REGROWTH OF *E. COLI* AND *S. FAECALIS* IN TREATED SEWAGE AFTER CHLORINATION IN A CONTINUOUS-FLOW REACTOR

by

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I. INTRODUCTION

It was more than eighty years ago that the first patent in the United States for a process covering the use of chlorine for sewage deodorization and disinfection was issued. Chloride of lime was the best commercial source of chlorine at that time, but because it was expensive and difficult to handle, its use progressed slowly in the United States. Today, because of the development of suitable equipment, chlorination is a major part of wastewater treatment (1).

Many questions are now being raised about the use of chlorine. Recent research points to adverse effects that chlorinated wastewater effluents might have on aquatic life. If these reports prove true, then it is time for increased management of wastewater chorination. Such management must ensure proper disinfection for the protection of public health while at the same time providing for the protection of aquatic life. Perhaps future research will show the best solution to the problem to be the substitution of another disinfectant for chlorine.

Virginia law states that the chlorine residual in sewage effluents must be two milligrams per liter (mg/l) after 30 minutes contact. This concentration requirement is based on data presented by Clarke *et al.* (1) in 1962, who compared the germicidal efficiency of hypochlorous acid, hypochlorite ion, and monochloramine against *Escherichia coli* at 2-6° C. in batch culture.

At the present time, there have been no published reports of

the efficiency of chlorine against sewage-borne organisms in continuous flow systems, a surprising fact as chlorination in practice is a continuous flow process. Therefore, a research project was designed to determine the effectiveness of chlorination in a system that simulated the chlorination process in sewage plants.

The emphasis in this project was not on the immediate effectiveness of chlorination on bacteria but rather on the potential of "indicator" organisms in sewage to regrow once the chlorine has been removed. The importance of the study lies in the fact that these organisms are used in sanitary engineering microbiology to indicate the presence of fecal contamination in water and to evaluate the efficiency of disinfection processes such as chlorination. If chlorination proved to be bacteriostatic instead of bacteriocidal, as regrowth studies would show, then the use of the coliforms and fecal streptococci as indicator organisms should be revaluated.

While others have studied the regrowth phenomenon, none have reported results of studies of the regrowth of indicator organisms that have been chlorinated in a continuous flow laboratory system where conditions could be carefully controlled. This project was a cooperative effort with a coinvestigator (2) who designed the laboratory treatment system and evaluated the effectiveness of chlorination for varying contact periods.

II. LITERATURE REVIEW

Chlorine Disinfection

History

Hugo (3) reported that one of the earliest reports of the use of chlorine compounds for the control of infections was published in 1827 in London by Thomas Alcock. In his 'Essay on the Use of Chlorurets of Oxide of Sodium and Lime', Alcock recommended chlorine compounds as disinfecting and deodorizing agents. Such compounds could be used in a variety of environments--hospitals, ships, workshops, stables, privies, reservoirs and sewers. He specifically recommended their use in deodorizing and disinfecting corpses and areas contaminated with blood or other body fluids.

Alcock's recommendations were not accepted by all and several publications of that time went so far as to ridicule Alcock himself. As reported by Hugo (3), the work of Semmelweis in 1847, which involved the use of chlorine in reducing the number of deaths from puerperal fever, helped dispel such critics. His results showed that hypochlorite solutions were successful in reducing deaths caused by puerperal infections from 12 per cent to 1.27 per cent.

Death from puerperal infections was prevalent during the 1800's, which explains the importance of Semmelweis' work. Still, there were many skeptics and the true value of disinfectants was not fully understood until Pasteur's germ theory (1861), involving putrefaction and infection, had been established. This understanding was enhanced when Koch, in 1881, was able to show the lethal effects of hypo-

chlorites on pure cultures of bacteria.

Today, the disinfectant uses of chlorine compounds can be classified into four groups: medical, household, food processing and catering, and public health (3).

Public Health Considerations

It was the concern for public health that helped create the need for wastewater chlorination. Currently, in the United States, 14 waterborne-disease outbreaks occur each year. This accounts for an average of 1600 illnesses and one death per year (4). While this is only a minute part of the total illnesses that occur, the threat to public health still exists.

Data gathered by Craun and McCabe (4) indicated that waterborne disease outbreaks were no longer declining in the United States. A substantial drop in outbreaks, from 45 to 10 per year, occurred in the period 1938-40 to 1951-55. Since 1951-55 there has been no noticeable decline, and none was foreseen for the near future.

During the ten year period 1961-70 there were 128 known outbreaks causing 46,374 illnesses and 20 deaths. Heading the list of illnesses was gastroenteritis that accounted for 39 of the outbreaks. Included in this number was what the authors referred to as "sewage poisoning" (4).

Chemistry of Chlorination

Before the principles behind wastewater chorination can be understood, it is necessary to understand the basic chemistry of chlorina-

tion itself. As a disinfectant, chlorine is used in the form of free chlorine or as hypochlorites. Both forms are extremely reactive and for effective disinfection to take place, the chlorine demand of the solution in question must be met.

One of the fundamental reactions is that of chlorine and water to form hypochlorous acid:

$$Cl_2 + H_2O \rightleftharpoons H^+ + Cl^- + HOCl$$

This reaction is displaced toward the right in dilute solutions and in solutions with a pH above 4.

The hypochlorous acid formed is a weak acid and will partially disassociate at pH levels above 6 to produce a hypochlorite ion:

$$HOCl \rightleftharpoons H^+ + OCl^-$$

At pH levels between 5 and 9 the reaction is incomplete and both the hypochlorous acid and hypochlorite ion will exist to some degree.

