine demand of a heterogeneous microbial suspension as a function of solids concentration and carbon–nitrogen ratio during cultivation. Initial chlorine concentration 50 mg l^{-1}.

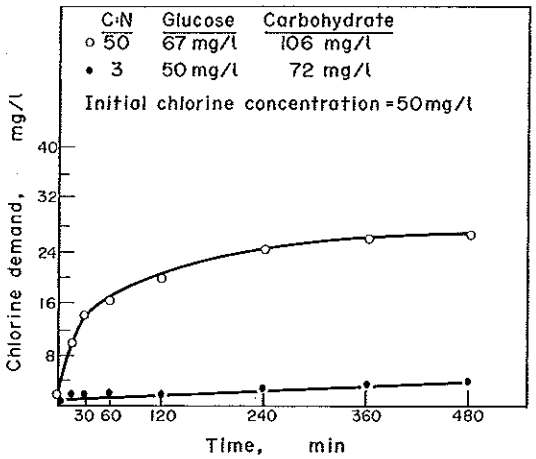

Fig. 4. Chlorine demand of culture filtrates employing different carbon–nitrogen ratios.

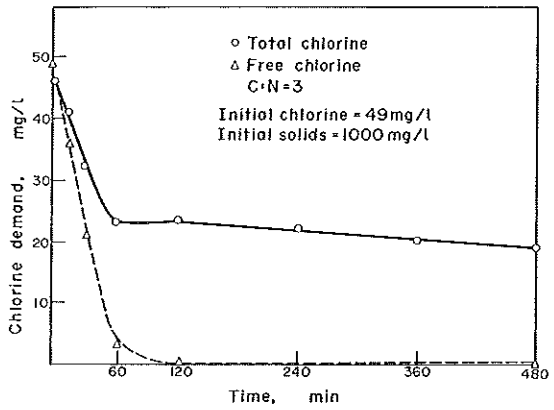

Fig. 5. Free and total chlorine demand of a heterogeneous microbial suspension.

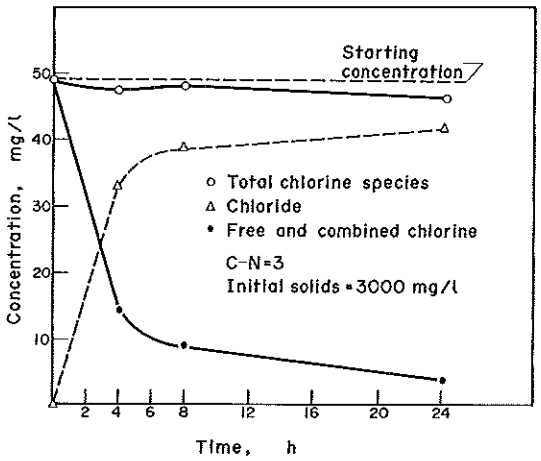

Fig. 6. Materials balance for chlorine species during a chlorine demand determination for a heterogeneous microbial suspension cultivated at carbon–nitrogen ratio equal to 3.

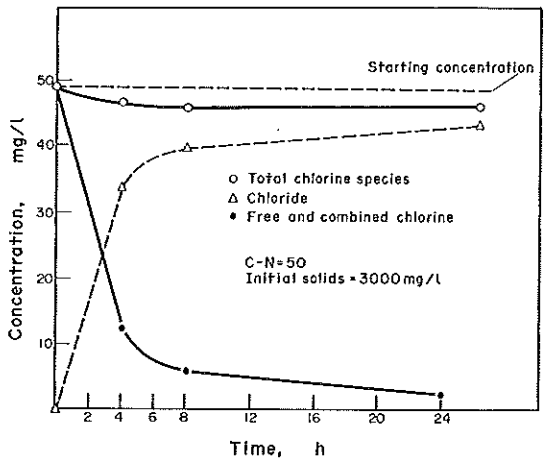

Fig. 7. Materials balance for chlorine species during a chlorine demand determination for a heterogeneous microbial suspension cultivated at carbon–nitrogen ratio equal to 50.

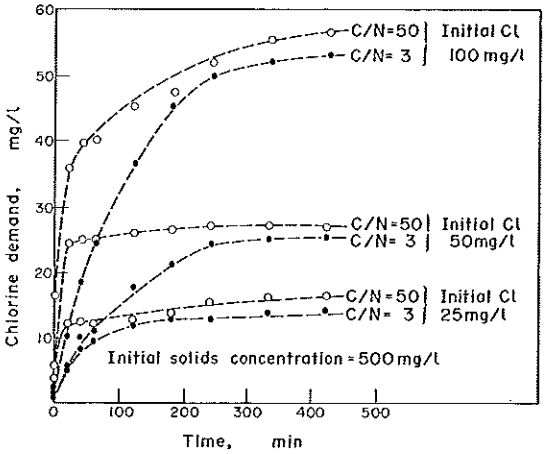

Fig. 8. Progression of chlorine demand as a function of initial chlorine concentration for heterogeneous microbial suspensions cultivated at carbon–nitrogen ratio equal to 3 and 50.

