A COMPARISON OF THE BIOCIDAL EFFICIENCIES OF FREE CHLORINE, CHLORAMINES, AND CHLORINE DIOXIDE ON THE HETEROTROPHIC IRON PRECIPITATING BACTERIUM, *Pseudomonas cepacia*

by

James Richard Rickloff

Thesis submitted to the Faculty of the
Virginia Polytechnic Institute and State University
in partial fulfillment
of the requirements for the degree of
MASTER OF SCIENCE
in
Environmental Sciences and Engineering

APPROVED:

W. R. Knocke

G. D. Boardman

R. C. Hoehn

June, 1982

Blacksburg, Virginia

ACKNOWLEDGEMENTS

This thesis was made possible through a grant awarded by the Office of Water Research and Technology through the Virginia Water Resources Research Center, whose funding and support was very much appreciated.
Dr. William R. Knocke and Dr. Gregory D. Boardman served as co-advisors on my committee; their patience, suggestions, and support were things I could always depend upon. Dr. Robert C. Hoehn was the third member on my committee, and he was always available to offer advice. I also appreciated his loaning a chlorine dioxide generator to my research. I was grateful to be working in a laboratory adjacent to Mr. Glenn Willard, who could always find or order materials which I desperately needed. Ms. Alice Kelling of the Microbiology Department must be thanked for offering her time and equipment when I required assistance in the identification of the isolated bacterium. Finally, I must thank H. W. "Buck" Cox for offering a supply of disposable test tubes which significantly reduced clean-up time.

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I. INTRODUCTION

Most annoying to all water utility personnel, as well as to the consumer, is deterioration of water quality that occurs between the treatment plant and the household tap. Once the potable drinking water leaves the treatment plant and enters the distribution system, substantial changes in water quality can occur. The observable effects of deterioration are numerous, including increased water discoloration, turbidity, and/or taste and odor problems. Such conditions, although not necessarily posing a threat to human health, still create "aesthetic" problems which require consideration and remedial action.

In the past, the problem of water quality deterioration within the distribution system has been investigated primarily with respect to chemical changes. It has only been in recent years that the potential role of microorganisms in mediating water quality problems has been studied and reported upon. A water distribution system is a sensitive, dynamic living "mini-ecosystem" which is conceptually well-suited to the development of bacterial growths. Sediment pockets in the distribution system and pipe sections encrusted with chemical deposits form a protected habitat for organisms surviving filtration and disinfection. Bacterial contamination introduced

from other sources into the distribution lines may be more sudden, resulting from plumbing cross-connections, loss of positive line pressure, or line breaks and their resulting repair. Regardless of point of entry, once the microorganisms are in the system, most are capable of long-term persistence in protected areas of the distribution network where they can undergo multiplication in the presence of trace concentrations of carbon and nitrogen containing nutrients.

Potable water of good bacteriological quality is generally associated with the attainment of less than one total coliform per 100 milliliters (ml) of water sample. Yet there are many other microorganisms common to the flora of finished water whose numbers far exceed those of the coliform group. It is doubtful whether anyone will ever be able to enumerate all of the organisms that may be found in distribution systems. However, selected members of the bacterial population in water can be measured accurately and their numbers related to water quality deterioration in the distribution system.

One common water quality problem, the bacterial deposition of iron, has been credited largely to the filamentous iron bacteria Crenothrix, Leptothrix, and the stalk-forming Gallionella (1-5). Over the past several years, it has become increasingly apparent that biologic iron deposits in water are probable even though iron

bacteria cannot be demonstrated. Starkey (1) stated that there were many different organic complexes of iron in natural waters, and that various bacteria were involved in the precipitation of iron. Many microbiologists who have microscopically examined iron precipitates in water have noted the presence of "zoogleal masses" of bacteria encrusted with iron, with no evidence of filamentous iron bacteria.

The major advantage of studying these bacteria, as opposed to the iron bacteria, are their relative ease of cultivation and enumeration. The use of a synthetic inorganic medium containing ferric ammonium citrate allows rapid growth (48 hours) and the resulting iron precipitation surrounding the bacterial cells permits fast, accurate counting on agar plates. Thus, study of the heterotrophic iron precipitating bacteria is not only feasible, but necessary when considering the various ways in which microorganisms can affect the concentration of iron in water distribution systems.

In determining remedial measures for controlling organism growth and water quality deterioration, disinfection is usually the first to be addressed. Disinfection involves an exponential decline in the concentrations of live organisms according to the laws of kinetics; however, the extent of the decline varies with the type of microorganism present and the particular disinfectant

in use. It has been shown that the presence of certain disinfectants in the bulk solution may not necessarily result in the control of water quality problems. If the problem is biologically mediated, it would appear that the bacteria are resistant or invulnerable to the disinfectant in the amounts generally applied. With chlorine being the disinfectant used most often in safeguarding water, it appears obvious that the mere presence of chlorine may not always provide the necessary safeguard against so-called nuisance organisms. Therefore, information related to the disinfecting capabilities of various chemical compounds in relation to organisms which have been isolated from water distribution systems possessing significant water quality problems would seem beneficial.

An examination of the literature shows that most information related to the efficiency of various disinfectants has dealt with pathogenic organisms. Little information is available regarding the applicability of various disinfectants to the control of microbial growths within water distribution systems, especially in relation to "nuisance" organisms.

As part of a research project which involved sampling a number of small municipal water distribution systems, a large number of sampling locations were found to be quite turbid, with many samples containing iron concentrations which far exceeded the Commonwealth of

Virginia potable drinking water standard of 0.3 milligrams per liter (mg/l). Microbiological examination ruled out the presence of the filamentous iron bacteria; however, heterotrophic iron precipitating bacteria were discovered in varying quantities.

The objectives of this research were to identify a heterotrophic iron precipitating bacterium which was isolated from a water distribution system within the Commonwealth of Virginia; determine the bactericidal efficiencies of chlorine, chloramines, and chlorine dioxide (ClO_2) on the isolated bacterium in order to examine their applicability in controlling or eliminating problems related to microbially mediated iron precipitation; and to investigate the environmental conditions that could alter the disinfectant's efficiencies against this bacterium.

II. LITERATURE REVIEW

Chlorination of water has long been an established method of disinfection for a majority of municipal water suppliers. Chlorine's pre-eminence has never been seriously challenged over the past 70 years, though efforts at finding a suitable alternative were reported in instances where chlorine-aggravated problems, such as the presence of tastes and odors, were encountered in the raw water. These and other problems have also occurred throughout distribution systems even though water quality was acceptable at the treatment plant. This is not to imply that chlorine should be abandoned as a disinfectant simply because of an increase in nuisance problems. Booster chlorination to guarantee a residual presence throughout the distribution system may be an answer; however, if this failed, secondary disinfection would most likely be accomplished by utilizing an alternative disinfectant.

Several criteria must be met before any disinfectant can replace chlorine as the primary disinfectant utilized within water distribution systems. Examples of applicable criteria are as follows:

- disinfectant stability during water distribution and storage;

- biocidal capabilities;
- effectiveness over a wide range of environmental conditions;
- fewer undesirable by-products;
- ease in residual measurement.

The importance of cost effectiveness and on-site generation difficulties should also be mentioned, but they shall not be discussed in this paper. Therefore, in this chapter, historical and technical critiques will be presented on these various criteria in order to examine their applicability in controlling and/or eliminating biologically mediated iron precipitation in water distribution systems. Specific details of iron precipitation by certain heterotrophic bacteria will also be investigated.

Disinfectant Stability

An important consideration in controlling nuisance problems is the stability of the disinfecting agent over a period of time and its ability to remain as a residual throughout the distribution system. Of the common disinfectants available today, a residual level can be maintained with all except ozone which, because of its reactivity, disappears very rapidly (6-7). Thus, if ozone is to be substituted for chlorine as a primary disinfectant, chlorine or another alternative must be

used together with ozone to provide for residual disinfection. Furthermore, Morris (8) reported that ozonation of polluted waters ruptured large organic molecules into fragments more easily metabolized by microorganisms. This fragmentation, coupled with the inability of ozone to maintain an active residual, led to increased slime growths and consequent deterioration of water quality during distribution. For these reasons, as well as the reported difficulty in residual measurement, it became apparent that ozone should not be investigated for controlling biologically mediated iron precipitation within distribution systems.

The relationship between chlorine residual and bacteriological quality in the distribution system has been a subject of great interest in the waterworks industry for years. As the classic bactericide, chlorine is added to most surface waters and to many ground waters before distribution to consumers. As widespread as the practice of chlorination is, however, agreement does not exist on how much and what kind of chlorine residual should be carried throughout the distribution system. The stability of chlorine is dependent upon the degree of organic contamination in the raw water and in the distribution system as well as the type and concentration of reducing agents present. The chlorine demand of the water must be satisfied before chlorine becomes available to

accomplish disinfection. Raw water demand is removed through the practice of breakpoint chlorination at the treatment plant. However, once the finished water leaves the plant and enters the distribution system, it is still subjected to a wide variety of contaminants which could significantly reduce the chlorine residual.

In the early days of disinfection, terminal treatment was the customary procedure for residual maintenance in the distribution system. Today, the use of booster chlorination at critical points in the distribution system has eased the burden of providing required minimum residuals (9, 10). As one would expect, few states have a fixed policy in this regard. Authorities usually relate requirements to local conditions and treatment objectives. Nevertheless, with the need for booster chlorination, chlorine's high reactivity is not one of its best selling points.

In some instances, unreliable free chlorine stability has led to the use of alternate disinfectants for distribution system protection. Sawyer and McCarty (11) indicated that the practice of ammonia addition to convert free chlorine to combined chlorine residuals was often preferred due to the quick dissipation of free chlorine in the distribution system.

Actually, in terms of secondary disinfection, chloramines have long been utilized due to their greater

stability. Vogt and Regli (12) reported that chloramines are commonly used as a secondary disinfectant, especially in waters with a high chlorine demand where they can provide protection against bacterial aftergrowth in lieu of free chlorine, which would be exhausted in the extremeties of the distribution system. This, in itself, would indicate that chloramines may be able to provide an environment within the distribution system which would not be favorable for biologically mediated iron precipitation.

Other investigators (13-15) have also recommended the use of chloramines in distribution systems (though not as primary disinfectants) to prevent secondary decomposition of heavily polluted surface water previously treated with pre- or super-chlorination. The use of chloramines would more likely be able to maintain a residual throughout the system, serving to prevent bacterial aftergrowths.

A number of water supplies are derived from rivers with high and often variable ammonia content. Chlorination of these supplies is always difficult. The quality of water is such as to make a free residual desirable due to its greater biocidal capabilities over chloramines. Inordinately high chlorine doses are often necessary to achieve breakpoint chlorination and constant feed rate adjustments are required to maintain a free chlorine

residual. Malpas (16) reported on two such circumstances that occurred in England where the feasibility of chlorine dioxide (ClO_2) was investigated. One feature of both trials was the stability of the ClO_2 residuals in the distribution systems. This was attributed to the lack of reaction between ClO_2, ammonia, and organic matter present within the systems.

Augenstein (17) reported on the use of ClO_2 in Hamilton, Ohio's treatment plant in order to meet United States Public Health Standards (USPHS) residual requirements for outlying areas of the distribution system. The chlorine demand of Hamilton's water supply was relatively low because the source of water was deep wells. However, chlorine residuals would have to be increased significantly to allow a 0.2 mg/l residual to be present in all points of the distribution system. Taste and odor problems were immediately a concern of the consumers, thereby allowing a study on the use of ClO_2. Subsequently, it was discovered that in addition to taste control, the ClO_2 residual was maintained in the system as far as fifteen miles from the point of application, equalling or exceeding the recommendations of USPHS. Therefore, a disinfectant such as ClO_2, with its good bactericidal capabilities and proven stability within the distribution system, would be a likely candidate for the control of a number

of nuisance problems affecting potable water quality.

Microbial Aspects of Disinfection

The primary reason for the use of disinfectants in potable water treatment is to kill or inactivate pathogenic microorganisms that may be present, thereby preventing the transmission of disease by drinking water. Secondarily, the presence of a disinfectant in the water distribution system helps maintain the quality of water by preventing the growth of nuisance organisms. The material presented in this section describes the relative merits of chlorine, chloramines, and ClO_2 as disinfectants from the microbiological standpoint, the second basic criterion of an acceptable secondary disinfectant.

Before examining the relative efficiencies of the disinfectants under consideration, it would be pertinent to mention the various means that disinfectants interact with microorganisms. Chemical disinfectant activity is dependent on contact between the disinfecting agent and the microorganism surface followed by reaction with the surface and/or penetration of and reaction with vital internal constituents resulting in death or inactivation of the microorganism. In the case of the disinfectants under consideration, the reactions that take place are oxidative in nature. Thus, oxidation potential is an important factor with regard to disinfection

capabilities. Hall (18) described hypochlorous acid (HOCl) as owing its power of oxidation to its components Cl and OH, which are both strongly electron-attracting and are in taut but fairly even balance. In comparison, the chlorine component of monochloramine has a greater share of the electron distribution; and its residual reactivity is, therefore, correspondingly less. With the dissociation of HOCl with increasing pH, the electron-affinity is largely satisfied and the resulting hypochlorite ion (OCl^-) is comparatively unreactive (18). The presence of the negative electrical charge also contributes to the low reactivity of OCl^- because it prevents its approach to and diffusion through cell membranes of microorganisms (19).

Theoretically, the oxidation capacity of ClO_2, in terms of available chlorine, is 2.5 times that of chlorine (20-21), assuming that all five electron changes are involved in oxidation. It has been shown, however, that ClO_2 does not oxidize to this extent in natural waters with pH ranging between 6 and 8. ClO_2 is an oxidant which reacts as follows:

$$ClO_2 + e^- \longrightarrow ClO_2^- \quad (1)$$

$$ClO_2^- + 4H^+ + 4e^- \longrightarrow Cl^- + 2H_2O \quad (2)$$

First, ClO_2 is reduced to chlorite; then chlorite, in the presence of sufficient hydrogen ions, is reduced to chloride (22). Thus, the entire oxidation-reduction of

ClO_2 is fundamentally tied to that of the chlorite. In waters ranging between pH 6 and 8, the reduction of ClO_2 is, for the most part, complete with the formation of chlorite which is further oxidized only in a medium of sufficient acidity. For this reason, ClO_2 is about as effective an oxidizing agent as HOCl which is illustrated in Equations 3 and 4:

$$HOCl + H^+ + Cl^- + e^- \longrightarrow H_2O + 2Cl^- \qquad E_o = +1.36\ V \quad (3)$$

$$ClO_2 + e^- \longrightarrow ClO_2^- \qquad E_o = +1.15\ V \quad (4)$$

Comparison of the oxidative properties of the disinfectants mentioned with the actual biocidal efficiencies is made difficult in the literature because of the lack of uniformity of test conditions among the various studies (23). By separating the environmental factors that can affect comparative disinfectants and discussing them in the following section, some generalizations and facts regarding the biocidal efficiencies of the disinfectants under consideration could be made. In spite of the differences in pH and temperature, a small group of data collected over the years were compiled into Table I for the purpose of critiquing the investigators' results.

From Table I, it can be seen that data accumulated during the 1940's produced varying results concerning the efficiency of ClO_2 when compared with HOCl. Bernarde *et al.* (21), citing Trakhtman (24), stated that ClO_2 exceeded or at least equalled free chlorine in biocidal

TABLE I. COMPARATIVE DISINFECTION EFFICIENCIES OF CHLORINE, CHLORAMINE, AND CHLORINE DIOXIDE

Year of Publication	Disinfecting Agents	Relative Efficiency	Conditions	Ref.
1946	ClO_2, HOCl	$ClO_2 \cong$ HOCl	pH 6	24
1947	ClO_2, HOCl	$ClO_2 >$ HOCl	0.1 mg/l, 30 min.	25
1949	ClO_2, HOCl, OCl^-	HOCl > $ClO_2 \gg OCl^-$	5° C	26
1958	HOCl, OCl^-	HOCl $\gg OCl^-$	viral, 0.1 mg/l	27
1965	ClO_2, HOCl, OCl^-	HOCl > $ClO_2 \gg OCl^-$	24° C	21
1966	HOCl, OCl^-, NH_2Cl	HOCl > OCl^- > NH_2Cl	5° C, 10 min.	28
1972	HOCl, OCl^-, NH_2Cl	HOCl > OCl^- > NH_2Cl	15° C, 40 min.	29
1974	NH_2Cl, $NHCl_2$, NCl_3	$NHCl_2 > NH_2Cl \gg NCl_3$		14
1977	ClO_2, HOCl, OCl^-, NH_2Cl, $NHCl_2$	$ClO_2 \cong$ HOCl > OCl^- > $NH_2Cl > NHCl_2$	viral, 15° C	30
1977	ClO_2, HOCl, OCl^-, NH_2Cl, $NHCl_2$	HOCl > ClO_2 > OCl^- > $NHCl_2 > NH_2Cl$	*E. coli*, 15° C	30

efficiency. Ridenour and Ingols (25) also reported that, on the basis of ortho-tolidine-arsenite (OTA) residuals, the bactericidal properties of ClO_2 were slightly greater than that of chlorine. In contrast to these statements, Ridenour and Armbruster (26) both agreed that HOCl definitely required less residual than ClO_2 for equal kill and that both disinfectants were superior to OCl^- on an OTA basis. Bernarde et al. (21) expressed doubts concerning the validity of these earlier investigations for a number of reasons, including the lack of knowledge on the physiochemical characteristics and kinetics of ClO_2, as well as the "now-known" interferences of the OTA residual measurement procedure.

Indeed, after examining the literature, the investigation of Bernarde et al. (21) appeared to be the first accurate examination of the disinfection efficiency of ClO_2. Using Escherichia coli (E. coli), the authors concluded that HOCl was somewhat more efficient than ClO_2 at concentrations less than 0.5 mg/l and that both compounds were equally efficient at an initial dose of 0.75 mg/l. They also agreed that OCl^- had little disinfectant value. Later investigations (8, 31) also agreed that ClO_2 was undoubtedly a powerful germicide with capabilities similar to HOCl against all known forms of pathogenic organisms. Little or no mention has been given to their efficiency in relation to the nuisance

bacteria.

Obvious from Table I are two facts relative to chlorine's disinfection efficiency. First, HOCl is definitely more effective than OCl^-; and second, HOCl is a more powerful disinfectant than any of its combined forms. Morris (28) stated that hypochlorite ion and chloramine are about 1% and 0.4% as effective as HOCl, respectively, when compared for different pathogenic bacteria. Data were also available for enteric viruses; again, chlorine efficiency data regarding nuisance organisms were lacking in the literature.

Although the literature is replete with information regarding the poor disinfecting powers of chloramine (14, 28-30), there are treatment plants that reportedly find chloramine disinfection to be effective and reliable (31-32), especially when the plants operate at high pH values. In fact, when the Environmental Protection Agency (EPA) proposed to "outlaw" the use of chloramines as a primary disinfectant (15), a number of water utilities took exception to the restriction. Subsequently, EPA concluded that the decision for chloramine use would best be made on a case-by-case basis.

When comparing the disinfection capabilities of the three chloramine species, Sletten (14) stated that dichloramine ($NHCl_2$) was the most effective followed by monochloramine (NH_2Cl), with minimal disinfectant

efficiency noted for nitrogen trichloride (NCl_3). In waters exhibiting a normal pH range of 6 to 8, NH_2Cl would predominate and, in fact, is preferred. Shull (15) stated that although $NHCl_2$ is a better disinfectant than NH_2Cl, its use should be avoided since it possesses a disagreeable chlorinous taste and odor.

Of particular interest to this research was the discovery in the literature that the City of Denver, Colorado, successfully applied a combination of chlorine and ammonia to its water distribution system to control nuisance bacteria (33). Though this fact has no bearing on chloramine's biocidal efficiency, a secondary disinfectant cannot rely solely on its efficiency capabilities when it becomes necessary to control nuisance problems within the distribution system.