The chlorine and hypochlorous acid will also react with a variety of substances, one of the more significant, in terms of disinfection, being ammonia. Ammonia reacts with chlorine and hypochlorous acid to form monochloramines, dichloramines, and trichloramines:

$$NH_3 + HOCl \rightleftharpoons \underset{\text{monochloramine}}{NH_2Cl} + H_2O$$

$$NH_2Cl + HOCl \rightleftharpoons \underset{\text{dichloramine}}{NHCl_2} + H_2O$$

$$NHCl_2 + HOCl \rightleftharpoons \underset{\text{trichloramine}}{NCl_3} + H_2O$$

As with the formation of hypochlorous acid and hypochlorite ion, the type of chloramine formed depends on the pH of the solution. Above pH 8.5 only monochloramine is formed. Between pH 5.0 and 8.5, vary-

ing mixtures of mono- and dichloramine are present, and at pH 4.4 to 5.0 only dichloramine is present. Below pH 4.4 trichloramine is formed.

The chlorine residuals formed in all of these reactions are of sanitary significance. Residuals of chlorine, hypochlorous acid and hypochlorite ion are referred to as "free chlorine residuals", and residuals of the chloramines are known as "combined chlorine residuals"(5).

The most effective disinfectant of all the chlorine forms is hypochlorous acid. Its effectiveness is due to the similarity in structure of hypochlorous acid and water, its low molecular weight, and its electrical neutrality. It is able to penetrate the bacterial cell wall with relative ease. The other free chlorine form, the hypochlorite ion, is a poor disinfectant. Unlike hypochlorous acid, the hypochlorite ion is negatively charged, preventing the ion from diffusing through bacterial cell walls (6). Overall, the chloramines are poorer disinfectants than the free chlorine residuals but their action continues longer (7). Of all the chloramines, dichloramine is the most lethal to bacteria (6).

Mechanism of Bactericidal Action

The exact mode of action of chlorine on microorganisms is still unknown. Several theories have evolved since the first use of chlorine as a disinfectant. Three of the earliest theories were: 1) The Nascent Oxygen Theory, 2) The Nascent Oxygen and Direct Chlorination Theory, and 3) The Formation of Toxic Substance Theory (8).

The Nascent Oxygen Theory stated that the disinfecting properties of chlorine were due to the formation of hypochlorous acid which liberated nascent oxygen. This nascent oxygen, in turn, oxidized the protoplasm of the microorganisms. Postulated reactions are:

$$Cl_2 + H_2O \rightleftharpoons HOCl + HCl$$

or

$$OCl^- + H^+ \rightleftharpoons HOCl$$

then

$$HOCl \rightleftharpoons HCl + O$$

The Nascent Oxygen Theory and the Direct Chlorination Theory furthered the first theory by stating that in addition to the nascent oxygen liberated by hypochlorous acid, microorganisms were destroyed by direct chlorination of the protoplasm of the organisms.

According to Chang (8), Baker, in 1926, formulated the Formation of Toxic Substance Theory, which stated that death of microorganisms was caused by an interference with cell division. Chlorine compounds chlorinated the lipoid-protein in the cell wall to form a toxic chloro-compound. This compound, in turn, caused the interference which resulted in death for the microorganisms.

All three of the theories led to much confusion concerning the destruction of microorganisms. Chang (8), in 1944, helped dispel some of the confusion. He showed that hydrogen peroxide and potassium permanganate also liberated nascent oxygen, but this nascent oxygen had no disinfecting power. In fact, he showed that no oxygen was even liberated in the chlorine reaction. The disinfecting agent was the hypochlorous acid.

Green and Stumpf (9), in 1946, added another theory to answer the question of the mode of action of chlorine. They showed that the

inhibition of the bacterial cell occurred by the disruption of glucose oxidation within the cell. This reaction they stated to be irreversible. The sulfhydryl group within the various enzymes was where the destructive oxidation reaction took place (10).

In 1953, Ingols (11) refined the theory of Green and Stumpf. While he agreed with their general principles, he also showed that other enzymatic groups may play an important role in the death of the bacterial cell. This he concluded after showing that bacterial activity could not be restored, even after the sulfhydryl groups had been restored.

Another contribution was made in 1962 by Wyss (12). Wyss also believed the destruction of the bacterial cell involved interference with the enzyme system. He called it the phenomenon of unbalanced growth in which the cell was thrown so out of balance that by progress of its own metabolism, it died before repairs could be made.

No matter what the actual mode of action, several factors have been agreed upon. The efficiency of the disinfecting compound is a function of the rate of diffusion through the cell. This efficiency is affected by several other factors: the type of disinfectant, concentration of the disinfectant, length of contact time with the disinfectant, temperature, pH, and the type and concentration of organisms (6).

Wastewater Chlorination

History

Chlorine's first practical use in wastewater treatment was as a

deodorant. Such use can be traced back to the pre-twentieth century idea that odors spread disease. The discovery in 1880 that certain bacteria cause specific diseases led to the subsequent discovery that chlorine could be of value in destroying such bacteria (6).

The objective of sewage disinfection is "to destroy the enteric pathogenic organisms that may be present in order to make the receiving body of water safe for the various uses to which it is put"(13). The pathogens which are the subject of such destruction include: 1) cholera vibrio, 2) typhoid and paratyphoid bacteria (Salmonella), 3) dysentery bacteria (Shigella), 4) amoebic dysentery (E. histolytica), 5) tuberculosis bacteria (Mycobacterium tuberculosis), 6) viruses causing infectious hepatitis and poliomyelitis and 7) parasitic worms, such as roundworms and tapeworms (Helminths), hookworm (Ascaris) and Shistosoma (13).