The results indicate possible diffusional limitations at the lower concentrations of chlorine (25–50 mg l^{-1}) but lack of data on aggregate size, shape and composition preclude further interpretation of the data in this regard.

Solids destruction

Measurements of solids concentration with time showed that both the rate and extent of solids destruction were dependent on the C/N during growth of the culture (Fig. 9). Solids "destroyed" were defined as those which passed through the 0.45 μm filter.

Since bacteria grown at high C:N produce large capsules while bacteria grown at low C:N produce small capsules, if any, these findings indicate that polysaccharides are degraded to a greater extent than the bacteria themselves. The results also suggest that

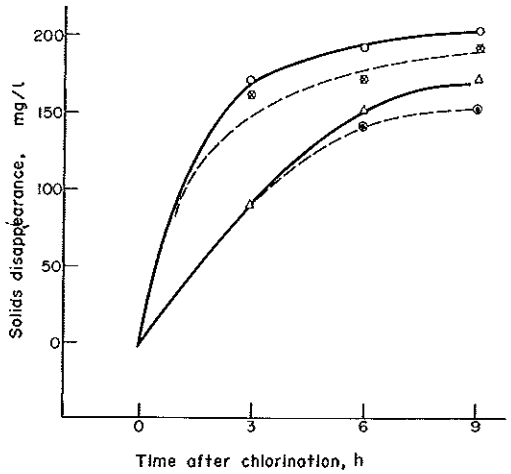

Fig. 9. Destruction of solids from heterogeneous microbial suspensions cultivated at various carbon–nitrogen ratios. Results are corrected for endogenous respiration.

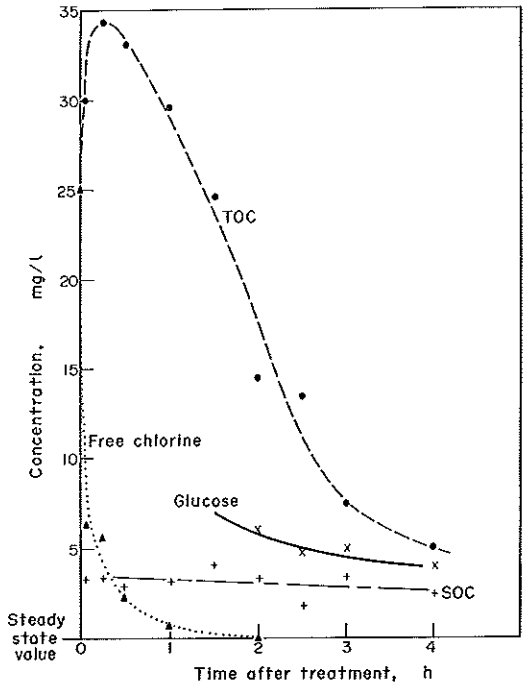

Fig. 10. Effect of hypochlorite addition on effluent TOC, SOC and glucose concentration from the heterogeneous reactor experiments.

more solubles pass through the filter after chlorination which could influence analyses of chlorinated effluents.

RESULTS AND DISCUSSION OF HETEROGENEOUS REACTOR EXPERIMENTS

Reactor performance prior to treatment

The % glucose removal by the attached biomass prior to treatment was independent of the glucose feed concentration and C/N in the feed. Average % removal was $26 \pm 8\%$ for sixteen experiments for a one hour detention time. Glucose removal was limited by available nitrogen, available surface area for growth or by diffusion of substrate to reactive sites in the film (Characklis, 1974). Soluble organic carbon (SOC) results indicate no significant difference in polymer excretion with change in C/N from 50 to 100. Turbidity data and batch tests with the reactor effluent indicate negligible amounts of active biomass suspended in the reactor.

Table 10. Varitations in dry weight of attached biomass (at 72 h) due to glucose feed concentration and carbon-to-nitrogen ratio during cultivation

Glucose Feed Concentration (mg/l)	C/N	Dry Weight (mg)
25	50	6.8
	100	10.0
50	50	16.6
	100	27.1

Dry weight of attached biomass increased with increasing glucose feed concentration and increasing C/N indicating higher yields due to increasing production of extracellular polysaccharides and decreasing production of carbon dioxide (Table 10).

Hypochlorite and scouring treatment

The results of the factorial experiment indicate that glucose concentration and C/N in the feed do not affect the response of attached biomass to hypochlorite addition. The effect of hypochlorite addition was quite drastic as indicated by its effect on dependent variables that were monitored e.g. turbidity, glucose, soluble and total organic carbon (SOC, TOC).