It is clear that none of the three disinfectants under consideration are optimal in all respects regarding disinfection efficiency. Varying test conditions among investigators over the years have produced conflicting results or at least they prevented accurate comparisons between them. Figure 1 is a composite of results obtained in one laboratory (34) over a period of years using consistent experimental methods and microorganisms. The results show that ClO_2 at pH 7 and HOCl at pH 6 produce similar rates of inactivation of E. coli. Hypochlorite ion (OCl^-) at pH 10 was less effective,

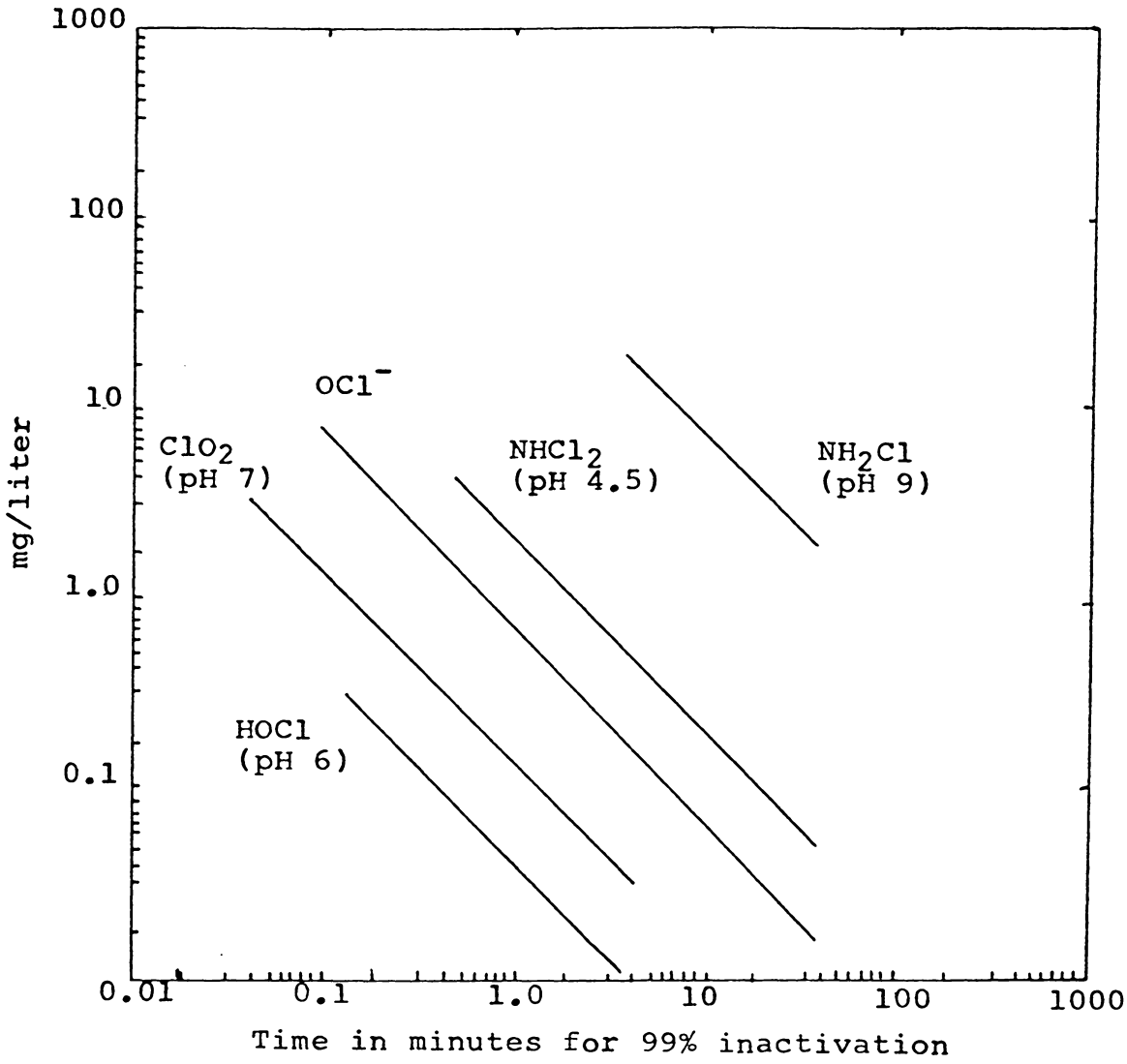

Figure 1. Inactivation of E. coli by free and combined chlorine species and chlorine dioxide at 15° C.

Adapted from: Scarpino et al. (34)

and chloramines were even less effective than OCl^-. Among the chloramines, $NHCl_2$ was found to be more effective against E. coli.

Environmental Conditions Affecting Disinfection Efficiency

The ability of a disinfectant to be effective over a wide range of environmental conditions is an important factor especially when water quality is variable (rivers) and where the climate is subject to change. More importantly, water quality may be altered with its passage through the distribution system.

The pH of the water being disinfected is a critical factor related to the efficiency of certain disinfectants, notably free chlorine, because of its influence on the nature of the existing species. In this case, disinfection efficiency declines rapidly as the pH is increased from 6 to 9 (31). This results from the change in disinfectant species present from predominantly HOCl at pH 6 to predominantly OCl^- at pH 9. If pH adjustment was ignored at a treatment plant where the pH of the water was variable, disinfection efficiency would likewise vary and permit possible contamination to by-pass treatment and enter the distribution system.

Studies (14-15) have indicated that increased pH values also have a pronounced effect on the bactericidal activity of chloramines. At a dose of 0.6 mg/l chloramine

and a pH of 7, a 100% kill would be expected in about 40 minutes. At pH values of 8.5 and 9.5, contact times would have to be increased 3 and 6 times, respectively, to produce a similar kill. This can be attributed to the presence of the more effective $NHCl_2$ at the lower pH. It should also be mentioned that too far a reduction in pH would impart objectionable tastes and odors to the water due to the formation of NCl_3. Also, as mentioned earlier, NCl_3 does not possess significant disinfectant qualities.

The pH of an aqueous solution of ClO_2 does affect the compound, but not in the same manner as chlorine (35). ClO_2 does not react chemically with water and does not dissociate as hypothesized in Equation 5, but can be forced to disproportionate to chlorite by raising the pH to 11 or 12 with caustic, as shown in Equation 6:

$$2ClO_2 + H_2O \rightleftharpoons ClO_2^- + ClO_3^- + 2H^+ \quad (5)$$

$$2ClO_2 + 2NaOH \rightleftharpoons NaClO_2 + NaClO_3 + H_2O \quad (6)$$

However, various authors (8, 23, 25, 36) have stated that the biocidal efficiency of ClO_2 is not affected by pH within the usual range for drinking water. Therefore, important potential applications are available for ClO_2 such as the disinfection of naturally alkaline waters and waters conditioned to high pH during softening or corrosion prevention treatment processes (25).

One investigation by Bernarde et al. (21) indicated an increase in efficiency of ClO_2 at a higher pH as shown

in Figure 2. However, the authors agreed that the pH of aqueous solutions did not directly affect the ClO_2, but rather, other factors probably contributed to the loss of efficiency. It may be that the rates of reaction of ClO_2 with substances found within the aqueous system are pH dependent, though such reactions have yet to be identified.

The presence of a strong temperature effect on the biocidal efficiencies of disinfectants suggests that the rate of kill of microorganisms is chemical by nature, as opposed to purely physical controlling steps (37). Ridenour and Armbruster (26) stated that usually the bactericidal effect of chlorine decreases somewhat with decreased temperature, as does that of ClO_2; however, small residuals were still adequate enough to kill the common water pathogens. Later studies (36, 37) also indicated that disinfection efficiency varied directly with temperature for a variety of concentrations of ClO_2.

The bactericidal activity of chloramine is also significantly reduced as the water temperature is lowered. Shull (15) reported that a 20^{o} C drop in temperature resulted in the need for a nine-fold increase in disinfectant exposure time or 2.5 times the chloramine dose to effect complete kill. Even though chloramine and the other disinfectants exhibit a strong temperature effect on their efficiencies of microbial kill, lower water

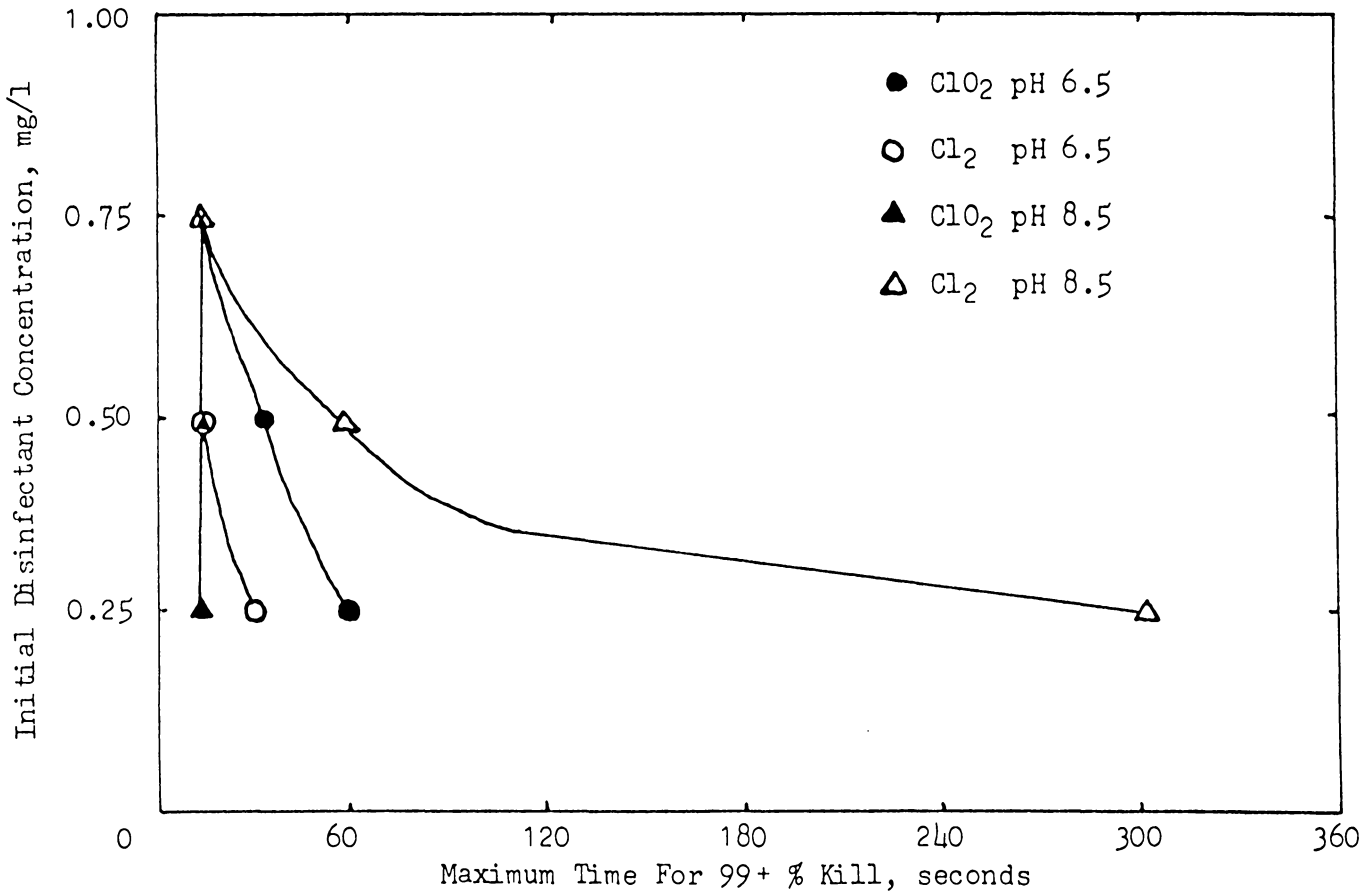

Figure 2. Effect of pH on kill of E. coli in organic free buffer. (24° C., 15,000 cells/ml)
Adapted from: Bernarde et al. (21)

temperatures are preferred in most instances even though disinfectant efficiency may be reduced. Biological activity within the distribution system is dependent upon the water temperature; and nuisance problems may be prevented by closely monitoring the water temperature and increasing the disinfectant residual when temperatures rise above a certain level.

For disinfection to be effective, contact must occur between the disinfectant and the microorganism to be inactivated or killed. It has long been considered that protection of microorganisms through their association with particulate matter could result in the shielding of microorganisms from disinfectant action (38-40). This, accompanied by problems with residual maintenance in distribution systems, has been the major consideration in establishing a turbidity limit for drinking water. The Interim Primary Drinking Water Regulations of 1977 set the maximum allowable turbidity level at 1 Nephelometric Turbidity Unit (NTU), except that up to 5 NTU may be allowed if it can be shown not to interfere with disinfection, residual maintenance, or microbiological determinations (40).

Little direct evidence of such protective effects has been available until recently. Scarpino *et al.* (30) reported that protection of bentonite-associated virus from various concentrations of ClO_2 was not found at turbidity levels at or below 2.3 NTU; protection did

occur at 3.2 and 14.1 NTU. Hoff (41) has shown that coliforms associated with washed primary effluent solids are inactivated by HOCl much more slowly than clean suspensions of E. coli (Figure 3). Comparable information for chloramines could not be found in the literature, but it would appear likely that these compounds would show the same limitations in efficiency when such solids were present.

Such evidence would seem to indicate that high turbidities in a distribution system could possibly help to aggravate biologically mediated iron precipitation by providing protection from a disinfectant residual. Also, the iron precipitate itself may provide some degree of protection since the precipitate is deposited around the cell sheaths (42).

By-product Formation

The practice of disinfecting drinking water with chlorine has come under scrutiny during the past decade. This increased scrutiny has resulted from findings that persons consuming water with high levels of trihalomethanes (THM's) have increased risk for experiencing carcinogenic effects (12, 23, 40). Viable alternatives for reducing THM's in drinking water subjected to chlorination include changing the point of chlorine application to permit the removal of precursor materials first, implementing process changes to minimize THM

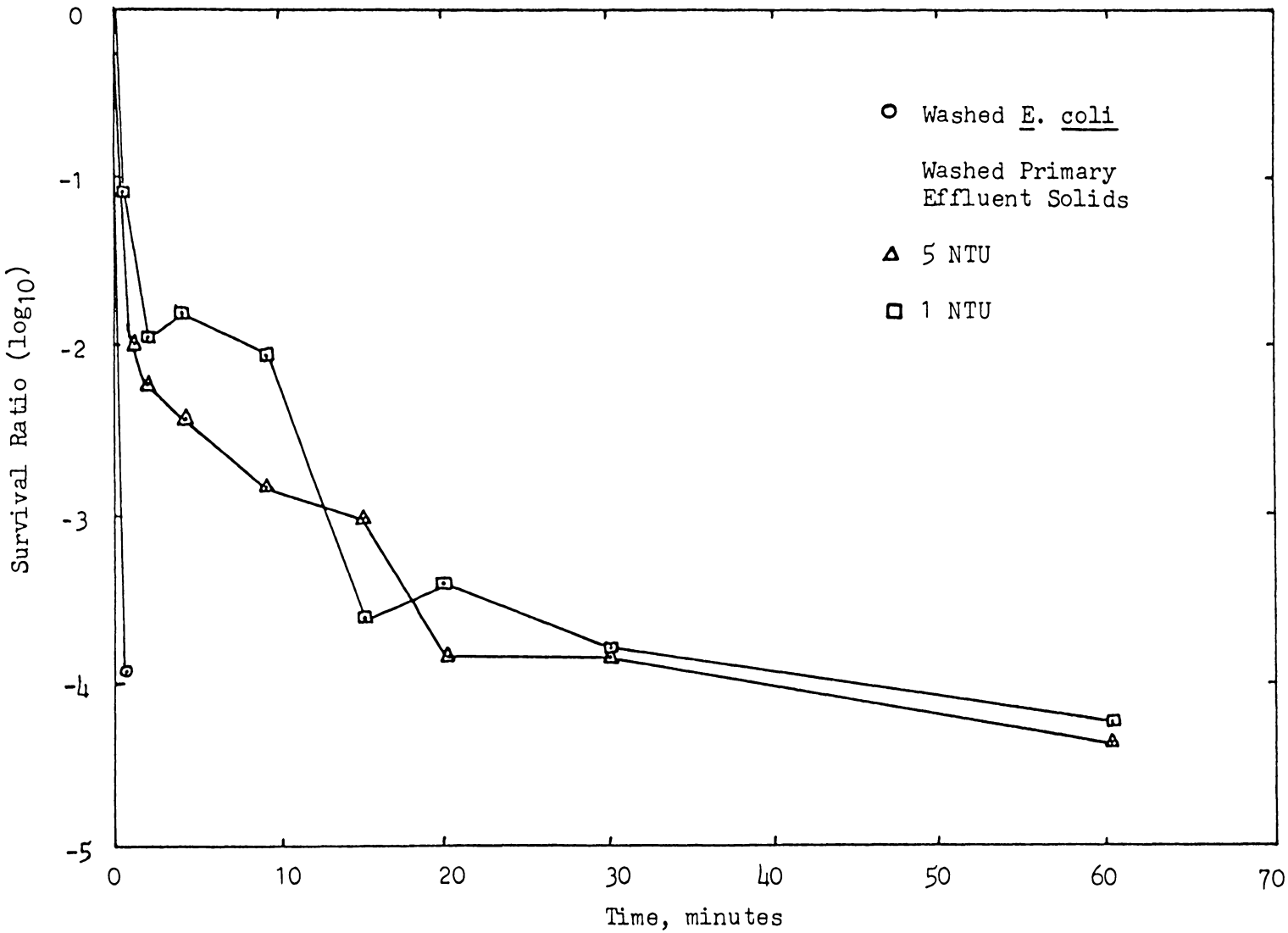

Figure 3. Inactivation of coliforms associated with primary effluent solids (pH 6, 5° C, 0.5 mg/l HOCl) Adapted from: Hoff (41)

formation or maximize precursor removal, THM removal after formation, and using an alternative disinfectant. If an alternative disinfectant is to be chosen to control nuisance problems in distribution systems, the disinfectant would naturally have to be safe for consumption. The purpose of this section is to present information exploring the health hazards associated with the use of alternative disinfectants. Naturally, chlorine should not be abandoned until or unless adequate substitutes, free from adverse side reactions, are available (6).

In the absence of free chlorine, all the alternative disinfectants under consideration do not produce THM's (12), but questions have been raised on their toxicology and the potential health effects of their by-products. In preliminary studies reported by Bull (43), there were no signs of overt toxicity in rats exposed to high concentrations of NH_2Cl. The only significant finding was a decrease in the amount of methemoglobin in the blood. Chloramines have been found, however, to be responsible for two epidemics of acute hemolytic anemia in dialyzed uremic patients (44).

The major research effort concerned with the direct toxicity of disinfectants has been directed at ClO_2. Inexperience with the use of ClO_2 in the United States at concentrations sufficient for primary disinfection in high demand waters dictated its investigation. Bull (43)

described exposure studies of rats that experienced increased mortalities at 100 mg/l ClO_2 and that the inorganic by-products of ClO_2, chlorite (ClO_2^-), and chlorate (ClO_3^-) were capable of producing methemoglobinemia. Other investigators (6, 22) indicated uncertainty about the toxicity of ClO_2, but they also reported that ClO_2^- and ClO_3^- produced symptoms of toxicity.

ClO_2 is also known for its high degree of reactivity with many aqueous organic compounds found within water such as phenol and humic materials. The human health effects of these compounds are unknown, although Bull (43) reported there was no increased incidence of skin tumors in mice treated with the organic concentrate obtained from ClO_2 disinfected Ohio River water.

It would appear that it is too early to draw any firm conclusions as to which alternative disinfectant, if any, would pose a reduced health hazard in comparison to chlorine. Though ClO_2 and its reaction products may pose a greater risk of toxicity than free or combined chlorine, its use may be advantageous if the only other choice is to produce a water containing possible carcinogenic by-products. In the near future, though, the use of alternative disinfectants to control THM formation likely will remain just an option instead of an answer to the problem.

Residual Measurement

The fifth basic criterion of an acceptable disinfectant is its ability at low concentrations to provide an easily measured, accurate residual. The accuracy and reliability of the amperometric titration method for solutions containing any form of residual chlorine was firmly established by Marks and Glass (45) and Palin (46). It was shown that HOCl quantitatively reacted with arsenite, while chloramine could not be titrated with this reagent unless iodide was present. By proper control of the pH and iodide concentration, free and combined chlorine could be precisely and quantitatively separated and measured with the use of a phenylarsine oxide titrant.

The measurement of ClO_2 residuals has been a subject of great interest over the years because of the difficulty of eliminating various interferences that made critical comparisons of ClO_2 and chlorine impossible. In a study conducted by Hodgden and Ingols (47), tyrosine was found to be selective for ClO_2; however, sensitivity was poor for ClO_2 concentrations less than 0.2 mg/l. Post and Moore (48) reported that o-tolidine and o-tolidine arsenite methods made no distinction between ClO_2 and chlorine which led to their recommendation of the indicator, 1-amino-8-napthol-3,6 disulfonic acid. This indicator proved to be most satisfactory in determining low concentrations of ClO_2. However, this procedure

did not distinguish between ClO_2 and ClO_2^-.

The basic Diethyl-p-Phenylene Diamine (DPD) Titrimetric method for free and combined chlorine was first published in 1957 (48). Since that time, a number of supplementary procedures have been added to meet the growing need for the differential analysis of other residual chlorine compounds, including ClO_2. In 1966, malonic acid was introduced to suppress any HOCl that may be present. Palin later reported (48) that glycine recorded superior results in suppressing chlorine by converting it to chloraminoacetic acid, which has no effect upon ClO_2. Chlorite interference was still a problem with the DPD method, and improved methods of differention were suggested by Myhrstad and Samdal (49). Palin initially suggested the use of extra disodium EDTA; however, further studies (50) preferred the addition of thioacetemide immediately to eliminate the ClO_2^-, thereby preventing color drift-back at the endpoints of the DPD titration.

Even with these modifications, investigators (51, 52) have experienced problems with low color production by ClO_2 with DPD, especially in low concentrations. This problem virtually eliminated the use of the titrimetric procedure. Spectrophotometric analysis was suggested as an alternate method, using standardized ClO_2 solutions to develop a reference concentration line. This method now offered a reliable and accurate determination of low

concentrations of ClO_2, thereby allowing the disinfectant to be measured not only at the treatment plant, but throughout the distribution system as well.

Microbially Mediated Iron Precipitation

In most discussions of iron precipitation by microorganisms, principal attention has been devoted to the iron bacteria. In fact, almost all other microbial activities which may be directly or indirectly related to iron precipitation have been excluded from investigation. This is an over-simplification of the subject which inadequately evaluates other important bacterial activities.

During the 1940's, it became apparent that biologic iron deposits in water were probable even though iron bacteria could not be demonstrated. Various investigators (1, 53) claimed that the involvement of the classical iron bacteria would not be necessary in many instances of microbially mediated iron precipitation. Certain non-specific bacteria could cause changes in the iron content of water by altering environmental conditions such as the decomposition of slightly ionized organic compounds of iron. The oxidation of these organics would ultimately lead to the precipitation of iron and the consequent deterioration of water quality. In such cases of decomposition by non-specific bacteria, the iron has no particular significance in the metabolism of the bacteria,

but is altered as a result of changes in the medium brought about by bacterial development (1).

Naturally, if this deterioration of water quality occurred during transport in a water distribution system, the observable effect would be a turbid water possessing a rusty color. Also, if the iron-precipitating bacteria were prolific enough to form a "zoogleal" slime, a measurable chlorine demand would be produced by oxidation of the organisms or of their metabolic products (55).