Chlorination of sewage on a large scale was first practiced in 1854 by the Royal Sewage Commission in London. Later, in 1879, chlorine was first used for disinfection purposes to treat the feces of typhoid patients before disposal into a sewer. It was not until 1893 that chlorination for disinfection purposes was practiced on a plant scale in Hamburg, Germany. During this period, all of the chlorination was accomplished by use of chloride of lime.

Meanwhile in the United States, studies on the use of chlorinated lime were being carried out by Phelps and Carpenter in 1906-07. Plants were set up in Red Bank, New Jersey, Baltimore, and Boston, and 1907-08 marked the beginning of effective chlorination practices

in the United States.

The use of chlorine in wastewater treatment was limited by the chloride of lime itself. It was hard to handle, deteriorated very rapidly in storage, and was expensive. It was not until the development of a gas chlorinator in 1913 that chlorination of wastewater was widely practiced (6).

Principles

The chlorination of wastewaters presents a different situation than the chlorination of water supplies due to the nature of the wastewater.

The factors affecting wastewater chlorination have been known and studied over the years. One of the earlier studies was that by Rudolfs and Ziemba (14), in 1934, who evaluated the importance of several conditions on disinfection. The first was the effect of chlorine dosage on bacterial kill. They also examined the effect of contact-time variation upon bacterial kill and attempted to explain what happens to chlorine when it is absorbed in the sewage and its ultimate effect. Other researchers have examined these and other questions.

Eliassen and Krieger (15), in 1950, developed the practice of maintaining a chlorine residual in sewage effluent after a given contact period. The relationship between chlorine dosages and chlorine residuals at various contact periods was developed, as was the measure of coliform bacteria density, known as the most probable number (MPN), in the sewage. Other studies which followed included

those of Kott and Ben-Ari (16), Buelow and Walton (17), Heukelekian and Faust (13), and Browning and McLaren (18).

Factors affecting chlorine disinfection are numerous. One factor, as previously mentioned, is the nature of the sewage itself. Sewage is composed of inorganic and organic components, and chlorine, being an extremely reactive chemical, oxidizes many of these components readily and irreversibly. Alkaline conditions, ammonia, and amino acids all serve to reduce the efficiency of chlorine disinfection. In fact, while ammonia interferes with disinfection by slowing down the process, the presence of amino acids may stop disinfection completely (19).

Some of these difficulties can be partially overcome by bio-oxidative treatment and clarification which, respectively, converts nitrogenous components into nitrate and removes large or clumped microorganisms that are resistant to chlorination (19).

Design factors also play a role in obtaining adequate sewage disinfection. An initial mixing of the chlorine solution and wastewater is a must. Rudolfs and Gehm (20) noted this as early as 1936. However, the usual practice in plants is to apply the chlorine solution directly to the contact basin without an initial rapid mix (20). Collins, Sellek and White (21) demonstrated that rapid mixing after chlorine application resulted in kills two orders of magnitude greater than when chlorine was introduced to a back-mixed reactor. According to the authors, the rapid mix allowed the formation of the more reactive and bactericidal portions of the chlorine residuals.

Another design factor is the contact basin which should be designed to approach plug-flow conditions. The contact time must be known in order to estimate the bacterial kill. This kill has been formulated by use of Chick's law:

$$\log N_o - \log N = kt$$

where N_o is the initial number of organisms, N the number of organisms after time t, and k is a constant varying with the disinfecting agent in use (22).

Other investigators have reported that Chick's law does not hold true in wastewater chlorination. Collins, Selleck and White (21) found that the survival rate of coliform bacteria in chlorinated wastewater never followed Chick's law. The graphical relationship between kill and contact time was approximately linear on log-log paper, whereas Chick's law expressed graphically is linear on semilog paper. This deviation from Chick's law might have been due to decreasing bactericidal activity of the chlorine residual and to an induced heterogeneity of the exposed bacterial populations (21).

Limitations

Along with the knowledge of the factors affecting disinfection, one must keep in mind the basic shortcomings of wastewater chlorination. The chlorination of wastewaters is a widely accepted practice in plants throughout the United States. Its ease of application and low cost have led to its acceptance. But, its shortcomings in disinfection are now becoming evident and need to be seriously evaluated. Two important limitations are the coliform test and the pres-

ence of compounds in wastewater that interfere with chlorination (23).

Coliforms serve as indicators of the possible presence of pathogenic organisms. However, limitations of their use as indicators presently are being debated. (These limitations are discussed in the following section.) Fecal coliforms were shown to be more reliable as indicators by Geldreich (24), but have not been recommended as indicators of the efficiency of wastewater chlorination.

In addition, the resistance of many other organisms (particularly pathogens) to chlorination should be noted. Butterfield (25) found that pathogens such as Salmonella typhosa, Shigella shiga and Shigella sonnei have the same susceptibility as the coliform group to free and combined residuals. But recent studies have shown that enteric viruses, protozoal cysts, and Mycobacterium tuberculosis are somewhat more resistant to chlorination (24). This could mean a serious threat to public health.

The presence of interfering compounds in wastewater presents another serious limitation. Included among such substances are ammonia, certain organic compounds, and reducing agents. Breakpoint chlorination can overcome this particular limitation but, unfortunately, it is not a usual practice in wastewater treatment plants (23).

The toxicity of chlorine to aquatic life has only recently been examined. Several such studies were reported by Brungs (7). Research now indicates that chlorinated effluent has a significant, adverse environmental impact.

The extent of toxicity to aquatic life depends on the concentration of the residual chlorine and on the relative amounts of free chlorine and chloramines. Merkens found in his study that the free chlorine was more toxic than chloramines and that residual chlorine was more toxic at a lower pH because more free chlorine was present at that pH.