The effect of hypochlorite treatment was quantified by comparing steady state values of the dependent variables before treatment to values at various time intervals following treatment. Glucose removal was a measure of the attached *active* biomass in the slime. An increase in effluent glucose concentration following hypochlorite addition indicated that a decrease in viable cell numbers occurs but that the film was never completely "killed" at an initial hypochlorite dose of 25 mg l^{-1} (Table 11). Addition of 250 mg l^{-1} hypochlorite completely removed the slime in 2 h. Visual observations suggest a continual dissolution process as opposed to a massive sloughing of film. Hypochlorite attacks the polymers (primarily polysaccharides) which are responsible for the film structure, causing depolymerization and solubilization. Evidence for such reactions has been discussed elsewhere (Characklis, 1973a,b).

Further evidence for this mechanism is the change in effluent TOC concentration following hypochlorite

Table 11. Results from the factoral experiment to determine effects of glucose feed, C/N feed, HOCl and scouring on CSTR effluent

Glucose Feed (mg/l)	C/N in Feed	HOCl Dose (mg/l)	Scour	% Change[1] in Effluent Glucose (mg/l)	Increase[2] in Effluent TOC (mg/l)
25	50	0	no	-3.8	6.5
50	50	0	no	4.2	11.8
25	100	0	no	-8.2	13.0
50	100	0	no	-0.4	15.0
25	50	25	no	13.5	30.0
50	50	25	no	31.0	38.0
25	100	25	no	43.3	31.5
50	100	25	no	23.2	34.8
25	50	0	yes	1.0	8.0
50	50	0	yes	-2.0	23.5
25	100	0	yes	9.7	25.5
50	100	0	yes	2.3	15.0
25	50	25	yes	7.7	31.3
50	50	25	yes	16.0	30.3
25	100	25	yes	34.4	36.0
50	100	25	yes	37.1	38.0

(1) $\frac{[\text{Glucose before treatment} - \text{Glucose 150 min after treatment}]}{\text{Glucose before treatment}} \times 100$

(2) TOC 30 min after treatment − TOC before treatment

Table 12. Results from the factorial experiment to determine effects of glucose feed, C/N feed and Hg^{2+} addition on CSTR effluent

Glucose Feed (mg/l)	C/N in Feed	Hg^{++} Dose mg $HgCl_2$/liter	% Change[1] in Effluent Glucose	Increase in[2] Effluent TOC (mg/l)
25	100	0	-8.23	13.0
50	100	0	-0.43	15.0
25	50	0	3.76	6.5
50	50	0	-4.24	11.8
25	100	500	-15.29	-3.5
50	100	500	6.23	12.5
25	50	500	-32.90	2.5
50	50	500	-10.90	6.5

[1] [Glucose before treatment − Glucose 150 min after treatment] ÷ Glucose before treatment × 100

[2] TOC 30 min after treatment − TOC before treatment

addition (Fig. 10). TOC represents soluble and particulate carbon whereas SOC is soluble organic carbon as defined by membrane filtration (0.45 μm). An increase in TOC represents an increase in particularte organics. Prior to treatment, TOC was essentially the same at SOC. It is obvious from the results that a substantial amount of particulate carbonaceous material is removed from the reactor surface by reaction with hypochlorite (Table 11).

Scouring treatment exerted no statistically significant effect on the microbial film as evidenced by analysis of the reactor effluent.

Mercuric chloride addition

Additional experiments were conducted to determine if the bactericidal nature of the chemical additive was important to slime removal.

Mercuric chloride, a non-oxidizing bactericide, was added to the CSTR to give an initial concentration of 500 mg l^{-1} $HgCl_2$. Ionizable mercury compounds poison biological systems by release of Hg^{2+} which reacts with sulfhydryl groups on proteins (Lamanna, 1965). The mercuric chloride treatment effectively stopped glucose metabolism in the reactor. A significant increase in effluent glucose concentration was detected due to inactivation of cells by $HgCl_2$ addition. However, no significant differences in TOC (due to compounds other than glucose) and turbidity occurred due to HCl_2 addition (Table 12). In addition, visual observation of the film indicated no morphological changes.

Twenty-four hours after mercuric chloride addition, hypochlorite was added (25 mg l^{-1}) and the observed changes in effluent quality correspond with data obtained on slimes not treated with $HgCl_2$.

SUMMARY

Extracellular microbial polysaccharides affect the rate of chlorine demand and, to a lesser extent, the total chlorine demand in a microbial suspension. Hypochlorite addition also significantly reduces the suspended solids concentration as determined by the membrane filter technique.