Clark et al. (42) isolated many heterotrophic iron-precipitating bacteria from drinking water in which iron problems were encountered. Enterobacter aerogenes was the most common organism isolated during their study, although various species of Serratia, Pseudomonas, and Bacillus were also frequently encountered. Two characteristics common to all of the isolated bacteria were their ability to utilize citrate as a sole carbon source and to produce a capsular material which was encrusted with iron. Lee et al. (56) reported isolating similar iron-precipitating organisms from a water distribution system in Columbia, Missouri. They concluded that these organisms were partially responsible for increases in the iron content of the water. Corrosion was generally not indicated because most of the water mains were lined with either bituminous materials or with cement.

These two investigations, as well as others (1, 57),

utilized a synthetic inorganic medium containing ferric ammonium citrate to cultivate their particular microorganisms of interest. Although it is doubtful that surface waters contain ferric ammonium citrate, they may contain other complexes of iron. Shapiro (58) studied the presence of unusually high concentrations of ferric iron in waters, describing "yellow acids" that could keep iron in a non-precipitable state for several weeks with the pH as high as 9.5. The author believed the acid compounds to be primarily aliphatic polyhydroxy, carboxylic acids, sometimes called tannins or humins, that are commonly found in surface water through the leaching of leaves, bark, and dead vegetation. With the capability of ferric iron to be soluble in nature, problems arise regarding its removal through conventional treatment. With the iron being in the ferric state, it cannot be removed by exposure to air and settling and it cannot be filtered from the medium because of its solubility.

Providing that an iron complex does by-pass treatment and enters the distribution system, it could come into contact with bacteria capable of decomposing the organics and allow soluble iron to be released into the water where it would precipitate out of solution as ferric hydrate (1). One possible treatment method of particular interest to this research was suggested by Masschelein (22). He stated that ClO_2 could be used in pre-treatment

to promote the micellization of protected colloids and the decomposition of organic molecules complexing iron and/or manganese. Treatment of the water with activated carbon could also eliminate the access of iron complexes to the distribution system by adsorption. However, many small utilities cannot afford this expensive treatment procedure.

In their investigation of iron-precipitating bacteria, Clark et al. (42) mentioned that cell growth was essential for iron precipitation. With water being continuously replenished throughout a distribution system, nutrient concentrations would be adequate enough to allow cell growth to occur if a proper energy source was available. The presence of small concentrations of organic iron complexes would facilitate the growth of many bacteria, with the result being iron precipitation within the capsular material of the organism.

As was mentioned earlier, aesthetic problems are the main concern when iron is precipitated out of solution. Yet, physical problems within the distribution system itself can also occur. MacRae and Edwards (54) indicated that colonization of the pipe surface by bacteria followed by precipitation of iron on the cells could lead to iron-plugging of water pipe lines. Characklis et al. (59) reported that such deterioration of pipeline capacity attributed to biofilm development

can be substantial. This would result in economic loss and inconvenience caused by shutdown of equipment and pipelines for cleaning. It is obvious from the literature that remedial measures for controlling biologically mediated iron precipitation should be investigated in order to assure a quality product for the consumer.

III. MATERIALS AND METHODS

The test microorganism selected for this study was a heterotrophic iron precipitating bacterium which was isolated from a water distribution system within the Commonwealth of Virginia. Morphologic and physiologic studies identified the isolate as Pseudomonas cepacia (P. cepacia). Specific details of the identification process will be discussed in a subsequent chapter.

Incubation and Growth of Cells

The culture medium used for the growth of P. cepacia was standard plate count broth which was manufactured by the Boston Biological Laboratories (BBL) and prepared in accordance with instructions given by the manufacturer. The recovery medium was ferric ammonium citrate agar. This medium was not available in dehydrated form and, thus, was prepared from the basic ingredients listed in Table II. The temperature of incubation for the growth of cells before disinfectant exposure and for recovery of survivors after exposure was 25° C.

Preparation of Cells for Exposure

Inoculum from the stock culture was subcultured onto separate slants of standard plate count agar (BBL)

TABLE II. INGREDIENTS COMPRISING FERRIC AMMONIUM CITRATE MEDIUM

Compound	Quantity/Liter
NH_4NO_3	0.5 g
K_2HPO_4	0.5 g
$MgSO_4 \cdot 7H_2O$	0.5 g
Ferric Ammonium Citrate	5 g
Distilled Water	1000 ml
Agar	15 g

and incubated for 24 hours. After this incubation period, the slants were stored at 4^{o} C to provide a stock of cells for future experimentation. In preparation of cells for exposure to a disinfectant, a 10 ml volume of sterile culture medium was inoculated with a loop of cells of P. cepacia taken from an agar slant. This was incubated at the appropriate growth temperature for a standard incubation time of 16 to 18 hours. After incubation, cells were harvested by centrifugation using a Fisher International Clinical Centrifuge (Model 28158H) at 3100 rpm for five minutes. After centrifugation, the supernatant was decanted and the cells were washed by redispersion of the cell pellet in 10 ml of sterile, demand-free, distilled water. This centrifugation and washing was repeated three times after which point it was felt that the cells were free of extraneous organic matter that might have been carried from the growth medium. After the final washing, the cells were resuspended in demand-free distilled water to yield a concentration of approximately 10^{7} to 10^{9} cells/ml. This was used as a stock cell suspension for the disinfection experiments. Such a stock cell suspension was prepared for each experiment such that cells in a similar phase of growth were used throughout the study.

Preparation of Disinfectants

The chlorine used in these experiments was prepared by dilution of a commercial bleach. Prior to each daily experiment, 0.25 ml of bleach was pipetted into 250 mls of demand-free distilled water which produced a stock solution containing approximately 240 mg/l free chlorine.

The formation of chloramine was accomplished by ammoniation of the reactor water with excess ammonium chloride (NH_4Cl) prior to the addition of the chlorine. The reaction between the NH_4Cl and the chlorine is not always instantaneous; therefore, the cells were not added to the reactors for at least 10 minutes to prevent exposure to free chlorine.

The chlorine dioxide (ClO_2) stock solution was generated using the sulfuric acid/sodium chlorite method recommended in Section 411A.2a of Standard Methods (60). The apparatus shown in Figure 4 was designed by McGhee (52); and for reasons of purity and safety, this generator was chosen for the project. The ClO_2 was swept out of the reaction chamber using air pulled by vacuum through the system instead of being introduced by positive pressure from an air compressor, as suggested in Standard Methods (60). McGhee's technique prevented the danger of having rubber stoppers blow out, and it eliminated the addition of oil droplets into the system.

The ClO_2 was collected in chilled, demand-free

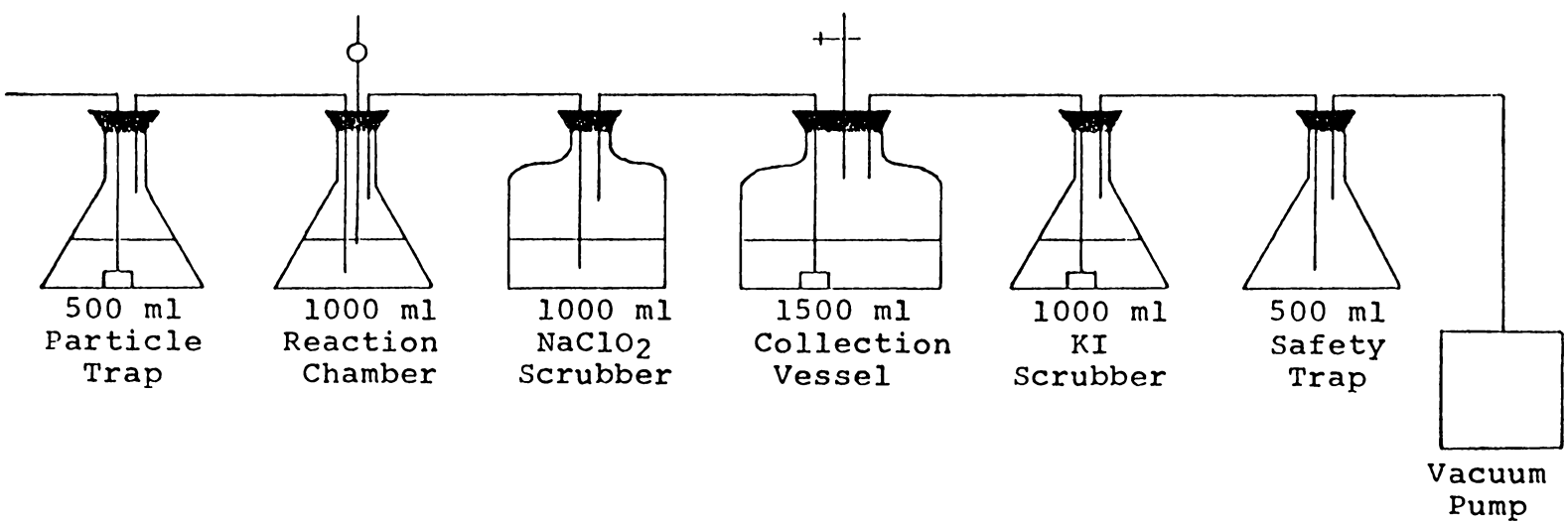

Figure 4. Schematic of chlorine dioxide generator.
Adapted from: McGhee (52)

distilled water for a period of 45 minutes, which produced a yellow-green stock solution containing about 200 mg/l ClO_2. The solution was transferred from the collection vessel to a one-liter, stoppered, amber bottle which was stored in a dark refrigerator until use. This procedure minimized the free space above the liquid and prevented the decomposition of the ClO_2 by light. Despite this protection, the stock solution was replaced within every three or four weeks with a fresh supply to maintain a constant titer.

Measurement of Disinfectant Residuals

Free and combined chlorine residuals were measured with a Fischer and Porter Amperometric Titrator (Model 17T1010) prior to and immediately following an exposure experiment. The titrator utilizes phenylarsine oxide as the titrant, the concentration of which is read directly in mg/l for chlorine. The procedure is explained in Section 409C.4 of Standard Methods (60).

The measurement of the ClO_2 residual was a two-step process. First, an aliquot of ClO_2 was removed from the stock solution and diluted to one liter with demand-free distilled water to produce a secondary standard ranging in concentration from 1 to 3 mg/l. The residual was measured three times using the N,N-diethyl-p-phenyldiamine (DPD) Method for ClO_2 as reported by Palin (50). McGhee (52) noted that the color developed in the DPD titrametric

procedure and was so light that it made titrations very difficult when low ClO_2 concentrations were present. Thus, residuals used in the exposure experiments were measured spectrophotometrically and a standard curve was generated to verify the ClO_2 concentration in the secondary standard. In this study, standards were prepared by dilution of the secondary standard solution that was standardized by the titrametric procedure. The dilutions, once made, were treated in the following manner:

1. 50 mls were transferred to a 125 ml flask containing 2.5 ml DPD indicator, phosphate buffer, and EDTA solution.

2. The sample was mixed and set aside for five minutes to allow for maximum color development.

3. The absorbance of the standard was measured at 552 nannometers (nm) on a Bausch and Lomb Spectronic 100 Spectrophotometer.

A standard curve was generated daily, and ClO_2 residuals were measured before and after each exposure with the use of this procedure.

Preparation of Demand-Free Distilled Water

The exposure substrate for this investigation was demand-free distilled water. A large glass carboy was filled with distilled water and 0.6 ml of chlorine bleach was added to produce a residual of approximately 5 mg/l. The chlorinated water was set aside for 24 hours to satisfy any demand that may have been present. A Pen

Ray Ultraviolet Lamp was then placed into the substrate and operated for at least six days to destroy the remaining chloro-derivatives. The final product was a sterile water free of any demand-causing substances. In order to obtain a demand-free turbid sample, the same procedure was followed, except kaolinite was added to produce a turbidity of 2 and 4 NTU and a Fisher Flexa-Mix magnetic stirrer was placed under the glass carboy to keep the clay in suspension. All disinfection studies were performed at 20^{o} C unless otherwise specified.

The substrate pH was adjusted to 6 with 1 N HCl or to 9 with 1 N NaOH prior to each respective experiment. The use of buffers was eliminated in order to reduce the addition of any demand-causing substances. The change in substrate pH over the reaction time was minimal, negating the potential for disinfectant species change during the experiment.

Reaction Vessels

Disinfection studies were accomplished in sterile, 1000 ml beakers that were previously acid- and chlorine-washed to remove all demand-producing substances. Throughout the entire exposure period, the beaker contents were stirred with acid- and chlorine-washed glass paddles which were sterilized by ultraviolet light. The paddles were operated at 50 rpm with the use of a

Phipps and Bird laboratory stirrer (Richmond, Virginia).

The ClO_2 reactor (shown in Figure 5) was constructed from Plexiglas pipe with dimensions 3.5 inches inside diameter and 7.5 inches high. The volume was slightly more than one liter, though only 500 ml was used during each test. One end was sealed with a Plexiglas plate. To minimize the loss of ClO_2 to the atmosphere during an experiment, a removable Plexiglas plate was fashioned for the reactor. A rubber gasket was placed inside a groove to permit a tight seal when the plate was screwed onto the reactor. To prevent decomposition of the rubber gasket, it was coated with a silicone compound. Near the center of the plate, a small hole was drilled and a piece of glass tubing inserted and siliconed into place. Tygon tubing was then placed on the outside end of the glass tubing. This exit hole was used to eliminate the vacuum created when samples were removed. It was immediately closed after sampling with a clamp.

For sampling purposes, a one inch diameter hole was placed in the side of the reactor at the 250 ml mark. A #6 rubber stopper, coated with silicone and having a glass pipet through its center, was placed into the side of the reactor. The outside end of the pipet was connected with Tygon tubing and a pinch clamp to a 10 ml Cornwall syringe. During each experiment, a magnetic

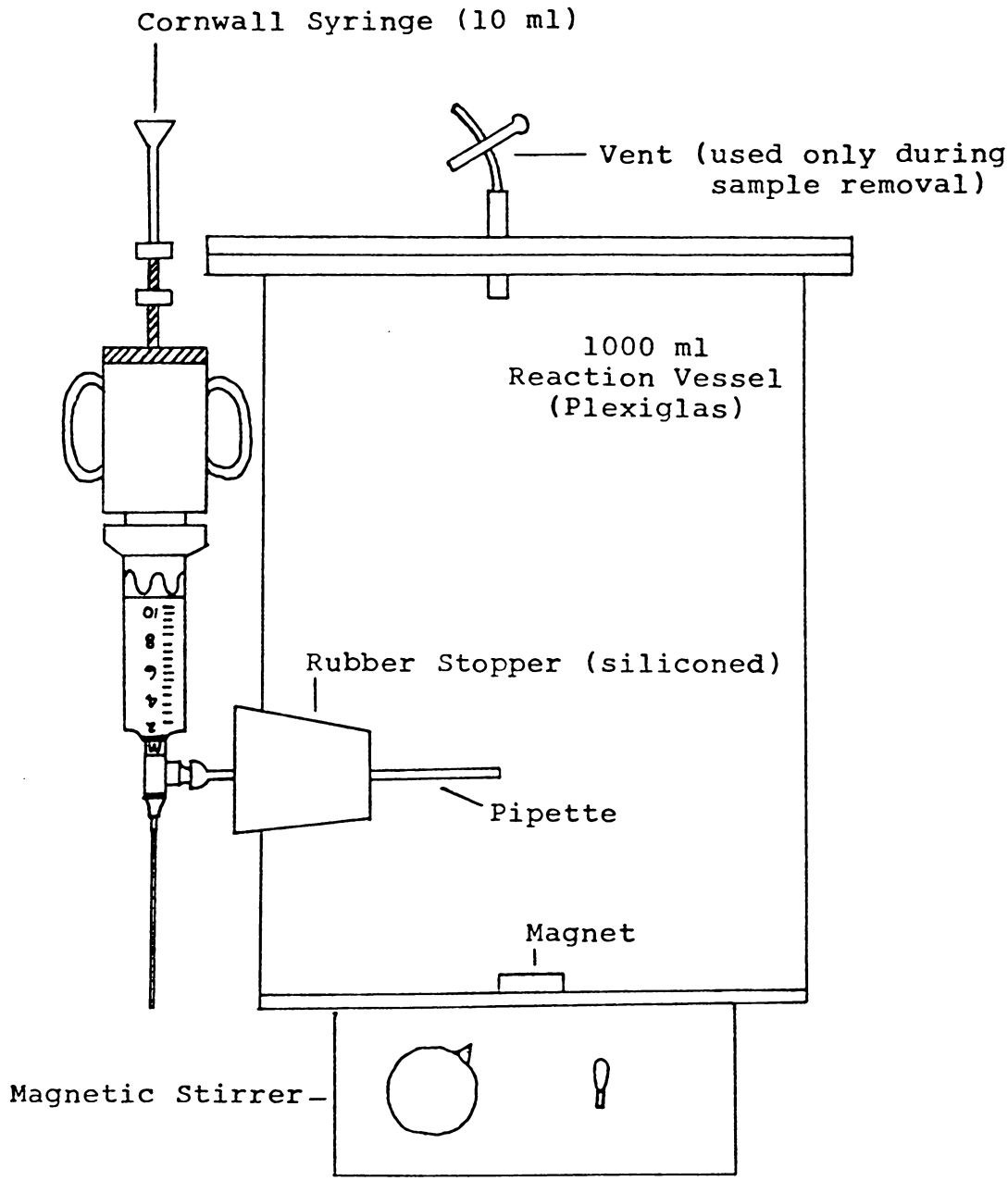

Figure 5. Chlorine dioxide reaction vessel and syringe.

stirrer was used to keep the contents of the reactor well mixed.

Because the reactor was made of Plexiglas, it could not be autoclaved. It was decided to acid wash the reactor prior to each experiment and to rinse it with demand-free distilled water. This was also done to the Cornwall syringe. Following the rinsing, an ultraviolet light rod was placed into the center of the reactor for at least five minutes to ensure sterilization. The syringe was exposed to the ClO_2 used in an experiment by removing 10 ml of sample from the reactor prior to the introduction of the bacterial suspension.

Exposure Procedure

A typical experiment began by washing the broth culture of P. cepacia as previously described. During the centrifugation periods, the test water was added to the beakers which, for chlorine disinfection studies, were then placed in a water bath that was thermostatically controlled with a Masterline Circulator (Model 2905) at 10° or 20° C. For ClO_2, the reactor was not placed in the water bath, but was instead wrapped with Tygon tubing that carried water to and from the circulator. The tubing was surrounded with cotton insulation to ensure a constant temperature inside the reactor.

Next, the solution was dosed with a known amount

of the appropriate disinfectant. The concentration was checked prior to the addition of the cell suspension and, if necessary, was adjusted to its desired strength. The pH of the reactor water was then adjusted to 6 or 9 with the use of a Fisher Accumet pH meter (Model 120).

Once the necessary conditions were reached in the reactor, 2 mls of the cell suspension was introduced. After the appropriate exposure times, a 5 ml aliquot of the solution was aseptically transferred into a sterile test tube containing sodium thiosulfate. The sample was immediately refrigerated at 4^{o} C to maintain bacteriostasis until dilution and plating could be made. At the end of the exposure period, the final disinfectant concentration and pH of the substrate were measured.

Once the disinfection experiments were complete, the bacterial samples were serially diluted and placed in sterile, disposable petri dishes. This was done in duplicate, and sterile recovery medium was then poured into the plates and swirled to dispense the cells. Colonies developing from surviving cells were counted after four days incubation time. The counts were averaged, and the logarithm of the survival ratio was plotted versus the disinfectant contact time.

Bacterial Adsorption Analysis

During this experiment, one bacterial suspension

was exposed to kaolinite (4 NTU) for approximately 10 minutes in a constantly stirred beaker. Another suspension was also stirred in another beaker for the same amount of time; however, it was not exposed to the clay. Both suspensions contained an equal concentration of P. cepacia.

Once the suspensions were adequately stirred, 10 mls of each were transferred to centrifugation tubes and both were then centrifuged at 1100 rpm for 30 seconds. Once the tubes were brought to a halt, the top 3 mls of the sample were carefully removed and immediately plated out in FAC agar. This procedure was then repeated at different centrifugation times up to 15 minutes. Following incubation of the cells, the plates were counted and the numbers indicated what percentage of bacteria remained in the top one-third of the sample during centrifugation. The results were then compared over time in order to show if an adsorptive effect was exerted on P. cepacia by kaolinite.

IV. RESULTS

In this section, specific details of the identification process regarding the iron-precipitating bacterium isolated from a water distribution system will be presented. The biocidal efficiencies of the disinfectants under consideration will then be compared in order to determine their potential for controlling microbially mediated iron precipitation. With the intention of providing information regarding disinfectant consistency over a wide range of conditions, microorganism and environmental test factors affecting disinfectant action will also be reported upon.

Identification of Isolated Bacterium

The Non-Fermenter System developed by Flow Laboratories, Inc., of Roslyn, New York, is intended for the simple and rapid identification of the most frequently encountered isolates of gram-negative, aerobic bacteria which do not belong to the family Enterobacteriaceae. The biochemical reaction chart illustrated in Table III shows that the isolated bacterium recorded positive results for the oxidase and growth at 42^{o} C tests associated with the N/F Screen. Thirteen biochemical parameters were tested using the Uni-N/F-Tek plate; the

TABLE III. N/F SYSTEM BIOCHEMICAL REACTION CHART*

Test		Test Results	Code Number	Biogram Number**
Oxidase	N/F SCREEN	+	4	
Fluoresence		-	2	4
Glucose Ferm.		-	1	
N_2 Gas Form.		-	4	
Pycocyanin		-	2	1
Growth at 42° C		+	1	
Glucose	UNI - N/F - TEK PLATE	+	4	
Xylose		+	2	6
Mannitol		-	1	
Lactose		+	4	
Maltose		-	2	4
Acetamide		-	1	
Esculin		-	4	
Urea		-	2	0
DNASE		-	1	
ONPG		-	4	
H_2S		-	2	0
Indole		-	1	
Motility		+		

Computer Code Book Identification - Pseudomonas cepacia (41-6400)

*Non-Fermenter System used for the presumptive identification of the oxidative gram-negative bacteria.