Rosenberger, in his research, came to the same conclusion. In addition, he found that larger fish will die faster than smaller fish because a large fish has less gill surface area per unit body than a small fish. Gills were the tissues attacked by chlorine.

Forbes, working with white suckers, had different results. He found that the gills were not the primary site of chlorine toxicity when he exposed the suckers to a 1.0 mg/1 chlorine residual without any effect.

Studies involving toxicity have also been conducted with chlorinated wastewater effluent. The Michigan Department of Natural Resources found that 50 per cent of their rainbow trout died within 96 hours at a residual chlorine concentration of 0.014 to 0.029 mg/1. Fish were found dead as far as 0.8 mile below the sewage outfall (7).

Tsai studied the effects of chlorination on streams below wastewater treatment plants in Maryland, Northern Virginia, and Southeastern Pennsylvania. Directly below the discharges, no living organisms were found, whereas the unchlorinated reaches were abundant with wastewater fungi. No fish were found in areas with a chlorine residual above 0.37 mg/1.

Additional studies could be cited, but the important finding cited by most all the researchers is that there are significant, adverse effects of chlorinated wastewater on aquatic life. In many cases, the problems result from overchlorination caused by poor control over chlorine-feed rates or by the application of poor analytical methods to determine the concentration of the chlorine residual.

Wastewater chlorination does have significant limitations and shortcomings. Dechlorination and the use of other disinfecting agents should be examined, keeping in mind the responsibility to public health and aquatic life.

Significance of the Coliform Bacteria and Fecal Streptococci

Fecal pollution is determined by the use of indicator organisms. Such indicators should be present in feces in large numbers, be absent from unpolluted environments, persist in the environment, and be easily detected. Two indicator organisms used today are the coliform bacteria and fecal streptococci (24). There are advantages and disadvantages to the use of both these groups as indicators.

Coliform bacteria are defined in Standard Methods for the Examination of Water and Wastewater (27) as "all of the aerobic and facultative anaerobic, gram-negative, non-spore forming, rod shaped bacteria which ferment lactose with gas formation within 48 hours at 35° C.".

As reported by Clark and Kabler (28), the coliform bacteria were first considered to be characteristic of human feces by Escherich in 1885. Their presence in water represented the possibility of patho-

gens also occurring and therein lies their sanitary significance. Escherich's bacterial species are now classified in the Escherichia-Aerobacter genera.

Later investigators isolated many other coliform bacteria from feces, soil and plants. Their isolation from such varied environments led to much confusion over their significance.

Clark and Kabler also reported that, in 1895, Smith stated that the presence of coliforms indicated fecal contamination in all of the environments because all coliform bacteria originate from the gut of warm-blooded animals.

Eijkman, about 1904, disputed the belief that the source and habitat of the coliform group had no significance in establishing the danger to public health. He recommended the use of an elevated-temperature incubation test that gave a positive reaction with fecal coliform organisms and a negative reaction with those of non-fecal origin (28).

This method has been modified by use of a lactose broth fermentation instead of a carbohydrate fermentation, a reduction in the elevated temperature from 46° to 44.5° C., the use of primary enrichment for the multiple tube procedure, and the development of a suitable procedure to be used with the membrane filter technique. Such changes have increased the sensitivity and selectivity of the test.

The source and habitat of coliform organisms varies from warm-blooded animals and freshwater fish, to soils, vegetation and insects. Of warm-blooded animals, human feces contain the greatest variety of

types although not the highest fecal coliform percentage. Livestock, with a fecal coliform percentage of 98.7 per cent, rates the highest. Humans are next with 96.4 per cent, followed by cats, dogs and rodents at 95.3 per cent and poultry at 93.0 per cent (24).

Because the feces of warm-blooded animals may at any time contain pathogens, they are significant as a source of pollution. Cold-blooded animals are quantitatively insignificant as a source of pollution (28).

Fresh-water fish, while they do not have a permanent coliform flora, can serve as carriers of pollution for up to seven days and could transfer a pathogen from a polluted area to a non-polluted one. Such potential depends on the feeding activity, bacteriological agents in the fish gut and the degree of contamination of the water (24).

The density of fecal coliforms in soil depends on the source of soil. Data collected by Geldreich (24) showed that the percentage of fecal coliforms in undisturbed soils was 9.2 per cent, while that of polluted soils was 82.9 per cent. These polluted soils included those from feed lots, from areas flooded with sewage, and from near heavily polluted streams. Several factors affected the presence of bacteria in soils, among them being temperature, soil moisture, pH, and the organic nutrients present.

Many of the bacteria found on plants might result from insect contact. Geldreich (24) found that the percentage fecal coliforms of the total bacterial population isolated was 10.9 on flowers, 18.5 on

foliage and 14.9 in and on insects. These percentages are low and, as with soils, have the same significance in terms of public health importance as those from feces when the soils and vegetation have been exposed to fecal pollution. They are less significant to public health when the coliform bacteria from soils or vegetation have not been exposed to recent fecal pollution. All of the preceding sources of bacteria are usually present in the surface water environment and contribute to the bacterial flora in it (28).

Another group of bacteria which has been shown to be of sanitary significance as an indicator are the fecal streptococci. Standard Methods (27) defines the fecal streptococci to include the intestinal streptococci from fecal wastes of all warm-blooded animals. These gram-positive cocci in chains of two or more organisms and are capable of growth in brain heart infusion broth at 45° and 10° C. (the enterococcus species) or at 45° C. only (S. bovis and S. equinus).