Experimental observations indicate that hypochlorite reacts with attached microbial films grown at high C/N causing disruption and partial detachment from the inert growth surface. Hypochlorite inactivates a portion of the active biomass in addition to disrupting the film. Experimental results from mercuric chloride addition indicate that the oxidizing characteristics of hypochlorite are responsible for slime removal and not its bactericidal effectiveness.

Acknowledgement—The authors gratefully acknowledge the U.S. Environmental Protection Agency (Research Contract No. 11010DUZ), The National Science Foundation (ENG74-11957), and the Merck Foundation for partial support of this study.

REFERENCES

American Public Health Association (1971) *Standard Methods for the Examination of Water and Wastewater*, 13th Edition.

Arnold G. E. (1936) *Crenothrix* chokes conduits. *Engng News Rec.* **116**, 774–775.

Black A. P. & Whittle G. P. (1967) New Methods for the Colorimetric Determination of Chlorine Residuals. *J. Am. Wat. Wks Assoc.* **59**, 607–619.

Busch A. W. (1971) *Aerobic Biological Treatment of Waste Waters*. Oligodynamics Press, Houston.

Chambers C. W. & Clark N. A. (1966) Control of bacteria in non-domestic water supplies. *Adv. Appl. Microbiol.* **8**, 105–139.

Characklis W. G. (1973a) Attached microbial growths. I. Attachment and growth. *Water Res.* **7**, 1113–1127.

Characklis W. G. (1973b) Attached microbial growths. II. Frictional resistance due to microbial slimes. *Water Res.* **7**, 1249–1258.

Characklis W. G., Pipes D. M. & Matson J. V. (1974) Diffusion and reaction in microbial aggregates. *Proc. A.I.Ch.E.-VTG Joint Meeting*, Munich, Germany.

Curtis E. J. C. (1969) Sewage fungus: Its nature and effects. *Water Res.* **3**, 289–311.

Derby R. L. (1947) Control of slime growths in transmission lines. *J. Am. Wat. Wks Assoc.* **39**, 1107–1114.

Dubois M., Gilles K. A., Hamilton J. K., Rebers P. A. & Smith F. (1956) Colorimetric method for determination of sugars and related substances. *Analyt. Chem.* **28**, 350–356.

Duguid J. P. & Wilkinson J. F. (1973) The influence of cultural conditions on polysaccharide production by *Aerobacter aerogenes*. *J. gen. Microbiol.* **9**, 174–189.

Freedman A. J., Miller R. M. and Robertson R. S. (1967) The chemistry of refinery and petrochemical wastewater. Presented at Gulf Coast Reginal Meeting, National Petroleum Refiners Assoc.

Hamer G. (1972) Discussion of entrained droplets in fermenters used for the cultivation of single-celled microorganisms. *Biotechnol. Bioengng* **14**, 1–12.

Horbund H. M. & Freiberger A. (1970) Slimefilms and their role in marine fouling; a review. *Ocean Engng* **1**, 631–634.

Howell J. A., Chi C. T. & Pawlonsky V. (1972) Effect of wall growth and scale-up problems and dynamic operating characteristics of the biological reactor. *Biotechnol. Bioengng* **14**, 253–265.

Jennings D. M., Littauer E. L. & Woodhouse J. R. (1967) *Marine Fouling and Anti-fouling Techniques.* Lockhead Aircraft Service Co., Marine Research Laboratories.

Lamanna C. & Mallette M. F. (1965) *Basic Bacteriology.* Williams and Wilkins, Baltimore.

Lee R. W. H. (1962) *Marine Fungi and Algae—Their fouling problems and Control.* Prevention of Deterioration Center, National Academy of Science, PDL 44131.

Minkus A. J. (1954) Determination of the hydraulic capacity of pipelines. *J. New Eng. Wat Wks Assoc.* **68**, 1–10.

Mueller W. S. & Litsky W. (1968) Effect of various chemical agents for the inhibition of *Sphaerotilus natans* in paper mill process water. *Water Res.* **2**, 289–296.

Pollard A. L. and House H. E. (1959) An unusual deposit in a hydraulic tunnel. *J. Pwr Div. Am. Soc. civ. Engrs* **85**, PO6, 163–171.

Renn C. E. (1956) Biophysical processes in slime film and floc formation. *Proc. Conf on Biological Waste Treatment Manhattan College.*

Sanborn J. R. (1944) Slime-producing coliform and coliform-like bacteria. *J. Bact.* **48**, 211–217.

Seifert L. & Kruger W. (1950) Unusually high friction factor in a long water supply line. *VDI*, **Z92**, 189–191.

Stundl K. (1963) Testing the effect of inhibiting substances on slime-producing bacteria in recirculating water in paper factories using stirred cultures. *Zentbl. Bakt.* **91**, 203–208.

Wilkinson J. F. (1958) The extracellular polysaccharides of bacteria. *Bact. Rev.* **22**, 46–73.