**Sum of the positive associated tests.

bacteria were capable of fermenting glucose, xylose, and lactose after a 24-hour incubation period at 35° C. Negative results were recorded for the remaining tests.

Occasionally, organisms will be isolated which require additional tests for definitive characterization. The isolated bacterium had to be examined microscopically to determine if the cells were motile. This examination did indicate motility, with the bacterium possessing a polar tuft of flagella, though flagella stains were not performed.

By summing the positive associated tests into a Biogram number and taking into consideration the additional motility information, the Flow Non-Fermenter computer code book identified the isolate as Pseudomonas cepacia.

Preliminary Investigations

Prior to initiating the disinfection study, the test environment itself was varied to simulate the conditions that would be used when the disinfectants were actually present and their effects on P. cepacia were recorded. As shown in Table IV, substrate pH never caused a consistent decrease in bacterial concentrations over a 45-minute exposure period. Log Survival Ratio's (LSR) were either positive, indicating an increase in population density, or were less than -0.1, except for the -0.149 LSR recorded at the 30-minute sample for

TABLE IV. EFFECT OF VARYING TEST ENVIRONMENTS ON *P. cepacia*

Time, minutes		Environmental Conditions			
		pH 6	pH 9	1.5mg/ml NH_4Cl	10° C
0	LSR*	0	0	0	0
	PD**	100	25	136	138
2	LSR	-	-	-0.023	0.003
	PD	-	-	129	139
5	LSR	-0.086	-0.095	-0.030	0.021
	PD	82	20.1	127	145
10	LSR	-	-	-0.003	0.072
	PD	-	-	135	163
15	LSR	0.021	-0.068	-0.037	0.012
	PD	105	21.4	125	142
30	LSR	-0.149	-0.052	-0.013	-0.003
	PD	71	22.2	132	137
45	LSR	-0.032	0.098	-	-
	PD	93	31.3	-	-

*Log Survival Ratio = Log $\left(\frac{\text{Surviving Population Density}}{\text{Initial Population Density}}\right)$

**Population Density expressed in cells/ml x 10^4

pH 6.0. Incidently, this value was the maximum encountered for any of these analyses.

The presence of NH_4Cl in the exposure substrate was analyzed in case any of this salt was present following chloramine formation. The LSR values over the 45 minute exposure period decreased inconsistently within hundredths of the original population, suggesting that a detrimental effect on P. cepacia was not present.

Additional temperature effects studies indicated that lowering the water temperature to 10^{o} C had the least effect on P. cepacia over the exposure period. The LSR's actually remained above the original sample during most of the analysis, with only a -0.003 LSR being recorded at 30 minutes exposure. It should be mentioned here that achieving such results appeared to indicate that sample removal and plating methods were being accurately reproduced.

Test reproducibility was naturally an important consideration during a disinfection study of this type. Figure 6 illustrates the range of data collected over a period of one hour for five replicate exposure experiments utilizing 0.4 mg/l free Cl at pH 9.0. Using this concentration and pH allowed a gradual decrease in cell density over the 60 minute exposure period. Note the fairly constant ranges recorded throughout the experiment. The results of one experiment spread the ranges further than

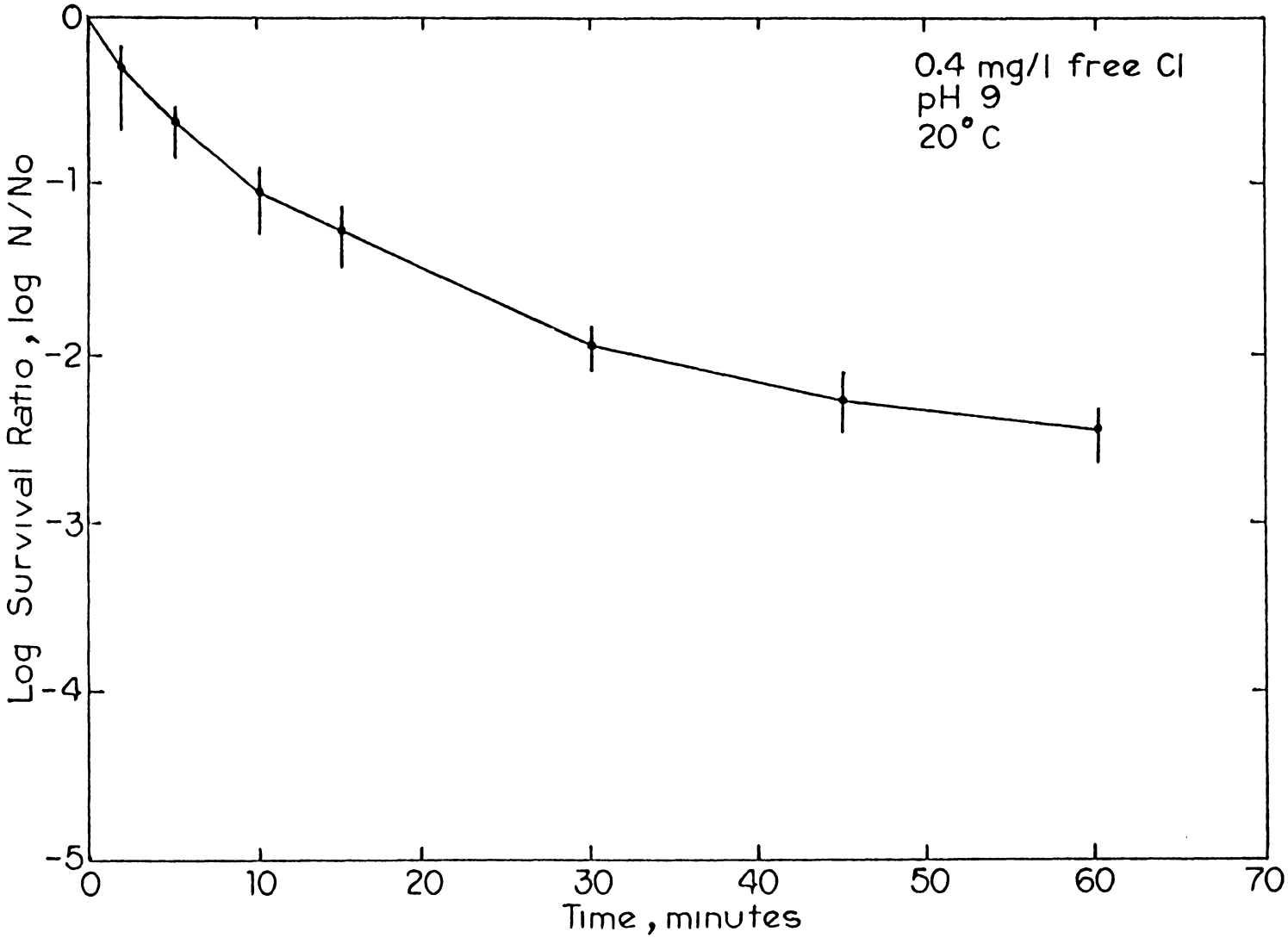

Figure 6. Statistical analysis (range and mean) of five separate exposure experiments utilizing free chlorine (95% OCl^-) as the disinfectant for P. cepacia inactivation.

what was hoped. The means (where the curve passed through the vertical bars) were initially off-centered, though this was less evident after the 15 minute exposure period. Initial cell concentrations were similar for four out of the five experiments studied, as experiment #1 had approximately a 140,000 cell/ml difference from the other experiments.

The standard deviations of these experiments are listed in Table A-1. The maximum LSR standard deviation of 0.223 occurred at two minutes exposure; however, values decreased over the exposure period, with log-deviations varying only between 0.106 and 0.154. With the sample size (5) being so small during this investigation, standard deviations for both LSR's and population densities were rather large, especially during the initial stages of disinfectant action. The population density standard deviation at two minutes exposure was 136,470, though this value was also reduced significantly for the remaining exposure times.

The study shown in Figure 7 was conducted to investigate the effect of cell concentration on the inactivation of P. cepacia with 0.5 mg/l free Cl at pH 9.0. Except for the steeper slope exhibited when the cell concentration was 8×10^3 cells/ml, the disinfection curves were similar throughout the exposure period. A difference of 1.5 logs (95% difference in remaining cells) was noticed after 10 minutes contact time; however, instead of continuing to

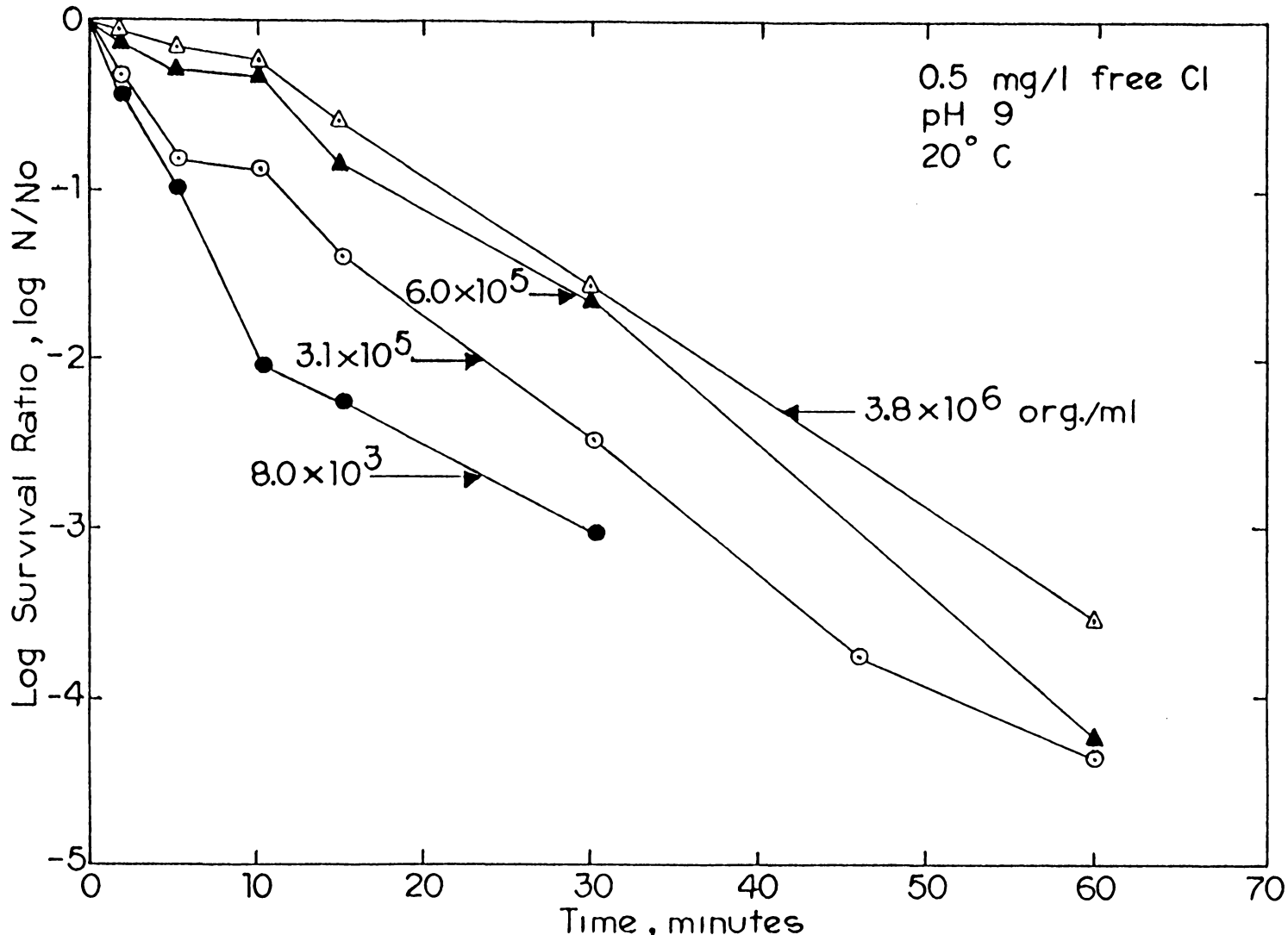

Figure 7. Effect of cell concentration on the inactivation of P. cepacia by free chlorine (95% OCl^-).

increase, the difference remained constant over the remainder of the experiment.

It would seem that bacterial iron ensheathment (Figure 8) does not affect disinfectant action. Iron ensheathment was facilitated with the use of ferric ammonium citrate culture medium in order to simulate P. cepacia's physical characteristics in the distribution system when iron precipitation was prevalent. Using 0.5 mg/l free Cl at pH 9.0, the difference between the two exposure curves was insignificant except at 30 minutes exposure where an LSR difference of 0.634 occurred, and the greatest kill occurred when the ferric ammonium citrate medium was used.

Comparative Disinfection Efficiencies

Figure 9 shows the effect of varying the disinfectant concentration on the efficiency of free chlorine at pH 6.0. It should be noted that, at pH 6.0, approximately 95% of the chlorine was in the HOCl form. It can be seen that the degree of efficiency for 0.1 mg/l free Cl was much less than the other concentrations studied. Within the first minute of exposure, a 99% kill (LSR of -2.0) was achieved for 0.25, 0.50, and 0.75 mg/l free Cl. When 0.1 mg/l free Cl was present, it did not achieve a similar kill until after approximately 40 minutes exposure. Incidently, an LSR of -5.0 was considered to be a 100% kill for this

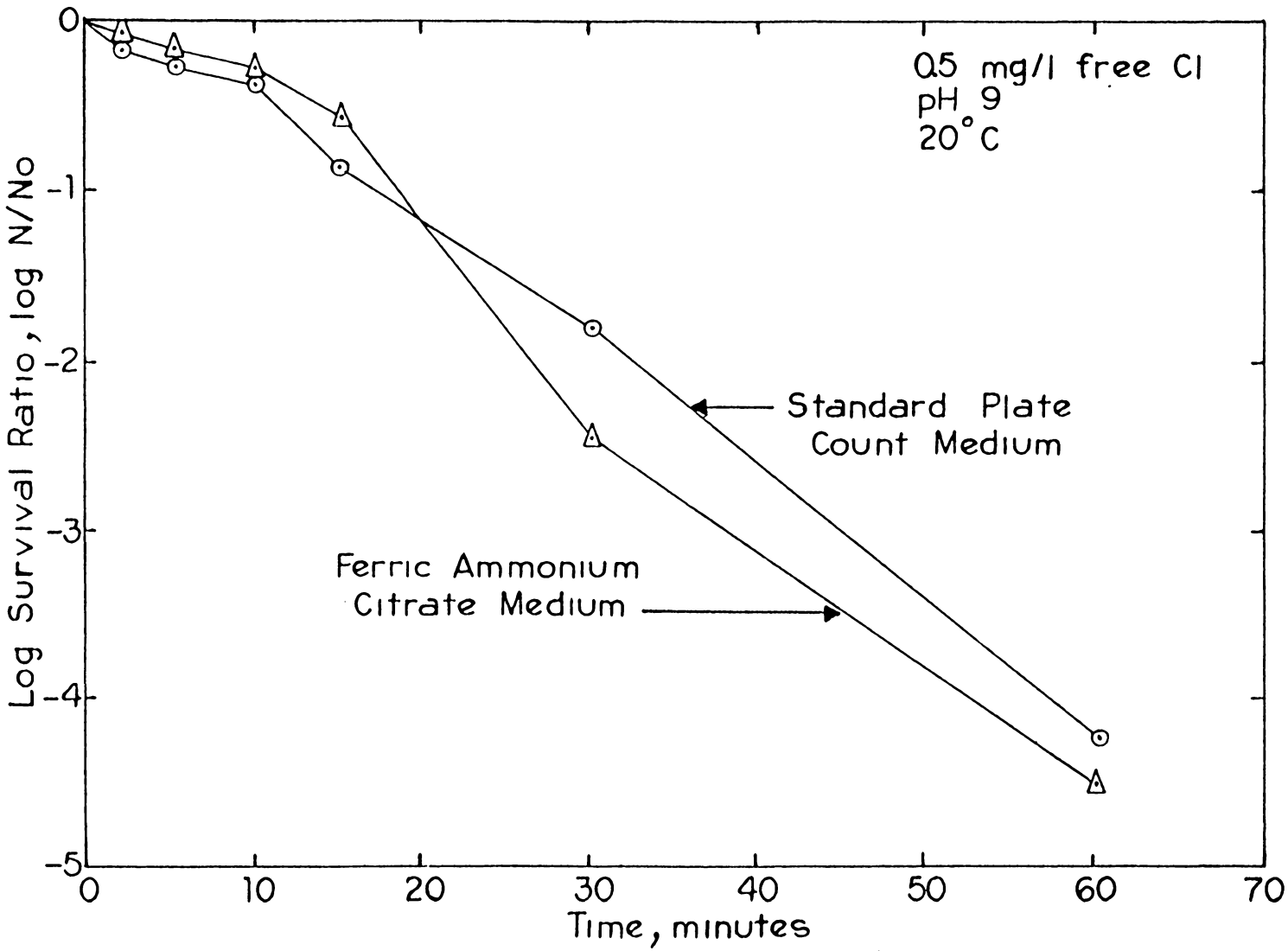

Figure 8. Effect of bacterial iron ensheathment (facilitated with the use of ferric ammonium citrate culture medium) on the inactivation of P. cepacia by free chlorine (95% OCl^-).

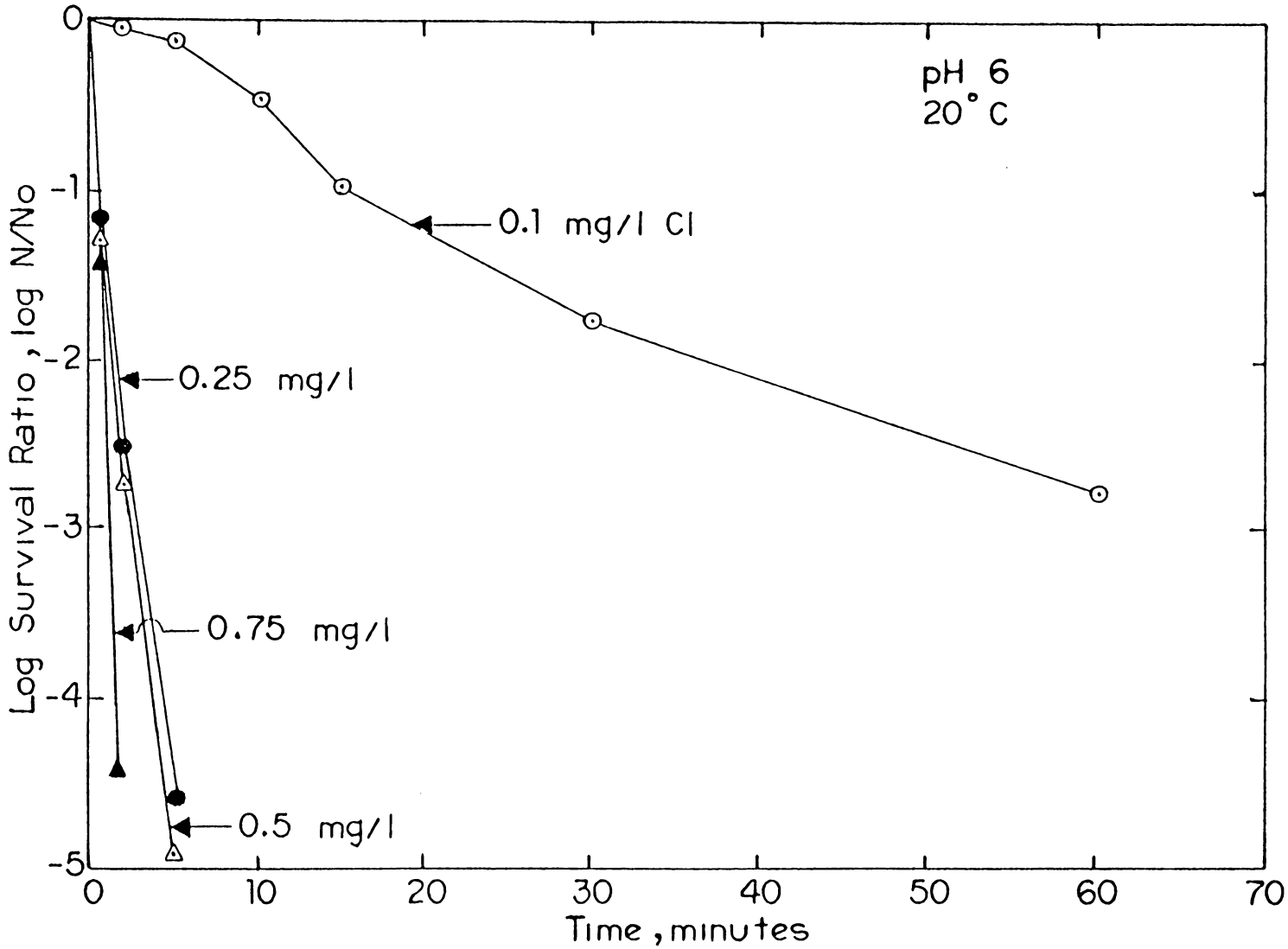

Figure 9. Inactivation of P. cepacia by free chlorine (95% HOCl).

study. By examining the final chlorine concentrations in Table A-2 of the Appendix, it was evident that each concentration decreased and changed to some combined Cl following 60 minutes exposure time; however, a residual was never completely absent. Also, the pH never varied by more than 0.2 units during the entire investigation. Figure 9 also indicated that only the 0.1 mg/l free Cl experiment recorded a bacterial concentration through the entire exposure period while a complete kill was evident for the other concentrations in well under 10 minutes exposure.