Geldreich and Kenner (29) reported some of the historical facts concerning the fecal streptococci. These organisms were observed in the feces of all warm-blooded animals and with associated waters as early as 1900 by bacteriologist Houston and others. In 1910, Winslow and Palmer noted that the significance of the fecal streptococci lay in their use to differentiate between pollution from humans and other warm-blooded animals. However, due to problems in methodology, it has been only recently that the group has been used as a bacterial indicator of pollution. Like fecal coliforms, fecal streptococci were found in a variety of habitats, including wastes from warm-blooded

animals, wildlife, freshwater fish, on vegetation, and in insects.

Geldreich and Kenner (29) showed the occurrence of fecal streptococci in warm-blooded animals and the correlation with fecal coliform. In all warm-blooded animals except humans, the density of fecal streptococci was higher than the fecal coliform density. In human feces, the ratio of fecal coliform to fecal streptococci was 4.4. In all other warm-blooded animals, the ratio was less than 0.7. In all animals except the cow, the enterococcus strain of fecal streptococci occurred most frequently.

Insects, vegetation, soils, and freshwater fish also exhibited the highest occurrence of the enterococcus strain of fecal streptococci. In addition, other strains of fecal streptococci showed higher occurrence than in the warm-blooded animals (30).

There are two valuable applications of fecal streptococci as indicators. First, S. bovis and S. equinus may be used as specific indicators of non-human pollution. These two organisms are the most sensitive of the fecal streptococci and, thus, show a rapid die-off outside of the body. The other application is in conjunction with fecal coliforms. The ratio of fecal coliform to fecal streptococci shows great promise in distinguishing sources of pollution (29), and it may prove useful as a tool in managing watersheds. However, even though coliforms and fecal streptococci are useful as bacterial indicators of pollution, limitations to their use should be realized.

Coliforms have an advantage of being the most conservative indicator. Their principal disadvantage lies within the fact that

they show little specificity and a tendency to regrow (the proliferation after chlorination of sewage effluent or upon dilution with clear water) (30).

Fecal coliforms overcome this disadvantage in that they are specific and show little regrowth. However, as compared to the total coliform group, they may not provide an adequate safety margin for judging water potability (30).

The fecal streptococci have the advantage of exhibiting little or no regrowth, and their detection enables one to distinguish between human and animal sources of fecal pollution on the bases of the streptococci species in water and of the fecal coliform to fecal streptococci ratio. One principal disadvantage is that they rapidly die outside the intestinal tract (30).

Despite such disadvantages, the coliforms and fecal streptococci have an important role in determining public safety. It is through future researchers that a more valuable indicator may one day replace or join the use of coliforms and fecal streptococci as indicators.

Regrowth Studies

Part of the confusion over interpretation of bacteriological data and questions concerning wastewater chlorination can be related to the regrowth of bacteria. Regrowth is the proliferation of bacteria after chlorination of sewage effluent or upon dilution with clean water. Such regrowth is known to occur in soil, water, and sewage (30).

Indicator organisms have a direct sanitary significance if they originate in the feces. But the true significance of bacteria that

are present due to regrowth is still unclear (26). This also means the use and efficiency of wastewater chlorination must be re-evaluated.

The regrowth of coliform organisms in partly and completely undiluted sewage was studied and established by Rudolfs and Gehm (20) in 1936. In their research, they used cotton-filtered sewage which had been chlorinated to 25, 50, 75 and 100 per cent of the demand. A 10 minute contact time was used. Densities of Bacterium coli (Escherichia coli), total bacteria, and total animals were determined every 24 hours for a period of 3 or 4 days in samples held at 20° C.

The results obtained with the total bacteria are shown in Table I. The numbers increased after a short lag in all cases. Aeration of the sewage after chlorination decreased the lag period. Samples receiving higher chlorine dosages also tended to show the greatest regrowth. A normal growth curve was obtained when total bacterial density was plotted as a function of time (Figure 1).

B. coli also regrew after chlorination. Regrowth in heavily chlorinated samples rarely resulted in population densities greater than those observed in unchlorinated samples. The authors concluded that this was due to the depletion of food by the other organisms during the longer lag period of B. coli.

Rudolfs and Gehm (20) explained also the reason for regrowth. They attributed it, in part, to the reduction by chlorine of fauna which feed on bacteria. The protozoan numbers, because of their lower initial population and greater sensitivity to the chlorine, are reduced to a greater extent. Their results, shown in Table II, show this rela-

TABLE I

Total Bacteria after Chlorination

(Thousands per cubic centimeter)

Cl_2 added	Cl_2 demand satisfied	Bacteria after chlorination of				
		0 hrs.	24 hrs.	48 hrs.	72 hrs.	96 hrs.
ppm	per cent					
0	0	5,000	8,000	6,000	1,600	400
2.4	25	4,000	15,000	30,000	5,000	1,500
4.7	50	300	21,000	50,000	20,000	3,000
7.1	75	40	26,000	50,000	50,000	18,000
0.4	100	5	25,000	50,000	53,000	20,000
0	0	1,500	25,000	30,000	75,000	
3.5	25	1,000	23,500	30,000	15,000	
6.9	50	100	13,000	18,000	20,000	
10.4	75	20	5,500	15,000	50,000	
13.8	100	5	2,000	32,000	60,000	

* After Rudolfs and Gehm (20).