To evaluate the effect of pH and the resulting formation of OCl^- on P. cepacia inactivation, a similar experiment was also performed at pH 9.0. Theoretically, approximately 95% of the chlorine should be in the OCl^- form at this pH. Figure 10 indicates that there was a significant difference in the time required for 99% inactivation for the various concentrations of free chlorine than when the experiment was performed at pH 6.0. In every case, the rate of inactivation at pH 9.0 was dramatically less than at pH 6.0, to the point where 0.1 mg/l free Cl was disregarded and 1.0 mg/l free Cl was added to the data. As an example of OCl's poor disinfecting capabilities, 0.25 mg/l free Cl did not produce a 90% kill of P. cepacia over the 60-minute exposure period. In order to achieve a 99% kill at pH 9.0 for free chlorine, a 0.5 mg/l residual required approximately 32 minutes exposure time. Even though the time

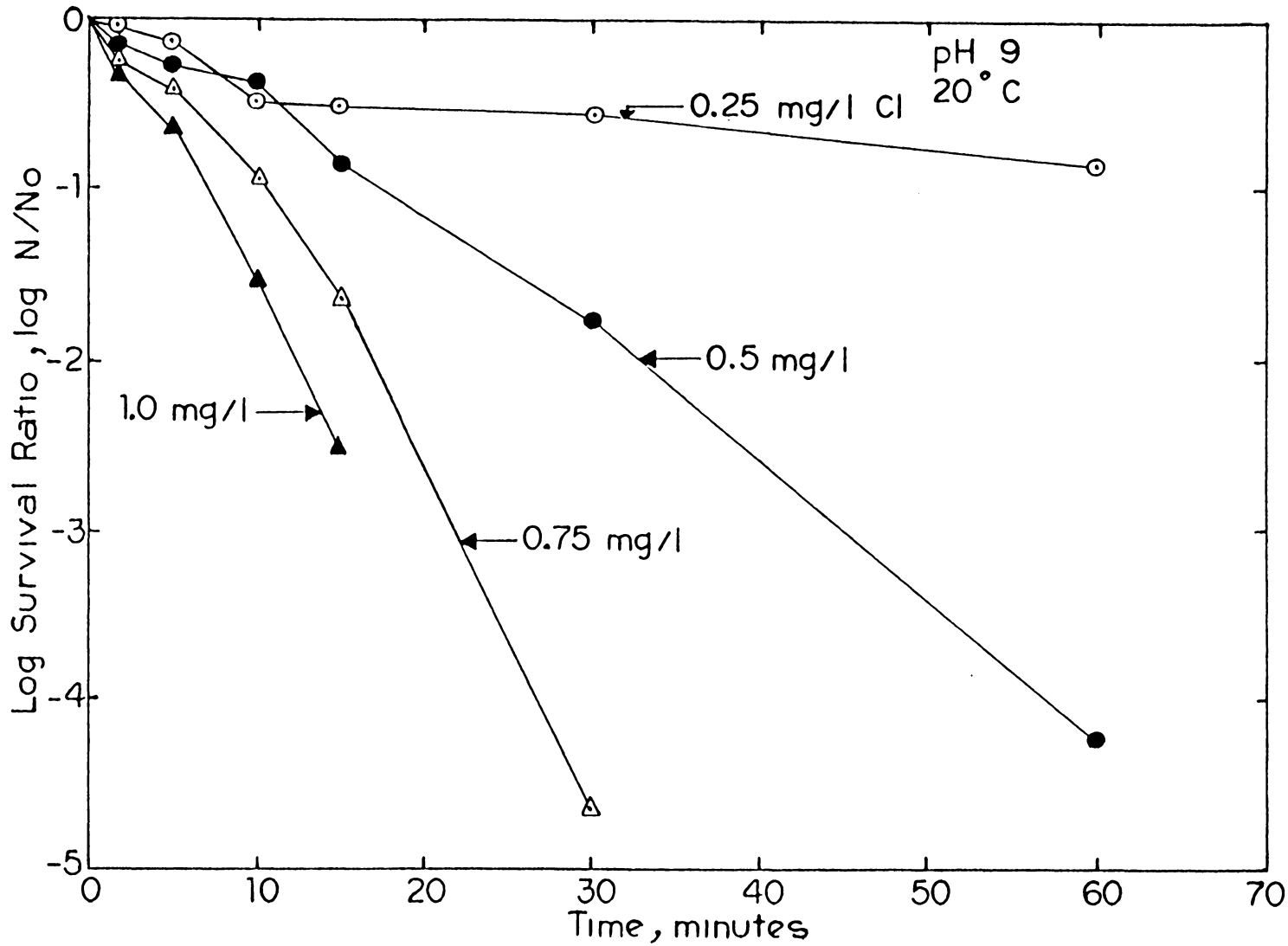

Figure 10. Inactivation of P. cepacia by free chlorine (95% OCl^-).

required for a 99% kill was reduced by increasing the disinfectant concentration, a relatively long exposure of 12 minutes was still required for a 1.0 mg/l free Cl residual.

By examining chloramine's effectiveness in inactivating P. cepacia at pH 6.0 (Figure 11), it was obvious that a residual greater than 0.25 mg/l combined Cl was required for a substantial kill. To achieve a 99% kill with 0.25 mg/l combined Cl, an exposure time of approximately 30 minutes was required even though the more effective dichloramine, which should predominate at pH 6.0, would be present. It was, therefore, evident that the combined chlorine species present were not as effective against P. cepacia as was HOCl. Exposure with larger concentrations of chloramine still did not produce significant kills rapidly, as 0.5 mg/l required 16 minutes contact for 99% inactivation, 0.75 mg/l required 6 minutes, and less than two minutes exposure facilitated a 99% inactivation for 1.0 mg/l combined Cl.

Figure 12 shows the inactivation of P. cepacia by combined chlorine at pH 9.0. With monochloramine being the predominant species at this pH, disinfectant efficiency was expected to decrease somewhat. Indeed, when 0.25 mg/l combined Cl was studied, a 90% kill was not achieved after 60 minutes exposure. Decreases in efficiency were also exhibited at every other concentration

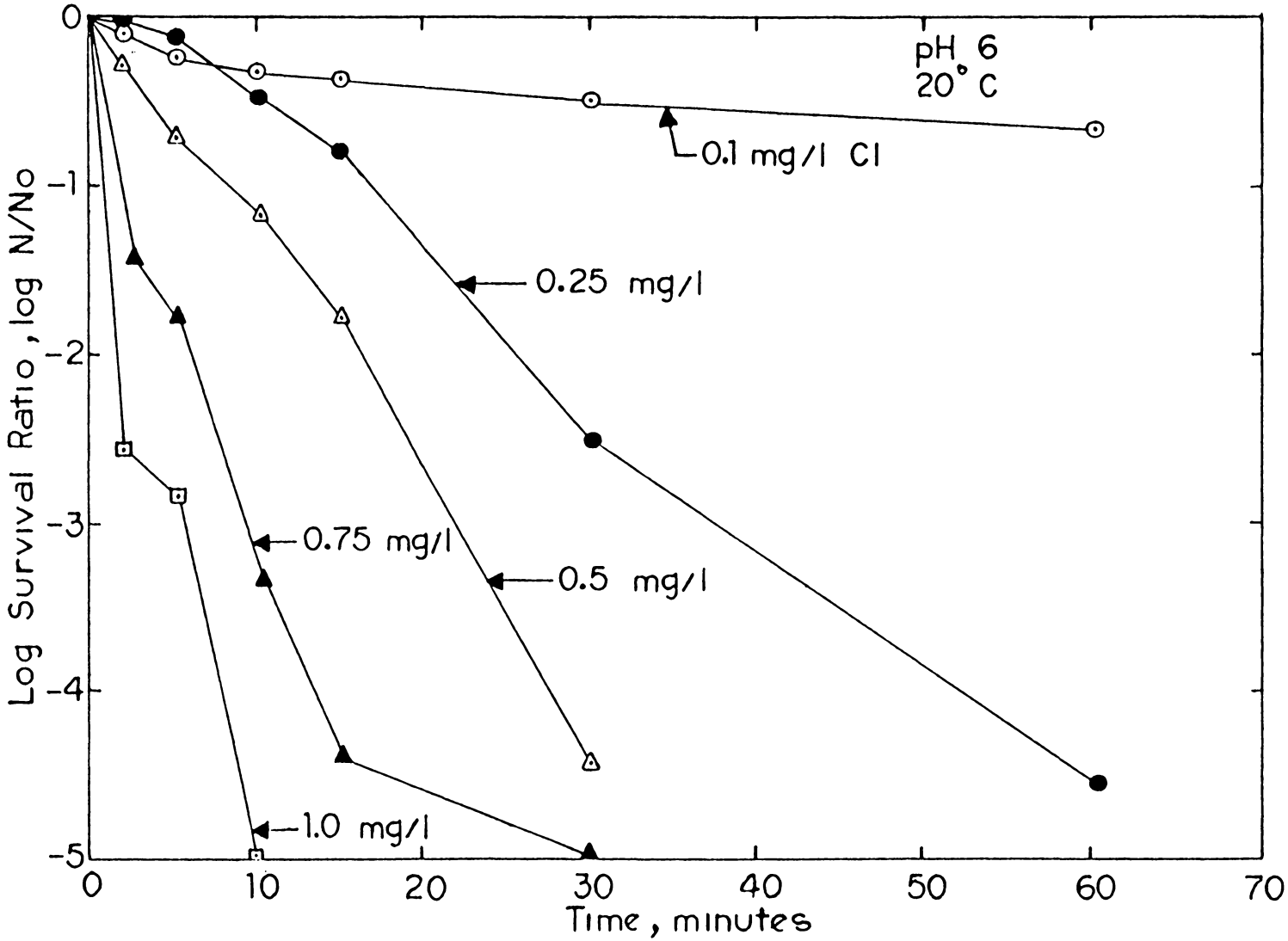

Figure 11. Inactivation of P. cepacia by combined chlorine species present at pH 6.0.

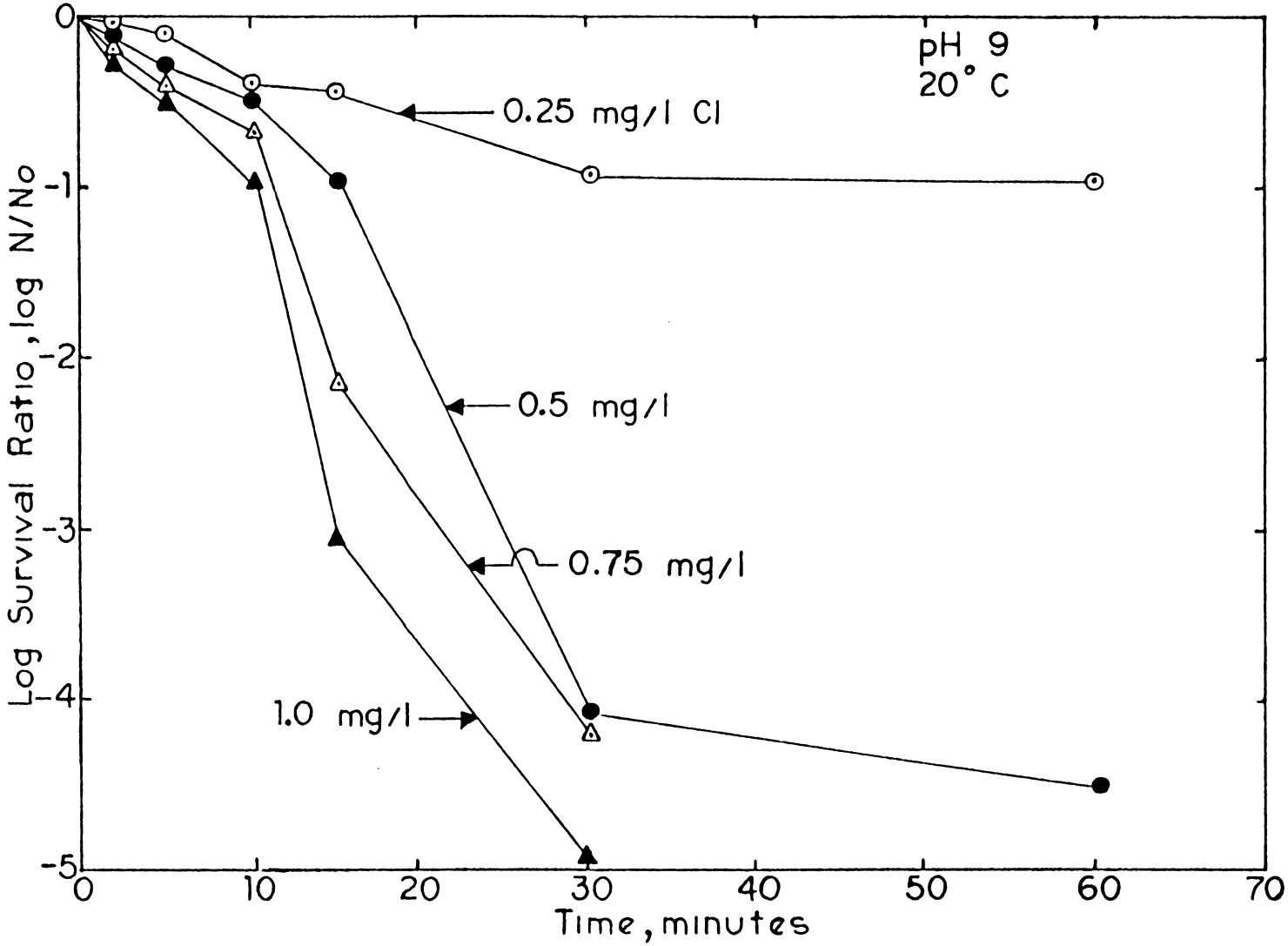

Figure 12. Inactivation of P. cepacia by combined chlorine species present.

utilized in this particular experiment when compared to inactivations at pH 6.0, suggesting that a strong pH effect was exhibited by chloramine. The data also indicate that a certain amount of time was required for the disinfectant to react with P. cepacia, as a similar rate of kill was observed over the first 10 minutes of exposure for every concentration studied. After that time, however, the 0.5, 0.75, and 1.0 mg/l disinfectant concentration produced significant rates of organism die-off within the following five minutes, with a 2-log inactivation occurring for the latter two concentrations.

Before presenting the results regarding ClO_2's disinfectant efficiency, it should be noted that the time axes for all the ClO_2 data are of a different scale than those used for the other disinfectants under consideration.

Figure 13 shows the inactivation of P. cepacia by ClO_2 at pH 6.0. Of particular interest was that the 0.1 mg/l residual produced a 99% kill in approximately 10 minutes, which was a 4-fold increase over HOCl. However, this concentration did lose much of its efficiency after 10 minutes exposure, with only a 2-log inactivation occurring over the final 50 minutes of the experiment. Similar results were not evident for the other ClO_2 concentrations studied, as organism inactivation was essentially complete before five minutes contact time. It would appear that the negative effect on efficiency

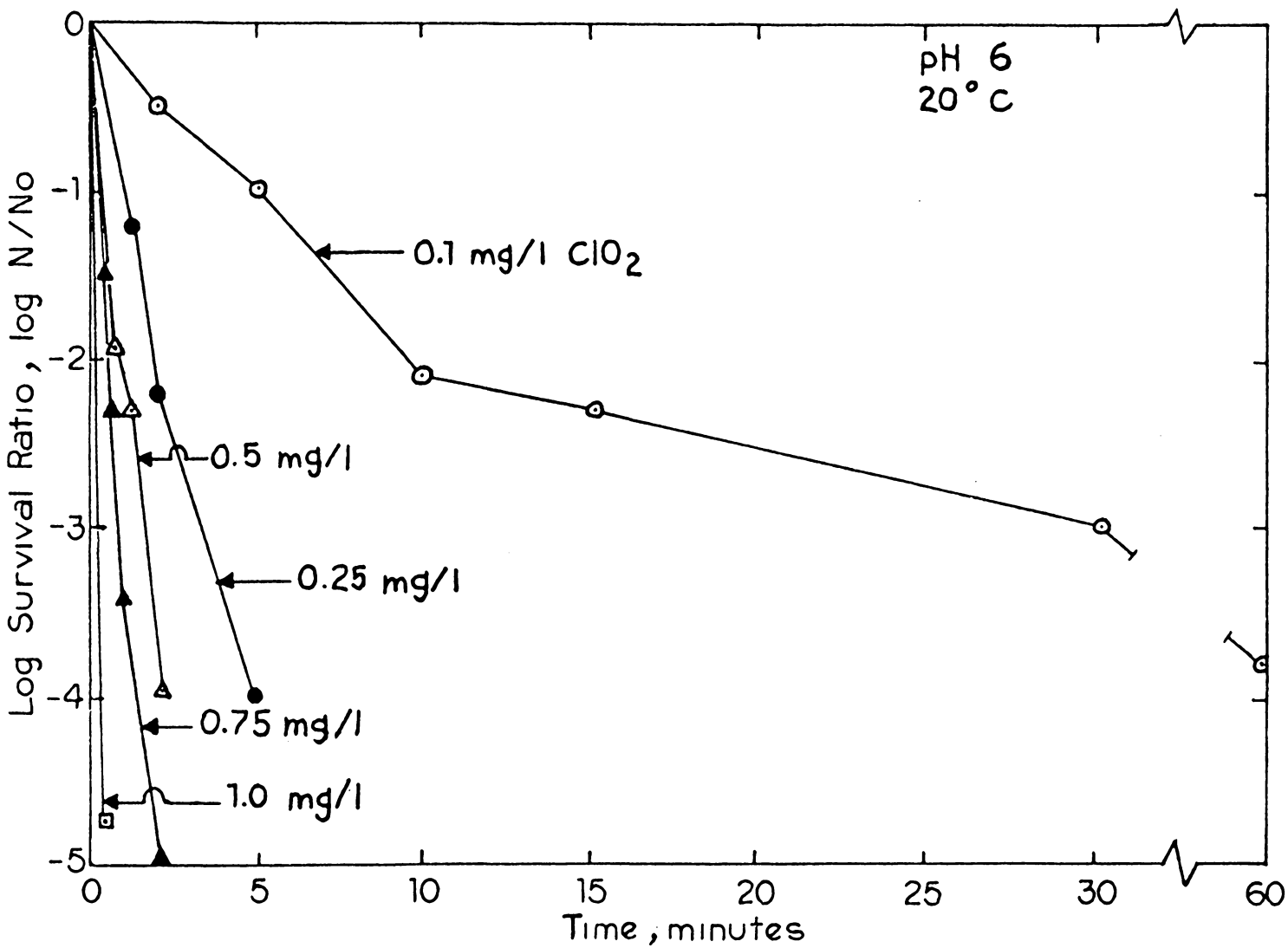

Figure 13. Inactivation of P. cepacia by chlorine dioxide.

caused by diminishing residuals occurred after this exposure time since such effects were only noticed for the 0.1 mg/l ClO_2 curve. Data presented in Table A-4 of the Appendix did not indicate any significant residual drop over the 60 minute exposure period for any ClO_2 concentration.

There was a significant difference in the time required for 99% inactivation of P. cepacia between 0.1 mg/l ClO_2 and the remaining concentrations examined. Under two minutes exposure was required for 0.25 mg/l ClO_2 while 0.50 and 0.75 mg/l residuals produced 99% kills after approximately 30 seconds exposure. A 99+% kill was obtained in 15 seconds when the ClO_2 concentration was 1.0 mg/l. Every concentration examined during this ClO_2 experiment produced a 99% inactivation level at a faster rate than did any previously mentioned disinfectant.

Raising the pH to 9.0 did have an effect on the efficiency of ClO_2, as can be seen by examining Figure 14. Only a 0.3-log inactivation occurred over the 60 minute exposure period when 0.1 mg/l ClO_2 was present, which was significantly different than the efficiency observed at pH 6.0. A similar pH effect was observed at 0.25 and 0.50 mg/l, as both concentrations failed to completely kill P. cepacia after 30 minutes exposure. The 99% inactivation levels were also not reached until 5.1 and 3.6 minutes, respectively. The sloping curves suggested a gradual decrease in ClO_2 concentrations was occurring; however, the majority

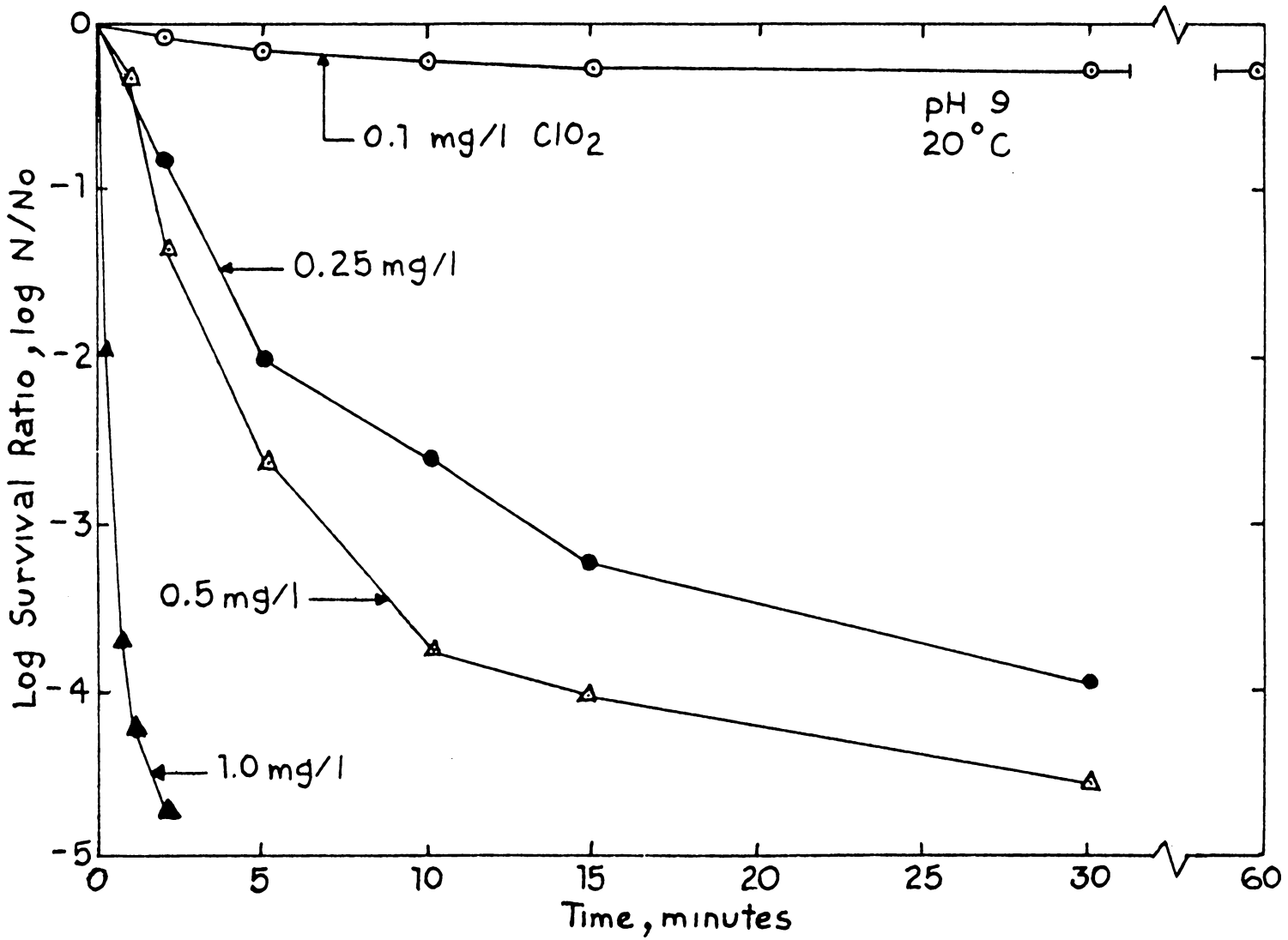

Figure 14. Inactivation of P. cepacia by chlorine dioxide.

of each residual was still present after the 60 minute exposure period (Table A-5). Nearly a 3-log difference in inactivation at 15 seconds exposure was noticed for 1.0 mg/l when the pH was elevated. A complete kill was still apparent, however, before five minutes exposure.

In order to better compare the rates of disinfection of P. cepacia by the disinfectants under consideration, new concentration-time plots (Figure 15) were constructed. The 99% inactivation or destruction points (which are the 1% survival points or an LSR of -2.0) were extrapolated from the survival curves, as shown in Table A-6 of the Appendix, to give the time necessary for 99% inactivation of the bacteria. These 1% survival points for each of the disinfectant levels utilized were then replotted on log-log paper to show the disinfectant concentration vs. the previously determined 99% inactivation times. The closer a concentration vs. time curve lies to the lower lefthand corner of the graph, the faster the reaction, i.e., the quicker the inactivation of the microbes. From the relative positions of these curves, it was found that ClO_2 at pH 6.0 inactivated P. cepacia 2.7 times faster than free chlorine (HOCl) at pH 6.0, 4.8 times faster than ClO_2 at pH 9.0, 18.1 times faster than chloramine ($NHCl_2$) at pH 6.0, 48.1 times faster than chloramine (NH_2Cl) at pH 9.0, and 63 times faster than free chlorine (OCl^-) at pH 9.0. It was also obvious that ClO_2 efficiency was the least

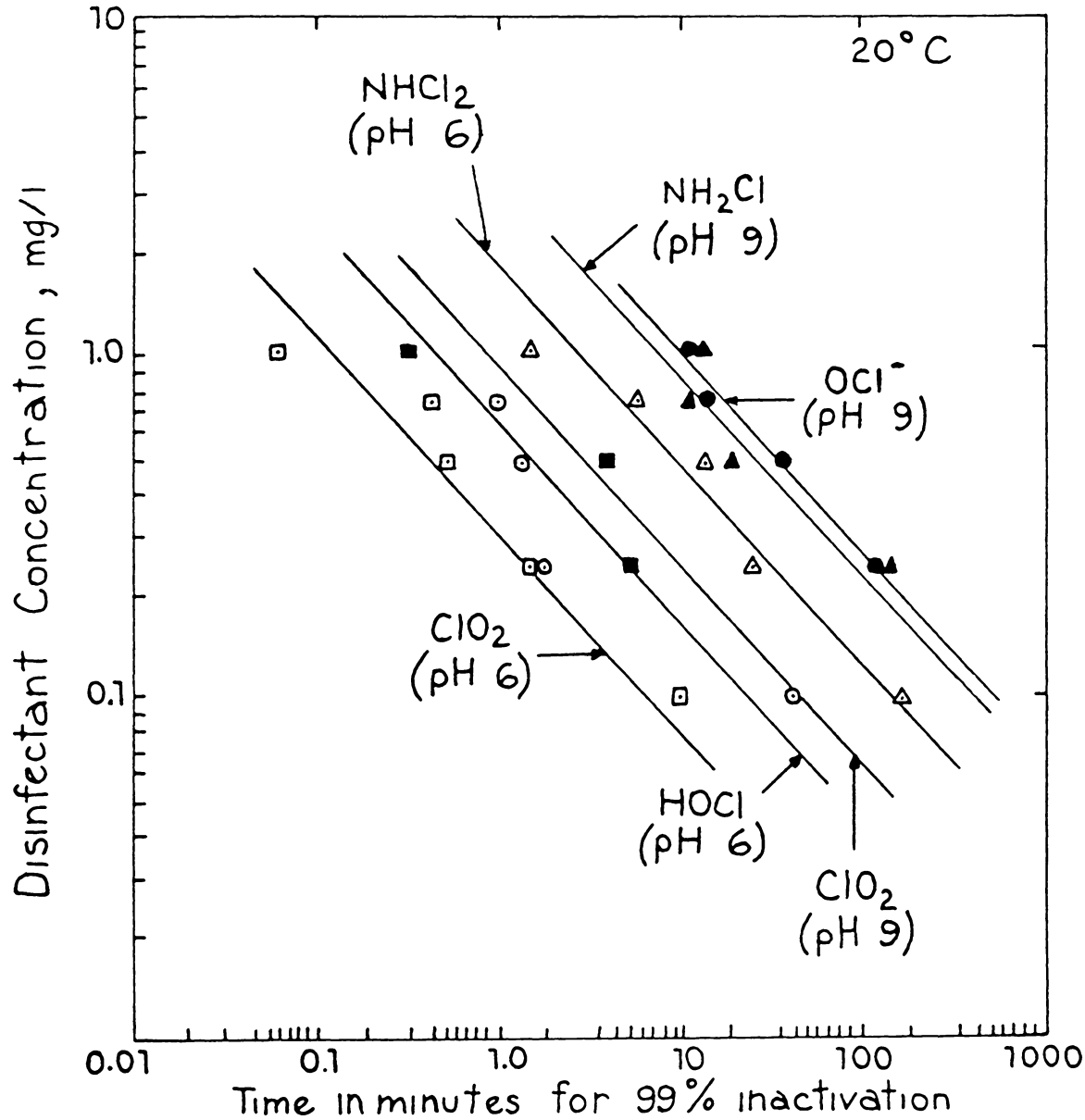

Figure 15. Inactivation of P. cepacia by free and combined chlorine species and chlorine dioxide.

effected by changing substrate pH while free chlorine experienced the greatest deviation in efficiency.

To determine whether or not OCl^- possessed any disinfectant characteristics at all or if they were due to the presence of 5% HOCl, the 99% inactivation times of 1.0 mg/l OCl^- and 0.05 mg/l HOCl were compared (Figure 15). The results show that 1.0 mg/l OCl^- inactivated P. cepacia approximately 8.8 times faster than 0.05 mg/l HOCl, which may suggest that OCl^- does possess some killing action toward this bacterium, though its efficiency was still relatively poor.

Temperature Effects

From the plot of survival with time and temperature, using 0.25 mg/l free Cl at pH 6.0 (Figure 16), it was apparent that biocidal efficiency did not vary directly with a drop in substrate temperature. To obtain a kill of 99%, 30 seconds were required at 20° C while only 15 seconds produced an equal kill at 10° C. A complete kill was also achieved quicker at the lower water temperature.

When 0.5 mg/l free Cl was studied at pH 9.0, an insignificant temperature effect was noticed over the first 30 minutes of exposure (Figure 16); however, during the remaining 30 minutes of the experiment, the rate of kill of P. cepacia was reduced at 10° C with a 2-log

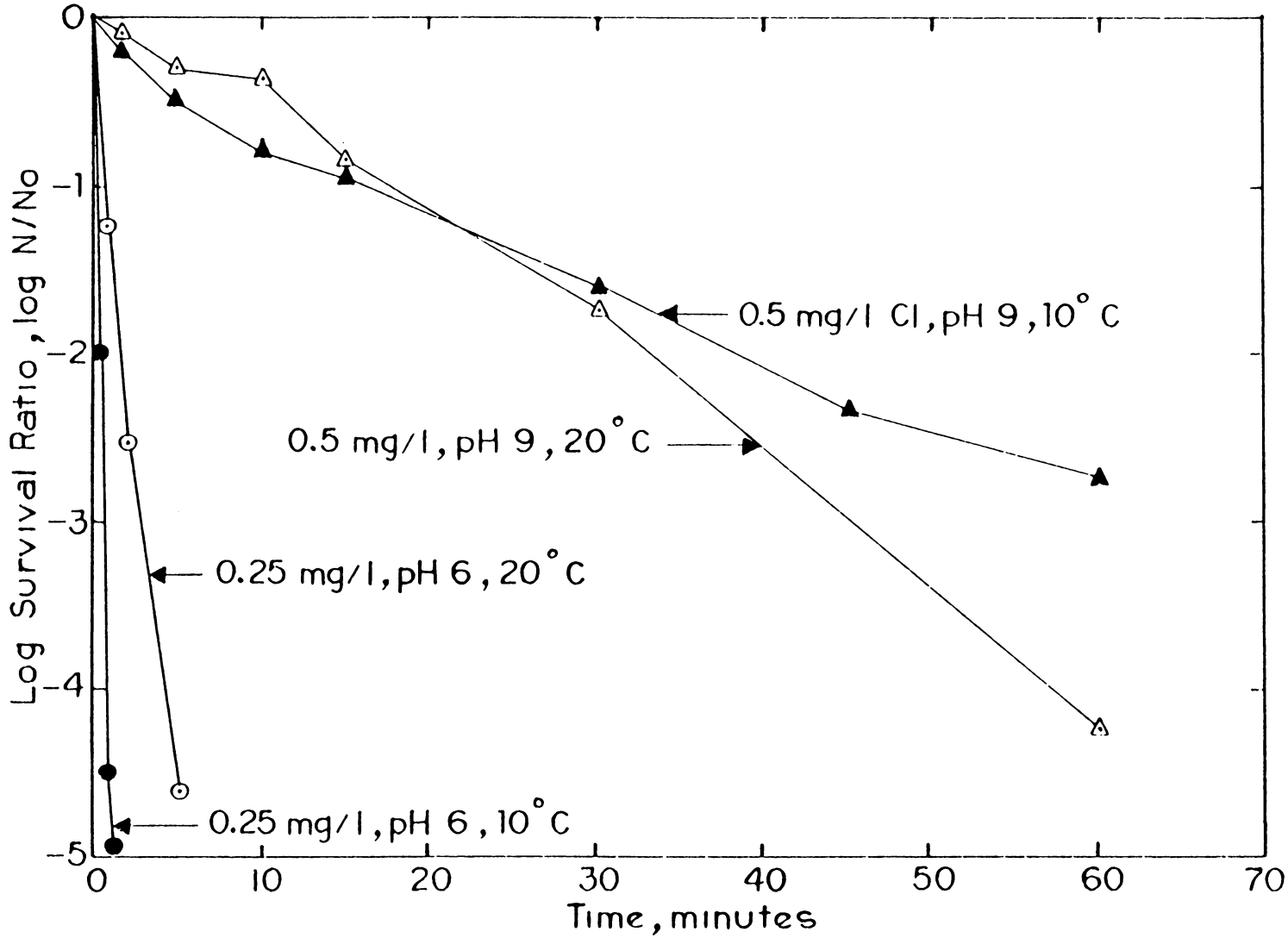

Figure 16. Effect of temperature on the inactivation of P. cepacia by free chlorine species.

difference in kill occurring after 60 minutes exposure. It should be noted, however, that such a difference was not as significant at 60 minutes exposure than it would be at the beginning of the experiment. For this experiment, an initial 2-log difference indicated a 99% difference in cell numbers while a 2-log difference at 60 minutes exposure indicated only a change of approximately 0.69% in cell densities. Both curves experienced similar 99% inactivation times, with 33 minutes required at 20° C and 38 minutes at 10° C, only a 1.15 fold increase at the lower temperature. This would indicate that a significant difference in efficiency was not present when water temperature was reduced to 10° C.

A noticeable temperature effect was not observed for chloramine either when 0.25 mg/l combined Cl was studied at pH 6.0 (Figure 17). Approximately 26 minutes were required for 99% inactivation at both 10° and 20° C; however, the rate of inactivation at 10° C was again less than 20° C over the final 25 minutes of exposure, with a 1.3-log difference occurring after 60 minutes. Again, this difference in terms of actual cell numbers was rather small.

As can be seen in Figure 18, a significant temperature effect was not evident throughout the experiment for ClO_2 at pH 6.0; however, the killing rate was reduced to some extent. With a ClO_2 concentration of 0.5 mg/l,

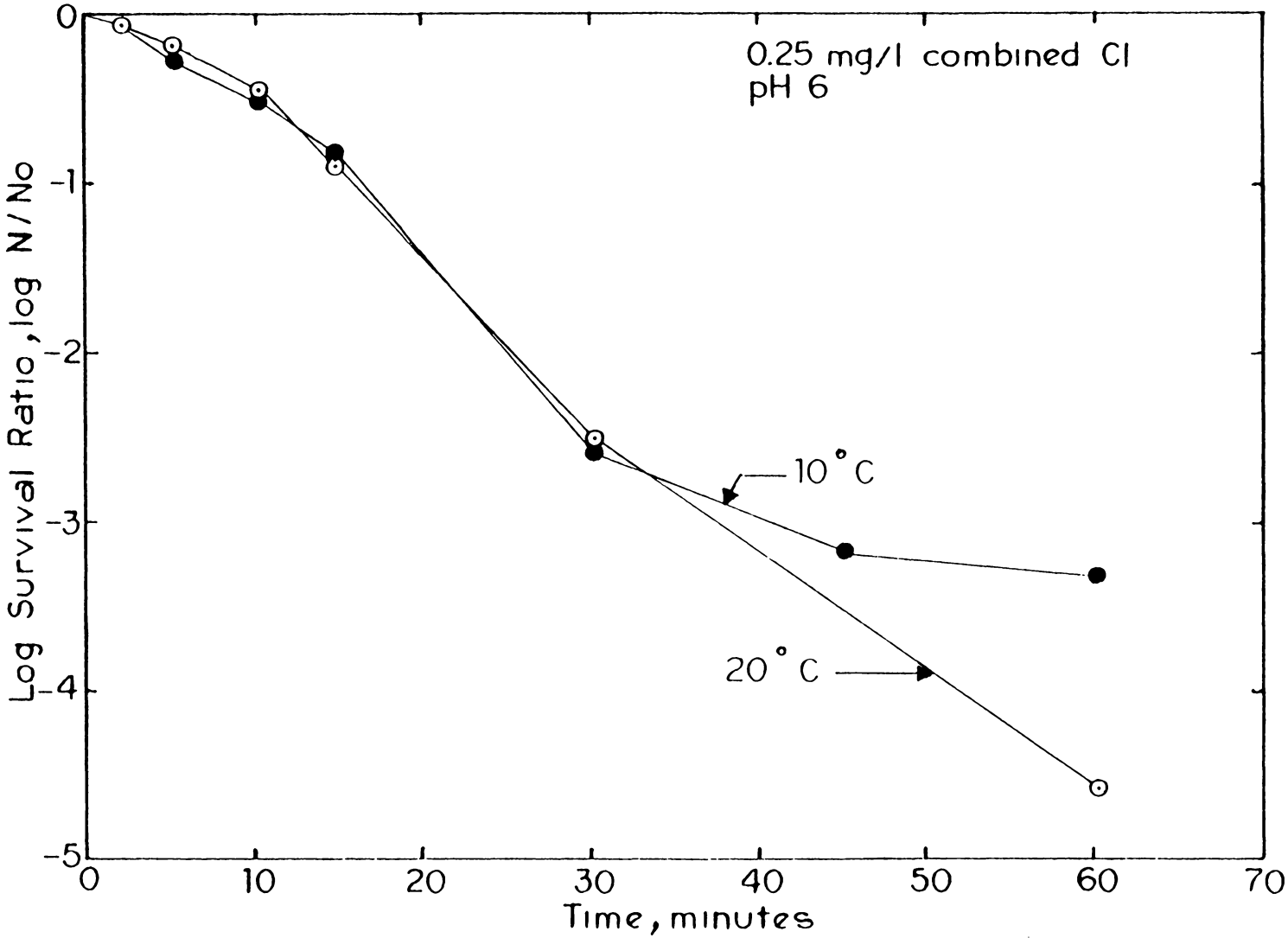

Figure 17. Effect of temperature on the inactivation of P. cepacia by combined chlorine.

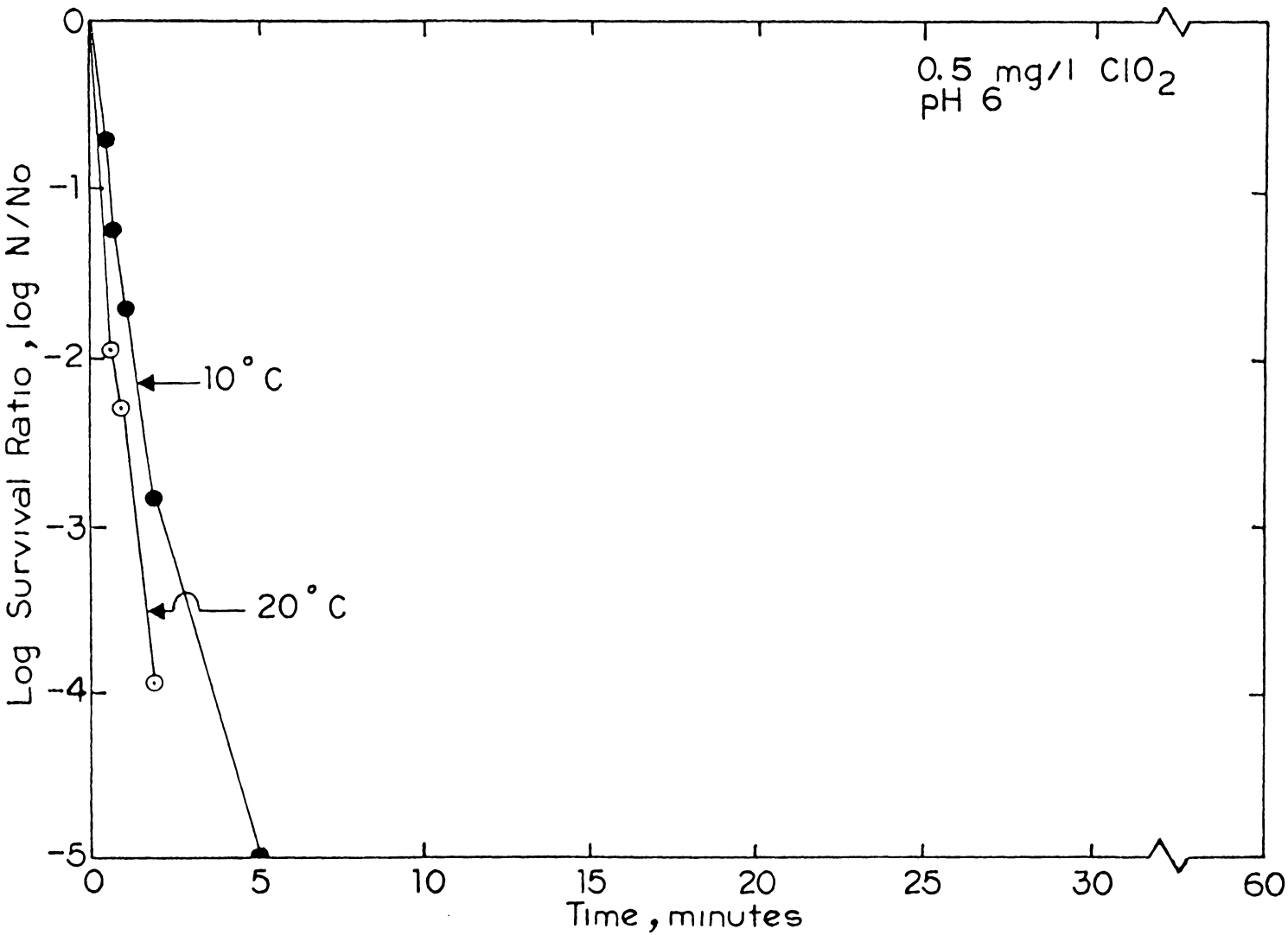

Figure 18. Effect of temperature on the inactivation of P. cepacia by chlorine dioxide.

99% inactivation occurred within 30 seconds at 20° C while 90 seconds was required when the temperature was lowered to 10° C; thus, the killing rate was three times as rapid at 20° than at 10° C.

Turbidity Effects

It has long been considered that protection of microorganisms through their association with particulate matter could result in the shielding of microorganisms from disinfectant action. Little direct evidence, however, of such protective effects has been available until recently. For this reason and the possibility that P. cepacia could increase turbidities by precipitating iron, the effect of kaolinite clay on P. cepacia inactivation was investigated.