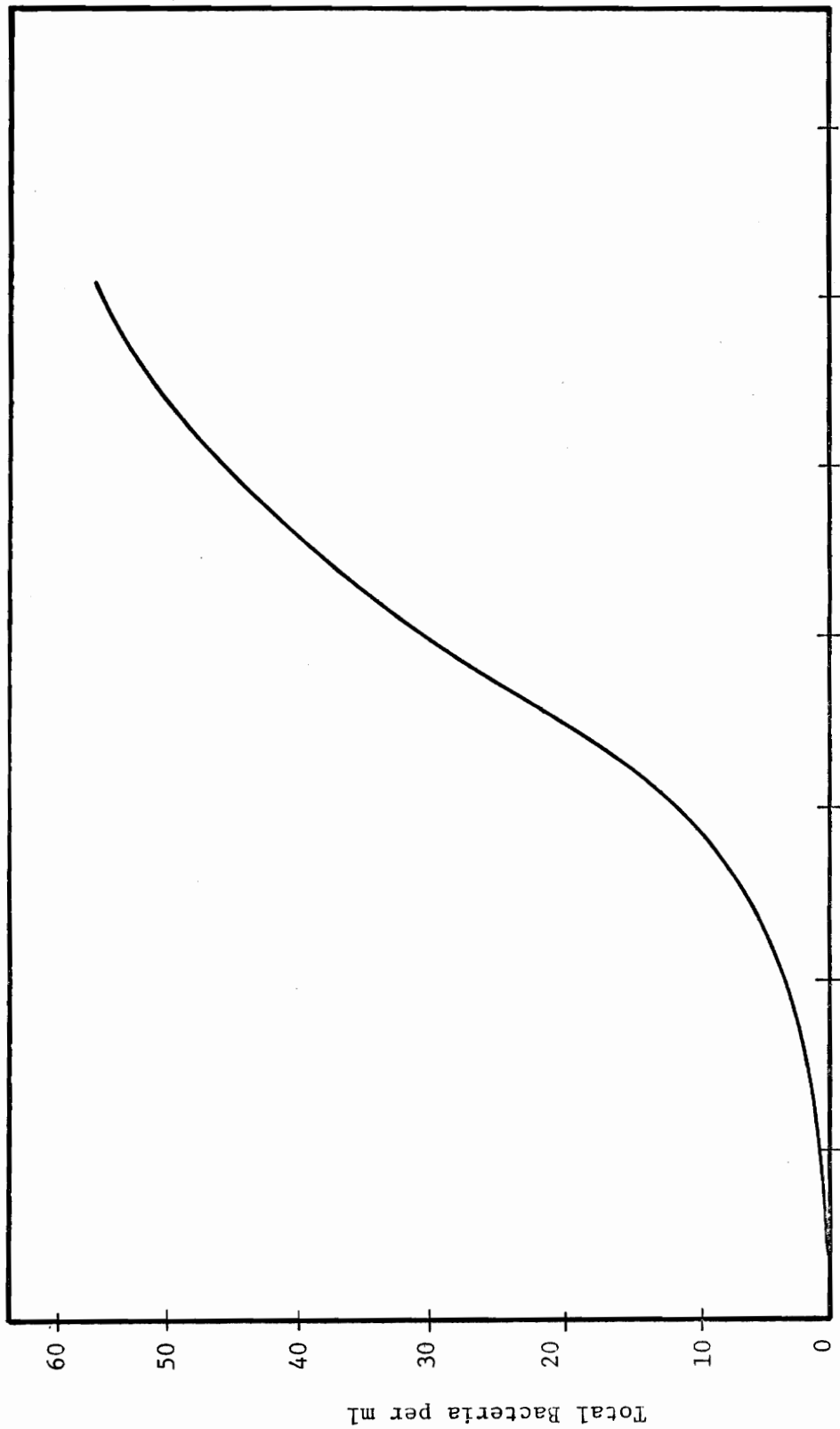

Figure 1. Relation Between Maximum Bacterial Aftergrowth Present in Partially and Completely Chlorinated Sewage and Time of Incubation. (After Rudolfs and Gehm, 20)

TABLE II

Relation Between Bacteria and Protozoa Remaining After Chlorination

Cl_2 demand satisfied	Organisms	Organisms remaining after incubation of							
		0 hrs.	24 hrs.	48 hrs.	72 hrs.	0 hrs.	24 hrs.	48 hrs.	72 hrs.
per cent									
0	Total bacteria 1,000/cc	3,000	12,000	10,000	8,000	1,500	25,000	30,000	7,500
25	Total bacteria 1,000/cc	2,500	15,000	25,000	20,000	1,000	23,500	30,000	15,000
50	Total bacteria 1,000/cc	250	50,000	70,000	20,000	100	13,000	18,000	20,000
75	Total bacteria 1,000/cc	10	11,000	40,000	70,000	20	5,500	15,000	50,000
100	Total bacteria 1,000/cc	1	1,500	30,000	50,000	5	2,000	32,000	50,000
0	B. coli 1,000/cc	2,500	4,500	9,000	2,500	950	950	1,400	1,500
25	B. coli 1,000/cc	450	1,100	11,000	9,500	450	2,500	1,500	1,500
50	B. coli 1,000/cc	95	14,000	24,000	14,000	4.5	1,100	1,500	2,500
75	B. coli 1,000/cc	0.04	2.5	250	400	2.5	1,100	1,700	2,500
100	B. coli 1,000/cc	0.004	0.0	250	200	1.4	250	450	950
0	Protozoa per cc	800	4,400	10,400	20,000	8,000	24,000	24,000	176,000
25	Protozoa per cc	1,200	9,600	8,000	20,000	6,000	20,000	60,000	24,000
50	Protozoa per cc	400	400	2,400	2,000	1,200	8,000	8,000	48,000
75	Protozoa per cc	0	0	0	0	0	0	0	0
100	Protozoa per cc	0	0	0	0	0	0	0	0

* After Rudolfs and Gehm (20).

tionship.