In order to determine if the bacteria actually became associated with the particulate matter, either through adsorption or some other mechanism, a centrifugation study was performed with and without kaolinite (Figure 19). It can be seen that at 1100 rpm, an immediate decrease in remaining cell density occurred in the top one-third of the centrifuged 4.0 NTU sample, as 45% removal occurred after only 30 seconds centrifugation time. In comparison, remaining cell density for the sample without kaolinite was 7% higher than its initial concentration after 30 seconds. Further analysis indicated

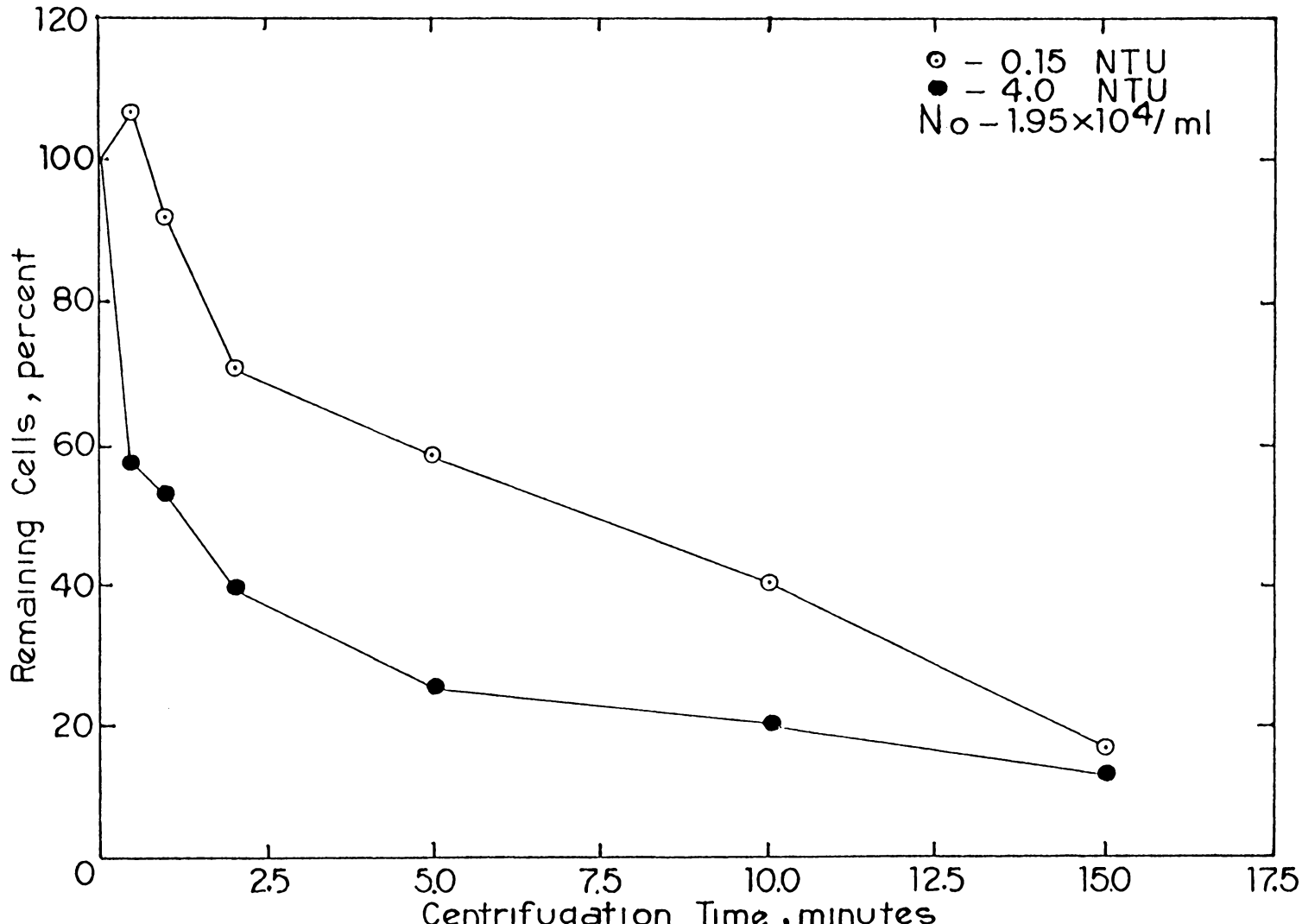

Figure 19. Removal of P. cepacia from the top one-third of centrifuged samples (1100 rpm) associated with and without kaolinite.

that 60% removal of the kaolinite-bacteria suspension occurred after approximately two minutes while a similar reduction did not occur for the bacterial suspension until after 10 minutes centrifugation. It should be noted that the 4.0 NTU curve displayed an asymptotic relationship forming after five minutes centrifugation. The use of a rather limited sized centrifuge tube required the removal of one-third of the sample, and poor sample removal may have resuspended some bacteria or a certain amount of bacteria may have not been able to adsorb to the clay.

Figure 20 shows the inactivation curves of P. cepacia associated with and without kaolinite when free chlorine species were studied at 20° C and at pH 6.0 and 9.0. The results indicated that at a turbidity level of 2.0 NTU, the disinfection curve was similar to that of the free chlorine inactivation curve without kaolinite-associated bacteria when 0.25 mg/l was studied at pH 6.0. In this case, there was no indication of a bacteria protective effect produced by association with the kaolinite. However, at a higher turbidity level (4.0 NTU), the kaolinite was protective in regard to P. cepacia survival. Twenty-seven minutes were required for 99% inactivation at 4.0 NTU while only 2 minutes were necessary when the bacteria were not associated with the kaolinite, indicating a 13.5-fold decrease in HOCl's effectiveness when the bacteria became

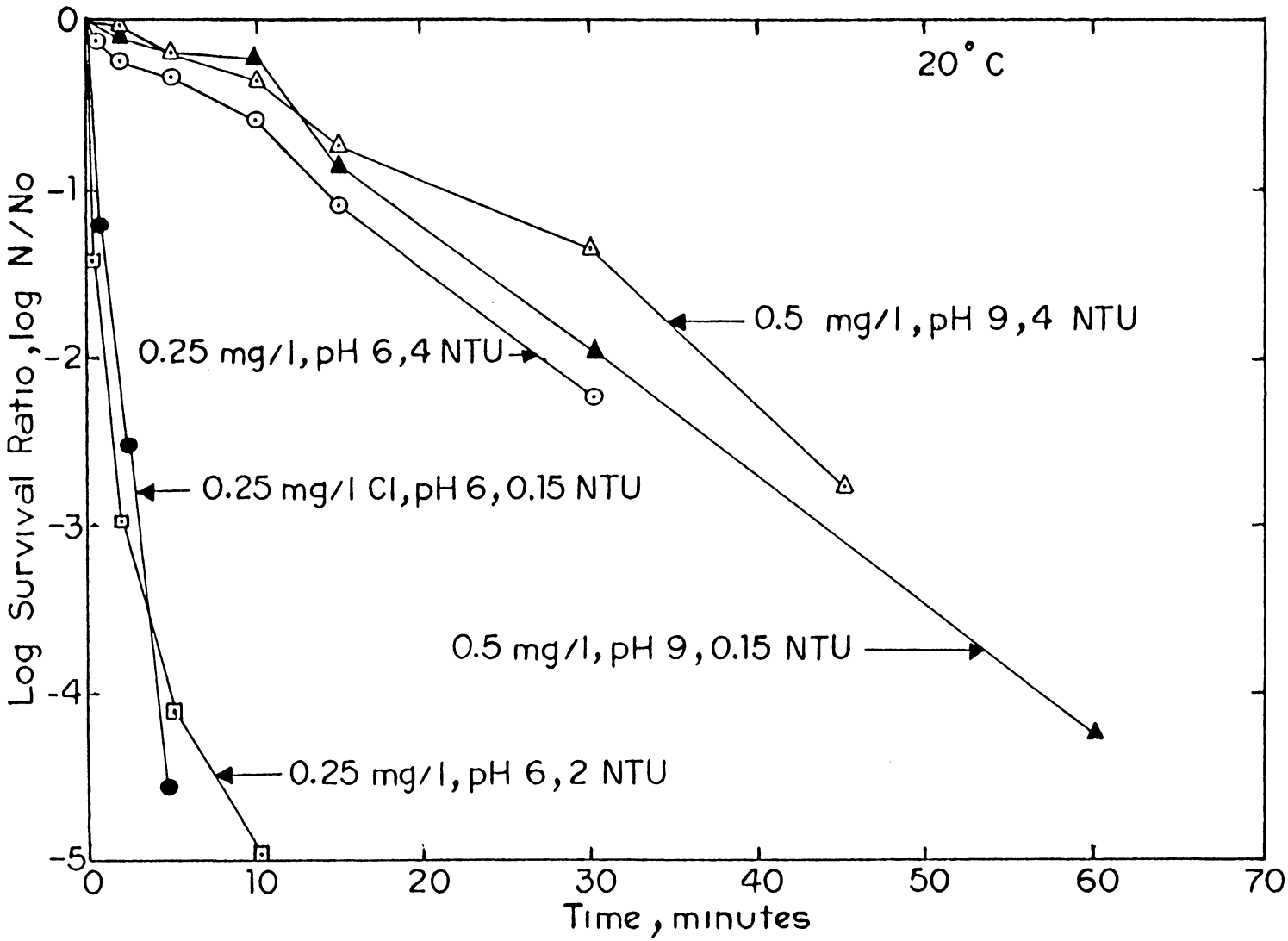

Figure 20. Inactivation of kaolinite-associated P. cepacia by free chlorine species.

associated with that level of turbidity. However, a lack of experimental replication regarding the turbidity study limits the validity of the particular investigation. Such a pronounced effect on efficiency was not indicated when 0.5 mg/l free Cl at pH 9.0 was used. The two inactivation curves (0.15 and 4.0 NTU's) were similar over the first 15 minutes of exposure, and a 0.5-log difference in inactivation at 30 minutes was the maximum spread between the two curves throughout the experiment.

Chloramine's efficiency was not affected with the presence of 4.0 NTU's of kaolinite, as can be seen in Figure 21. The kaolinite-associated P. cepacia inactivation curve showed only slightly less effectiveness than did the curve not associated with the particulate matter (0.15 NTU) when 0.75 mg/l combined Cl at pH 6.0 was studied.

Kaolinite was also not protective in regard to P. cepacia survival when ClO_2 was the disinfectant under consideration (Figure 22). The results showed that at the turbidity levels of 2.0 and 4.0 NTU's, their disinfection curves were similar to that of the ClO_2 inactivation curve which was not associated with kaolinite when 0.5 mg/l ClO_2 was studied at pH 6.0. Complete kill required a slightly longer exposure time (5 minutes) when the turbidity level was 4.0 NTU. Complete kills for the other two curves required about half that exposure.

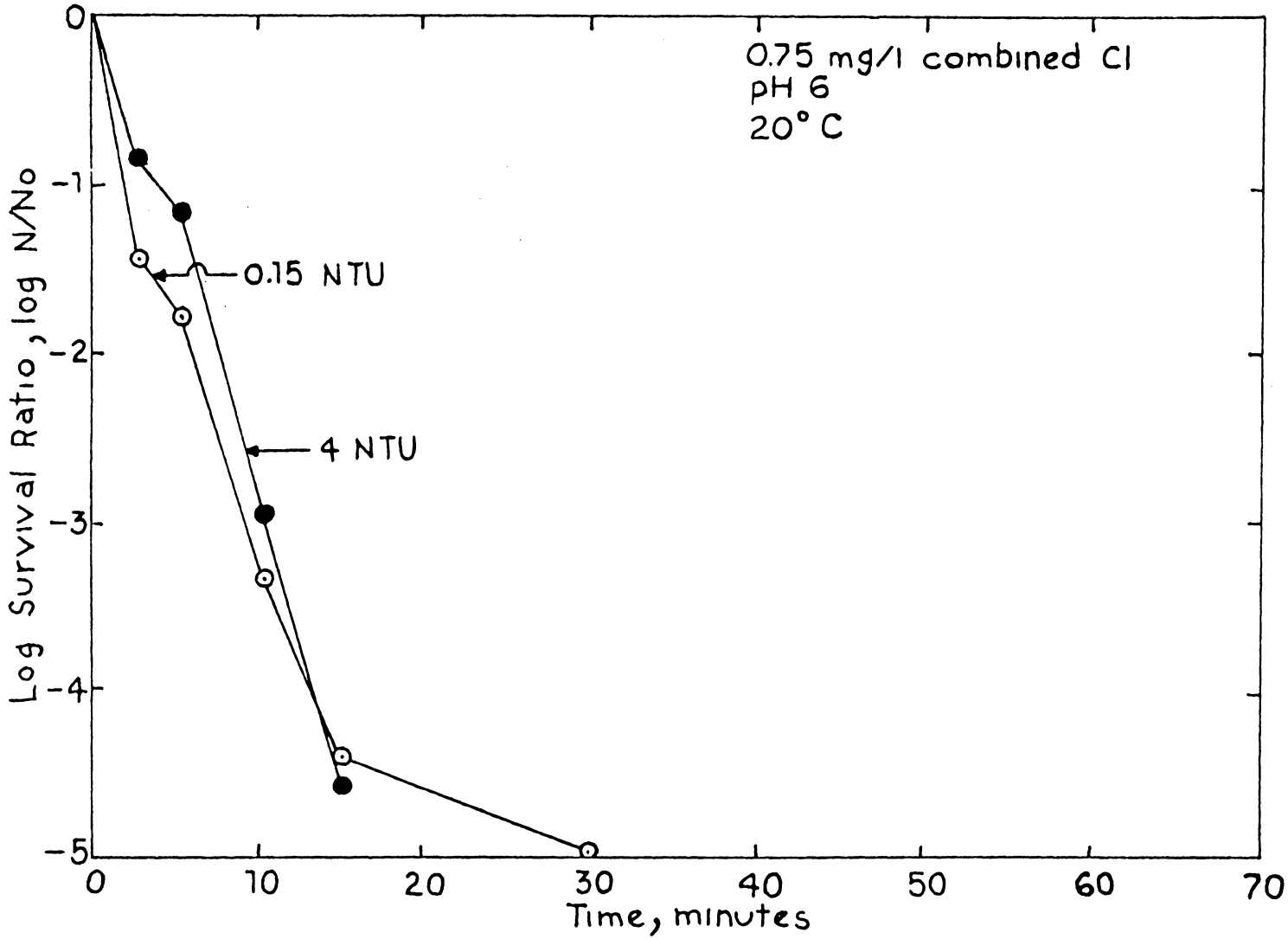

Figure 21. Inactivation of kaolinite associated P. cepacia by combined chlorine.

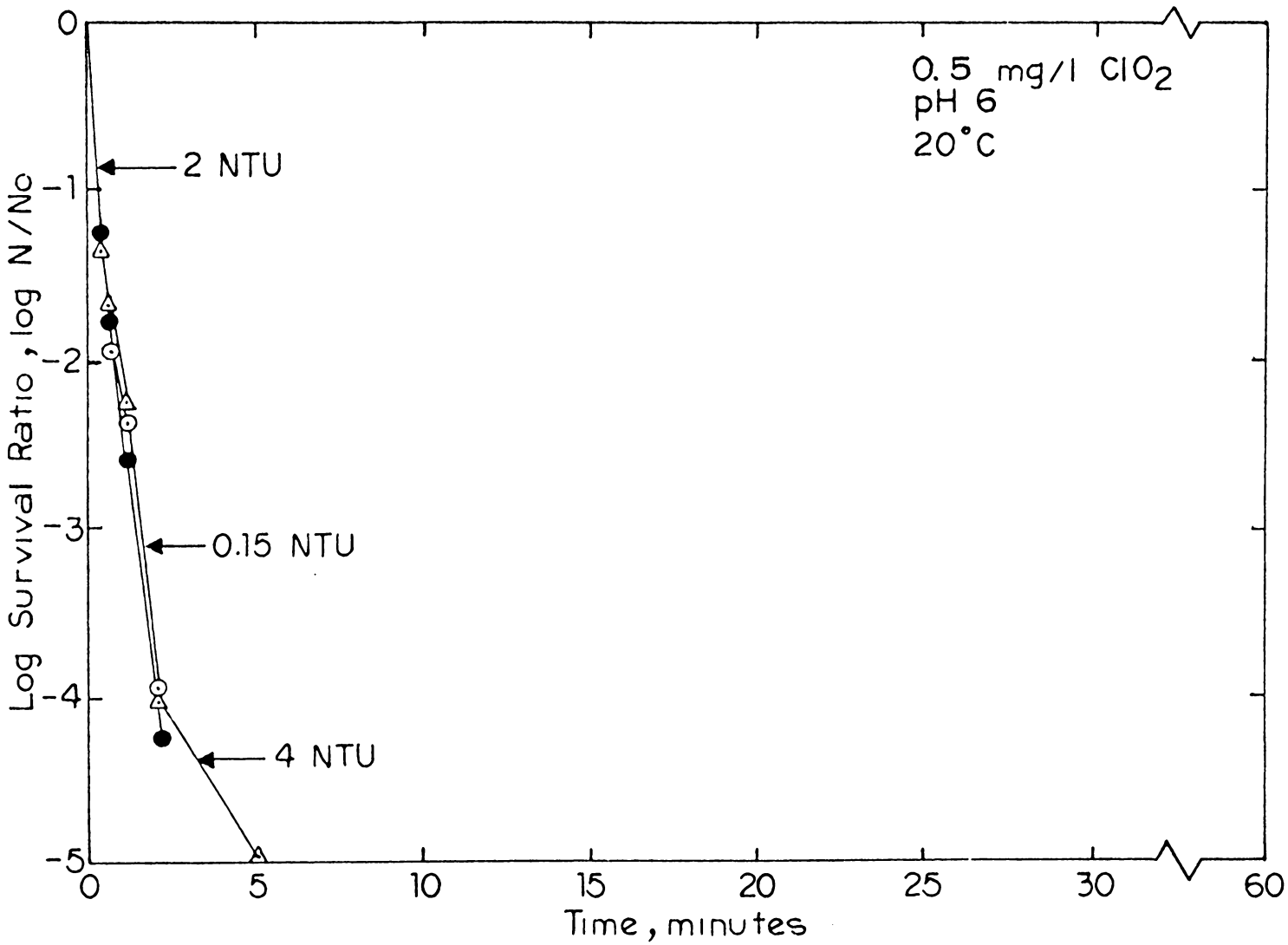

Figure 22. Inactivation of kaolinite-associated P. cepacia by chlorine dioxide.

V. DISCUSSION

The N/F (Nonfermenter) System, which was developed for the rapid identification of frequently encountered nonfermentative or oxidase-positive gram-negative rods, provided an easy and accurate method for identifying the isolated bacterium. Barnishan and Ayers (61) reported that the N/F System's results were consistent with expected conventional test reactions with both the tubes and plates. Use of tubes permitted the identification of 90% of the strains of Pseudomonas aeruginosa in 24 hours and 97% in 48 hours. Use of the plates permitted the identification of 95% of the other oxidative nonfermenters, of which P. cepacia is included, within 24 hours and 96% within 48 hours. The authors also mentioned that, when 1298 nonfermentative gram-negative rods were studied, there were no misidentifications based on discrepancies of observed individual test reactions with expected results.

Bergey's Manual of Determinative Bacteriology (62) indicated that P. cepacia appeared to be widely distributed in soil which would allow the bacterium to enter the distribution system during pipeline repair and/or construction or through the raw water supply itself.

Growth factors are not required for this species and, nutritionally, they are very versatile, with most individual strains capable of using approximately 100 different organic compounds as sole carbon sources for growth. It would appear that a water distribution system would provide an ideal environment for the survival and growth of P. cepacia.

During a study of the Salem and Beverly (Massachusetts) water distribution systems, P. cepacia was identified at various locations within each system (63). These microorganisms, as well as others, were able to survive a variety of conditions by being encapsulated. Their dense polysaccharide coat, generally absent from the pure laboratory cultures but not from environmental strains, enabled the organism to protect itself from the "hostile" conditions of a distribution system by collecting useful materials and binding harmful ions and molecules. The relatively high resistance to chlorine of Pseudomonas sp. was reported by Poynter et al. (7). It was mentioned that strains capable of growth at 22° C were commonly encountered at sample points immediately after chlorination and that, in the absence of residual chlorine, their numbers increased in the distribution system. The isolated bacterium was incubated at 20° C throughout the study, which could suggest that the encapsulated P. cepacia isolate possessed a resistance to chlorine. An evaluation

of alternate disinfectants would, therefore, seem justifiable especially since this bacterium has also been shown to be involved in the precipitation of iron from organic complexes.

Washed preparations of P. cepacia did not decline to any measurable extent when the test environment pH, temperature, or salt concentration was varied. Sharp et al. (64) have reported that thermal degradation occurs more rapidly in the alkaline region, which was noticed only slightly in this experiment. Thermal effects would naturally be minimal since the maximum substrate temperature was only 20° C. The lower water temperatures were studied because of the desire to simulate the range of temperature conditions commonly encountered within the distribution system. Many investigators (27, 30, 34, 64) have studied the effects of salt concentration on disinfectant efficiency. In this study, however, the only concern was with the possible effect of the salt (NH_4Cl) itself on the bacteria in case the compound did not completely react with chlorine to form chloramines. The data showed no direct effect on P. cepacia, which could suggest that inactivation of the bacteria would be solely due to the presence of chloramines.

Test reproducibility appeared to be acceptable as long as initial population densities did not vary to a significant degree. One experiment had an initial density

that was 140,000 cells/ml lower than the next highest density, which led to markedly different LSR values than were recorded for the four other experiments. Despite this problem in the difference of efficiency for smaller population densities, standard deviations over the exposure period were less drastically affected because of the similar kills recorded for the other four experiments.

Further substantiating the need for similar initial population densities was the experiment studying the use of various cell concentrations on the efficiency of free chlorine. The microbial process is an interaction between the disinfecting agent and the organism. Therefore, the properties or state of the microbe are of equal importance to the nature and properties of the disinfectant in determining the pattern of biocidal activity. The results showed that the inactivation of P. cepacia was independent on the concentration of organisms, at least at levels similar to the ones used during this investigation. The use of small bacterial densities were, therefore, avoided during the study to hopefully insure test reproducibility.

Various studies (42, 54) have indicated that adsorption of colloidal iron by bacteria was possible through a number of mechanisms, including contact between a ferric iron sol or the use of organic iron complexes for a carbon source. Pseudomonas sp. were among the bacteria capable of such colloidal iron adsorption. One study (42)

indicated that when the cells were tested for iron by the hydroxylamine method, no reaction occurred; however, when the cells were treated with concentrated hydrochloric acid, a strong iron reaction occurred, which further confirmed that the iron was bound to the cell. It was, therefore, of interest to examine if such iron adsorption to its capsular material could offer some protective effect for P. cepacia against disinfectant action. Microscopic analysis of washed P. cepacia grown on ferric ammonium citrate culture medium indicated the presence of encapsulated iron prior to disinfection. Subsequent exposure to free chlorine indicated that the adsorbed iron offered no protective effect from the disinfectant.

The experimental results obtained during the disinfection portion of the study demonstrated that HOCl was effective against P. cepacia, as long as its residual was at or above 0.25 mg/l. Various investigators (8, 14, 28, 29) have previously reported on the substantial disinfection capabilities of HOCl, which is the main reason for its use by the majority of water-treatment facilities in the United States. It was the intention of this study to indicate that, even though a disinfectant may possess good biocidal properties, proper residual maintenance in the distribution system is also critical for controlling biologically mediated water quality deterioration. The

literature has indicated that a minimum free chlorine residual of 0.05-0.10 mg/l should generally be maintained at distant points in the distribution system (9). The data presented in this study would suggest that higher residuals would be warranted, especially in areas experiencing water quality deterioration such as microbial iron precipitation.

The data regarding OCl^- disinfection clearly show the effect of pH on free chlorine. The role of pH in affecting the efficiency of free chlorine has been well documented (27-31, 66). Twelve minutes were required to achieve a 99% kill of P. cepacia when using 1.0 mg/l OCl^-. In circumstances where iron precipitation was evident, a large OCl^- residual would be required to lessen the problem. From the data, it would appear that when such conditions do exist, the pH should be lowered to ensure that the more disinfective HOCl was prevalent. Control of the pH would most likely be less expensive than it would be to maintain large residuals of OCl^- in the distribution system.

According to the data, the biocidal efficiency of preformed chloramines at pH 6.0 was also poor. This finding compares well with studies found within the literature (15, 28-30). There is evidence, however, that the chlorammoniation process has been used successfully for years by a number of utilities. It is important to note that the

way chloramines are formed and used in the field differs from the procedures used in the laboratory (31). In experimental work, chloramines are preformed, and this is then followed by the addition of the microorganisms. In conventional chlorammoniation, as practiced in the field, ammonia is added to the water first and then chlorine gas is pumped into the solution. The rate of conversion of free chlorine to chloramines is dependent on pH, temperature, and the chlorine to ammonia ratio present. Therefore, under certain conditions, free chlorine could be present for several minutes, resulting in the rapid inactivation of microorganisms particularly at lower pH values. This was suggested by Hoather (67) as an explanation for the much faster bactericidal action observed in ammonia-chlorine treatment than could be shown using preformed chloramines.

Such findings, no matter what the mechanism was, still cannot offer a deterrent to biologically mediated iron precipitation that would occur at points in a distribution system. Biocidal efficiency may be greater at the plant, but once the ammonia-chlorine reaction was complete, residual chloramines would be the only disinfectant present within the system. With the findings determined in this study and others, chloramines would not be recommended for the control of biologically mediated deterioration of water quality within distribution

systems.

To further substantiate these claims, data collected at pH 9.0 indicated that the biocidal efficiency of chloramines was less than what was recorded at pH 6.0. Various investigators (14, 15) have reported similar results for chloramines. A greater percentage of the more powerful disinfectant, dichloramine, is present at lower pH's, while the weaker monochloramine predominates in alkaline conditions. Although dichloramine is a better disinfectant than monochloramine, its use should be avoided since it possesses a disagreeable chlorinous taste and odor. Not only are residual chloramines poor in disinfecting ability, but their weaker forms are required to be used which further hampers their effectiveness in controlling water quality deterioration problems.

The biocidal effectiveness of ClO_2 has been shown in the literature to be equal to or better than free chlorine (8, 17-22). Similar results were recorded for ClO_2 during this study on the inactivation of P. cepacia. At pH 6.0, residuals greater than 0.1 mg/l significantly killed the bacteria in less than 5 minutes exposure. Ridenour and Armbruster (26) reported that a residual of 0.1 mg/l ClO_2 would destroy the common water pathogens though more resistant forms required slightly higher residuals for complete kill. With the isolated bacterium being an environmental strain, its encapsulation should be able to

offer some protection against ClO_2, or for that matter, against any disinfectant. Besides ClO_2's noticeable effectiveness as a disinfectant, the literature has also indicated that it does not react with ammonia and only slightly with organic matter (22, 23, 51). Consequently, its stability can be advantageous where the ammonium content of the water is significant. More important is the strong potential for maintaining a ClO_2 residual throughout the distribution system. The maintenance of such a biocidal residual could greatly assist in preventing the outbreak of biologic iron precipitation problems.

Another important property of ClO_2 as a disinfectant is the failure of pH to materially affect its biocidal efficiency. Many investigators (8, 23, 25, 36) have mentioned that ClO_2 does not ionize in water, which allows its efficiency to remain substantially constant over the normal range of pH values in natural waters. The ClO_2 study performed at pH 9.0 was in marked contrast to these findings, however, as each concentration exhibited an increase in the amount of exposure required to achieve similar kills that were recorded at pH 6.0. One explanation for these results could be that a pH effect may be substantiated for P. cepacia. Also, using NaOH to raise the pH may have forced the ClO_2 to disproportionate to ClO_2^-. Efficiency was, therefore, reduced because ClO_2^- possesses a lower biocidal effect than ClO_2 (49).

By comparing the disinfection results together (Figure 15), it was obvious that ClO_2 at pH 6.0 was the most effective disinfectant for the inactivation of P. cepacia. The data indicated that ClO_2 was 2.7 times more effective than HOCl at the same pH. This could be attributed to the resistance of free chlorine by P. cepacia since this environmental strain was most likely encapsulated. The encapsulation apparently affected ClO_2 less than HOCl because when laboratory cultures of E. coli were exposed to these disinfectants, HOCl was found to be more effective (31). The test bacterium was isolated from a distribution system utilizing free chlorine as the disinfectant, and the organisms may have built up a resistance solely to it.

As was mentioned previously, the ability of a disinfectant to be effective over a wide range of environmental conditions is an important factor. One such condition would naturally be substrate pH, and the results indicated that ClO_2 experienced the smallest change in efficiency when the pH was elevated from 6 to 9. Free chlorine recorded the greatest change in efficiency as OCl^- (pH 9) was the least effective disinfectant under consideration for the inactivation of P. cepacia. A 60-fold decrease in efficiency was noticed when compared to HOCl. Hoehn (23) mentioned that the difference between these chlorine species was actually somewhere between 70- to 80-fold.

Such findings would justify the use of ClO_2 in areas where pH fluctuations were common and nuisance problems a concern.

The use of chloramines for the control of biologically mediated iron precipitation in distribution systems would have to be questioned since the data clearly show that rapid P. cepacia inactivation was restricted to only concentrations near or above 1.0 mg/l. The use of NH_2Cl would also be required to prevent the occurrence of taste and odor problems with the presence of $NHCl_2$ in concentrations exceeding 20% of the residual (15). The effectiveness of NH_2Cl was comparable only to that of OCl^-, which would most likely limit its use on a wide scale for the control of the nuisance bacteria.

It was apparent that disinfection efficiency did not directly vary with temperature for the disinfectants under consideration. Temperature effects did not appear to be uniform throughout the duration of contact, suggesting that any differences present were most likely due to bad samples. Various investigators (15, 26, 36, 37) reported that, by decreasing the substrate temperature, bacteriological activity would also be subsequently decreased for any disinfectant. However, substantial changes were only recorded when a 20° C drop in temperature was studied. During this investigation, however, it was decided to simulate water temperatures most commonly

encountered by microorganisms in a water distribution system and, thus, inactivations of P. cepacia were compared only at 10° and 20° C. Consequently, the data obtained over this temperature range still suggest that ClO_2 would be the most effective disinfectant for controlling water quality deterioration.

Of particular interest with respect to disinfection is the turbidity contaminant level because it is believed that it often interferes with the disinfection of water supplies and the maintenance of effective disinfectant levels throughout a water distribution system. This study concentrated on its interference on disinfection only by utilizing an inorganic clay (kaolinite) that was considered demand-free following its exposure to ultraviolet light. Clark et al. (68) reported that clays exert adsorptive action against biological flocs which allow them to be an effective coagulant aid. Such an adsorptive effect was hoped to be shown in this study for P. cepacia; and if this was affirmative, the possibility of protective effects would then be investigated. The centrifugation results showed that P. cepacia probably did adsorb to the clay, though a common decrease in efficiency for all the disinfectants under consideration was not noticed. The largest decrease in efficiency occurred for 0.25 mg/l HOCl when the turbidity level was 4.0 NTU's. Geldreich et al. (32) reported that turbidities as low as 3.8 NTU's

offer protection from the effects of chlorination even after exposure to residuals as high as 0.5 mg/l for 30 minutes. This finding regarding a chlorine protective effect may be suspect, however, since the experiment was not replicated. Minor differences were recorded for OCl^- and chloramine while the ClO_2 results showed that at turbidity levels of 2.0 and 4.0 NTU's, the disinfection curves were very similar to that of the ClO_2 inactivation curve without kaolinite-associated P. cepacia. Consequently, there was no indication of a protective effect produced by association with the kaolinite when ClO_2 was the disinfectant, at least for turbidities at or below 4.0 NTU's. These findings would, therefore, offer further support for the use of ClO_2 in instances where biologic iron precipitation was prevalent in the distribution system.

VI. SUMMARY AND CONCLUSIONS

Continual problems in developing useful techniques for eliminating water quality deterioration in distribution systems have prompted consideration of alternate treatment methods, including the evaluation of alternate disinfectants. Upon identification of one bacterium responsible for mediating microbial iron precipitation, a comprehensive investigation of free chlorine, chloramines, and ClO_2 was undertaken to examine their applicability in the control and/or elimination of this type of deterioration in water quality. Environmental conditions were then varied to simulate the possible changes that could occur within a distribution system, and their effects on the disinfectants were discussed.

From the results described in this thesis, significant conclusions include:

1. There is a widespread ability of many species of bacteria to precipitate iron from salts of organic acids. Using ferric ammonium citrate as the recovery medium, one such isolated bacterium was identified as Pseudomonas cepacia.

2. Adsorbed iron on P. cepacia did not alter free chlorine's effectiveness in inactivation when the

majority of the precipitate was washed from the bacteria.

3. Hypochlorous acid was effective in inactivating P. cepacia at concentrations greater than 0.1 mg/l. An increase in pH affected this disinfectant's efficiency the greatest, suggesting that the use of hypochlorite ion (OCl^-) as a primary disinfectant should be avoided.

4. Chloramine effectiveness against P. cepacia was poor, suggesting its use should be avoided when microbial iron precipitation needs to be controlled.

5. The results suggest that chlorine dioxide offers a serious challenge to chlorine as a secondary disinfectant on the basis of biocidal capabilities and stability.

6. Lowering the reaction temperature from 20° C to 10° C minimally affected the efficiencies of all the disinfectants under consideration.

7. Turbidity, at or below 4.0 NTU, showed a minimal effect on the rate of inactivation of P. cepacia for all the disinfectants under consideration.

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VIII. APPENDIX

TABLE A-1. STATISTICAL ANALYSIS OF FIVE SEPARATE EXPOSURE EXPERIMENTS UTILIZING FREE CHLORINE (95% OCl^-) AS THE DISINFECTANT FOR *P. cepacia* INACTIVATION

Time, minutes		Experiment Number 1	2	3	4	5	Standard Deviation
0	LSR*	0	0	0	0	0	0
	PD**	33.0	58.0	56.0	48.0	47.0	9.864
2	LSR	-0.655	-0.130	-0.204	-0.125	-0.195	0.223
	PD	7.30	43.0	35.0	36.0	30.0	13.647
5	LSR	-0.820	-0.557	-0.628	-0.469	-0.647	0.130
	PD	5.00	16.10	13.20	16.30	10.60	4.675
10	LSR	-1.268	-0.951	-0.879	-1.000	-0.924	0.154
	PD	1.78	6.50	7.40	4.80	5.60	2.153
15	LSR	-1.462	-1.272	-1.168	-1.229	-1.116	0.133
	PD	1.14	3.10	3.80	2.83	3.60	1.054
30	LSR	-2.084	-1.860	-1.879	-2.028	-1.859	0.106
	PD	0.272	0.80	0.74	0.45	0.65	0.218
45	LSR	-2.432	-2.286	-2.168	-2.163	-2.320	0.113
	PD	0.122	0.30	0.38	0.33	0.225	0.101
60	LSR	-2.536	-2.316	-2.621	-2.341	-2.493	0.130
	PD	0.096	0.28	0.134	0.219	0.151	0.073

*Log Surviving Ratio = $\text{Log} \left(\frac{\text{Surviving population density}}{\text{Initial population density}} \right)$ **Population Density Expressed in cells/ml x 10^4

TABLE A-2. INACTIVATION OF *P. cepacia* BY FREE CHLORINE AT pH 6.0 and 20° C

Time, minutes		Disinfectant Concentration (mg/l)			
		0.1	0.25	0.5	0.75
0	LSR*	0	0	0	0
	PD**	40	37	37	37
0.25	LSR	-	-1.226	-1.351	-2.422
	PD	-	2.2	1.65	0.14
1	LSR	-	-	-	-2.869
	PD	-	-	-	0.05
2	LSR	-0.058	-2.471	-2.755	-4.489
	PD	35	0.125	0.065	0.0012
5	LSR	-0.097	-4.614	-5	-5
	PD	32	0.0009	0.0003	0
10	LSR	-0.438	-5	-5	-
	PD	14.6	0	0	-
15	LSR	-0.912	-5	-5	-
	PD	4.9	0	0	-
30	LSR	-1.803	-5	-5	-
	PD	0.63	0	0	-
60	LSR	-2.824	-	-	-
	PD	0.06	-	-	-
Final pH		6.0	6.0	6.1	6.2
Final Cl Conc.***		0.03/ 0.02	0.1/ 0.17	0.16/ 0.27	0.26/ 0.33

*Log Surviving Ratio = Log $\left(\frac{\text{Surviving population density}}{\text{Initial population density}}\right)$

**Population Density expressed in cells/ml x 10^4

***Expressed as mg/l free Cl (x) and combined Cl (y), (x, y)

TABLE A-3. INACTIVATION OF P. cepacia BY COMBINED CHLORINE AT pH 6.0 and 20° C

Time, minutes		Disinfectant Concentration (mg/l) 0.1	0.25	0.5	0.75	1.0
0	LSR*	0	0	0	0	0
	PD**	123	120	123	120	120
2	LSR	-0.094	-0.038	-0.270	-1.511	-2.574
	PD	99	110	66	3.7	0.32
5	LSR	-0.251	-0.150	-0.780	-1.824	-2.864
	PD	69	85	20.4	1.8	0.164
10	LSR	-0.334	-0.456	-1.203	-3.380	-5
	PD	57	42	7.7	0.05	0.001
15	LSR	-0.350	-0.862	-1.830	-4.477	-5
	PD	55	16.5	1.82	0.004	0
30	LSR	-0.427	-2.535	-4.488	-5	-5
	PD	46	0.35	0.004	0.001	0
60	LSR	-0.789	-4.602	-5	-5	-5
	PD	20	0.003	0	0	0
Final pH		6.1	6.0	6.2	6.0	6.2
Final Cl Conc.***		0/0.03	0/0.14	0/0.38	0/0.58	0/0.91

*Log Surviving Ratio = Log $\left(\frac{\text{Surviving population density}}{\text{Initial population density}}\right)$

**Population Density expressed in cells/ml x 10^4

***Expressed as mg/l free Cl (x) and combined Cl (y), (x, y)

TABLE A-4. INACTIVATION OF P. cepacia BY CHLORINE DIOXIDE AT pH 6.0 and 20° C

Time, minutes		Disinfectant Concentration (mg/l) 0.1	0.25	0.5	0.75	1.0
0	LSR*	0	0	0	0	0
	PD**	42	44	67	39	67
0.25	LSR	-	-	-	-1.409	-4.747
	PD	-	-	-	1.52	0.0012
0.5	LSR	-	-	-1.981	-2.363	-5
	PD	-	-	0.7	0.169	0
1	LSR	-	-1.199	-2.321	-3.632	-5
	PD	-	2.78	0.32	0.0091	0
2	LSR	-0.516	-2.152	-3.969	-5	-
	PD	12.8	0.31	0.0072	0.0002	-
5	LSR	-1.055	-3.971	-5	-5	-
	PD	3.7	0.0047	0	0	-
10	LSR	-2.192	-5	-5	-5	-
	PD	0.27	0	0	0	-
15	LSR	-2.361	-5	-	-	-
	PD	0.183	0	-	-	-
30	LSR	-3.032	-5	-	-	-
	PD	0.039	0	-	-	-
60	LSR	-3.838	-	-	-	-
	PD	0.0061	-	-	-	-
Final pH		6.0	6.1	6.1	6.0	6.2
Final ClO_2 Conc. (mg/l)		0.08	0.2	0.33	0.61	0.9

*Log Surviving Ratio = Log $\frac{(\text{Surviving population density})}{(\text{Initial population density})}$

**Population Density expressed in cells/ml x 10^4

TABLE A-5. INACTIVATION OF P. cepacia BY CHLORINE DIOXIDE AT pH 9.0 and 20° C

Time, minutes		Disinfectant Concentration (mg/l)			
		0.1	0.25	0.5	1.0
0	LSR*	0	0	0	0
	PD**	153	46	153	42
0.25	LSR	-	-	-0.198	-1.924
	PD	-	-	97	0.5
0.5	LSR	-	-	-	-3.674
	PD	-	-	-	0.0089
1	LSR	-	-	-0.266	-4.243
	PD	-	-	83	0.0024
2	LSR	-0.074	-0.850	-1.365	-4.720
	PD	129	6.5	6.6	0.0008
5	LSR	-0.159	-2.050	-2.605	-5
	PD	106	0.41	0.38	0
10	LSR	-0.226	-2.623	-3.766	-
	PD	91	0.109	0.0262	-
15	LSR	-0.255	-3.251	-4.035	-
	PD	85	0.0258	0.0141	-
30	LSR	-0.245	-3.973	-4.561	-
	PD	87	0.0049	0.0042	-
60	LSR	-0.271	-	-	-
	PD	82	-	-	-
Final pH		8.8	8.9	8.9	9.0
Final ClO_2 Conc. (mg/l)		0.07	0.18	0.32	0.73

*Log Surviving Ratio = Log $\frac{\text{(Surviving population density)}}{\text{(Initial population density)}}$

**Population Density expressed in cells/ml x 10^4

TABLE A-6. COMPARISON OF *P. cepacia* INACTIVATION BY FREE AND COMBINED CHLORINE SPECIES AND CHLORINE DIOXIDE AT 20° C

Disinfectant	Conc. (mg/l)	Minutes for 99% Inactivation	
		pH 6.0	pH 9.0
Free Chlorine	0.1	36.1	-
	0.25	2.0	120.0
	0.5	1.5	32.4
	0.75	1.0	16.5
	1.0	-	12.0
Combined Chlorine	0.1	180.0	-
	0.25	26.5	160.0
	0.5	16.0	20.5
	0.75	5.6	14.0
	1.0	1.7	12.8
Chlorine Dioxide	0.1	9.5	-
	0.25	1.9	5.0
	0.5	0.5	3.6
	0.75	0.4	-
	1.0	0.06	0.3

TABLE A-7. *P. cepacia* REMOVAL FROM THE TOP ONE-THIRD OF CENTRIFUGED SAMPLES ASSOCIATED WITH AND WITHOUT KAOLINITE AT 1100 rpm AND 20° C

Centrifugation Time (min)		Turbidity (NTU) 0.15	4.0
0	%*	100	100
	PD**	19.9	19.5
0.5	%	107	55
	PD	21.3	10.7
1	%	91	50
	PD	18.2	9.8
2	%	63	37
	PD	13.5	7.3
5	%	56	21
	PD	11.1	4.0
10	%	36	15
	PD	7.1	2.92
15	%	12	8
	PD	2.41	1.49

*Percent cells remaining in top one-third of centrifuged samples

**Population Density expressed in cells/ml x 10^3

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A COMPARISON OF THE BIOCIDAL EFFICIENCIES OF FREE CHLORINE, CHLORAMINES, AND CHLORINE DIOXIDE ON THE HETEROTROPHIC IRON PRECIPITATING BACTERIUM, *Pseudomonas cepacia*

by

James Richard Rickloff

(ABSTRACT)

Little information is available regarding the applicability of various disinfectants to the control of microbial growths within water distribution systems, especially in relation to "nuisance" organisms. With regards to microbially mediated iron precipitation, an isolated heterotrophic iron precipitating bacterium was identified. An investigation of free chlorine, chloramines, and chlorine dioxide was undertaken to examine their applicability in the control and/or elimination of this type of deterioration in water quality. Environmental conditions were then varied to determine their effects on the disinfectant's efficiencies.

The isolated bacterium was identified as *Pseudomonas cepacia*. It was determined that chlorine dioxide offered a serious challenge to chlorine as a secondary disinfectant on the basis of its biocidal capabilities and stability. Solution pH affected free chlorine's efficiency

the greatest, while chloramine's poor efficiency suggested that its use should be avoided in areas of microbial iron precipitation.

Water temperature and turbidity showed a minimal effect on the rate of inactivation of P. cepacia for all the disinfectants under consideration.