The authors also stated that chlorine-resistant bacteria also may be partly responsible for regrowth. Survivors were identified by pigment and colony type. The majority belonged to the Pseudomonas group, which are not spore formers. Spores are known to be resistant to chlorine; however, conditions in sewage are not favorable to abundant spore formation. Temperature and food concentration promote vegetative forms rather than stimulate spore formation.

A number of other studies concerning regrowth have been carried out over the years. Two early studies (31), (32) were involved with the regrowth phenomenon that occurs in polluted waters when they are diluted with clean water. Butterfield (31), in conjunction with the U. S. Public Health Service, helped establish the effect of dilution of non-chlorinated sewage on the subsequent rise in bacterial numbers in a stream. This was one of several stream surveys conducted. Streeter (32) was involved in a similar survey. Each researcher reported a rise in the number of bacteria following dilution.

Heukelekian (33) studied the regrowth of coliform organisms in streams receiving chlorinated sewage. Fresh sewage was chlorinated to an orthotolidine residual of 0.1 and 0.5 parts per million (ppm). Different types and percentages of dilution water were used. Results were expressed as MPN per ml. Table III shows the results he obtained using sewage diluted and chlorinated to a 30-minute residual of 0.1 ppm. The undiluted sewage remained relatively constant for seven days following the initial increase. In the stream water, the

TABLE III

Aftergrowth of Coliform Organisms in Diluted Chlorinated Sewage[1]

	Sewage Conc. (%)	Type of Dilution Water	Chlorine Residual[2] (p.p.m.)	Coliforms (M.P.N./ml.)[3]						
				0 Days	1 Day	2 Days	3 Days	4 Days	5 Days	7 Days
1.	100	-	0.1	2.5	60	25,000	20,000	25,000	11,500	25,000
2.	5	Stream	0	2.5	2.5	0.6	2.5	2.5	2.5	2.5
3.	10	Stream	0.1	2.5	25	600	60	1,150	25	25
4.	25	Stream	0.1	2.5	3.5	2,000	1,150	950	250	95
5.	5	Sea	0	2.5	2.5	0.2	2.5	2.5	2.5	2.5
6.	10	Sea	0	2.5	2.5	2.5	2.5	2.5	25	2.5
7.	25	Sea	0	2.5	2.5	0.2	2.5	2.5	2.5	2.5
8.	5	Distilled	0	2.5	2.5	2.5	2.5	2.5	2.5	2.5
9.	10	Distilled	0	2.5	2.5	2.5	60	35	3.5	2.5
10.	25	Distilled	0.1	2.5	6.0	600	250	95	200	250

[1]Sewage chlorinated to an ortho-tolidine residual of 0.1 p.p.m. after 30-min. contact.
[2]Residual after dilution; residual absent in all dilutions after 4 hr.
[3]On basis of confirmed counts.

* After Heukelekian (33)

higher the sewage concentration the higher was the maximum number of bacteria obtained. Populations in sea water changed little, regardless of the concentration of sewage. Results in distilled water were similar to those observed in stream water.

When the sewage was chlorinated to an orthotolidine residual of 0.5 ppm the results were somewhat different, as can be seen in Table IV. Coliforms attained a much higher level in the undiluted sewage than when the residual was only 0.1 ppm. In addition, there was no definite increase in numbers in the diluted samples. Again, populations in sea water showed little change. Heukelekian concluded that food may be a controlling factor. This explained the difference in numbers using different dilutions. Another controlling factor was the residual chlorine in the sewage. The higher the residual chlorine and the fewer the coliform organisms remaining in sewage, the greater was the regrowth.

Kittrell and Furfar (34) explained the effects of environmental factors on coliform bacteria in streams. The general effect of temperature was to cause a more rapid decrease in summer than in winter. They concluded from this that the wastewater from a given population contributes approximately three times as many coliform bacteria to a stream in summer as it does in winter. The slower rate of decrease in winter allows the coliforms to persist longer in the stream. Rainfall and surface runoff were factors in the increase of coliforms in the stream. The contribution from runoff is a problem, the extent of which is just being realized today, and much research is now being

TABLE IV

Aftergrowths of Coliform Organisms in Diluted Chlorinated Sewage[1]

	Sewage Conc. (%)	Type of Dilution Water	Chlorine Residual[2] (p.p.m.)	Coliforms (M.P.N./ml.)[3]						
				0 Days	1 Day	2 Days	3 Days	4 Days	5 Days	7 Days
1.	100	-	0.5	0.2	1,400	16,000+	160,000+	160,000+	160,000+	160,000+
2.	1	Stream	0	0.2	0.2	3.5	0.2	0.3	0.2	0.2
3.	5	Stream	0.1	0.2	0.2	0.2	0.2	2.5	0.2	2.5
4.	10	Stream	0.15	0.2	0.2	0.2	0.2	0.2	0.2	2.0
5.	25	Stream	0.3	0.2	1.3	1,600+	350	350	70,000	11
6.	1	Sea	0	0.2	0.2	0.2	0.2	0.2	0.2	0.2
7.	5	Sea	0.1	0.3	0.2	0.2	0.2	0.2	0.2	0.2
8.	10	Sea	0.1	0.2	0.3	0.2	0.2	0.2	0.2	0.2
9.	25	Sea	0.15	0.2	0.2	0.2	0.2	0.2	0.2	2.5
10.	5	Distilled	0	2.5	2.5	3.5	2.5	2.5	2.5	2.5
11.	10	Distilled	0	2.5	2.5	11.5	2.5	55	3.5	2.5
12.	25	Distilled	0	2.5	2.5	350	400	6,000	5.5	165

[1] Sewage chlorinated to ortho-tolidine residual of 0.5 p.p.m. after 30-min. contact.
[2] Residual 3 hr. after dilution; residual absent in all samples 24 hr. after dilution.
[3] On basis of confirmed counts.

*
After Heukelekian (33)

conducted to determine its importance. Other factors mentioned were stream channel characteristics, pH, turbidity and nutrients.

Regrowth is usually associated with non-fecal coliforms. Non-fecal coliforms are able to grow in an environment with minimal nutrients. Fecal coliforms require more favorable conditions. Deaner and Kerri (35) studied the regrowth of fecal coliforms in a stream below a sewage treatment plant. No regrowth of fecal coliforms occurred. Several inhibitory factors may have been the reason. There was a short travel time of bacteria within the study area, a possible lack of available nutrients, and the stream in question was shallow, swift, and low in turbidity.

The regrowth of coliforms in stormwater runoff was studied by Evans, et al. (36). Regrowth of total coliforms occurred despite high chlorine dosages. The regrowth occurred within 24 to 72 hours after chlorination. Fecal coliforms and fecal streptococci showed no significant aftergrowth.

Eliassen (37) also showed a significant increase in coliforms in storm water following chlorination. However, the chlorination markedly decreased the initial population of coliforms compared to that in unchlorinated water.

One of the more recent studies concerning the regrowth of coliforms was that of Shuval, et al. (38). Their research involved the regrowth of coliforms in a chlorinated effluent held in a storage reservoir for approximately three days. Their results indicated that such regrowth was inversely related to the residual chlorine

concentration in the storage reservoir and to the number of coliforms surviving chlorination.

Braswell and Hoadley (39), in a recent study, examined the recovery of Escherichia coli from chlorinated secondary sewage using standard procedures for the enumeration of fecal coliforms in chlorinated effluents. Spread plates were prepared on Trypticase soy agar, MPN's were determined in lactose broth and E.C. broth, and membrane filters were incubated on M-FC medium according to Standard Methods (27).

Chlorine-injured E. coli failed to produce colonies on the membrane filters or to grow and produce gas in lactose broth. Such results prove the inadequacies of presently used methods for the enumeration of total and fecal coliforms in chlorinated effluents. More reliable methods of enumeration must be developed.

All of the research cited points to the need for more studies in the field of wastewater chlorination. Such research is needed in the areas of equipment design, management, and chlorine efficiency. Also, there is a need for a better understanding of the regrowth phenomenon and its significance in terms of public health.

III. METHODS AND MATERIALS

The basic experimental plan called for the inoculation of filter-sterilized, secondary-treated sewage (unchlorinated) with bacteria representative of those found in sewage. The inoculated sewage then was chlorinated in a flash-mixer and pumped through a system designed to approach plug flow conditions. Samples were collected after several contact periods and were evaluated immediately for viable organisms and at intervals for six to seven days afterward. Prior to these studies, several preliminary studies were conducted to insure that the procedures to be used were sound and that they could be reproduced. These preliminary studies are described in subsequent sections.

Preliminary Studies

Selection of Organisms and Culturing Techniques

Three bacteria: Escherichia coli (ATCC 9723), Streptococcus faecalis (ATCC 8043), and Staphylococcus epidermis (VPI&SU strain) were selected for use in these studies. The latter was discarded later for reasons that will be explained. Their appearance and growth characteristics on selective media were evaluated prior to actual studies with chlorine to determine if the individual species could be distinguished from one another. These evaluations were made with bacteria in pure and mixed cultures suspended in buffered water and in sterilized sewage. Selective media used were: desoxycholate agar for E. coli, azide dextrose agar for S. faecalis, and

mannitol salt agar for S. epidermis. All media were manufactured by Baltimore Biological Laboratories.

Viable population density evaluations were made by the spread plate procedure wherein a small aliquot (0.1 ml) of sample is placed on the surface and spread with a sterile, glass rod. A properly and thoroughly mixed sample was essential for accurate results (40).

This method was preferred over the membrane filter technique because inactivated cells deposited on a membrane with a limited nutrient availability are unable to rid themselves of monochloramine which inhibits their growth. Thus, the use of the membrane filter would have meant a serious error in the results of this research (41). Nutrient agar allows reactivation by neutralizing cell bound chlorine more effectively than thiosulfate by providing preformed metabolites essential for recovery (42). Colonies were enumerated by the standard plate count of 30-300 colonies. Counts were made after incubation periods of 24 and 48 hours with the aid of a Quebec Colony Counter.

All bacterial cultures were maintained on nutrient agar slants and transferred a minimum of once a week. Cells grown on nutrient agar are much less affected by chlorine than are cells from minimal agar (42). Inocula for the studies in sewage were cultures of the bacteria in liquid culture media. Aseptic techniques were maintained throughout the research. Cultures were checked periodically for purity.

Enrichment Studies

There was concern that the bacteria exposed to chlorine in the

laboratory chlorination system might be injured to the extent that the selective media would be toxic to them if samples were plated immediately after collection. Therefore, a study was undertaken to determine if the samples should be placed in an enrichment medium (nutrient broth) to allow recovery (but not growth) prior to plating on selective agar medium.

The maximum time cells could be maintained in the enrichment medium before reproduction occurred was determined by plating suspensions of the pure cultures in nutrient broth at intervals of 5, 10, 15 minutes after inoculation. At the end of each of these intervals, serial dilutions were made in other tubes containing the enrichment medium, and aliquots of these dilutions were treated by the spread-plate technique. A similar test was conducted with distilled water as the dilution medium. Once these data were available, the maximum time the treated effluent should be left in nutrient broth to allow for recovery would be known.

Turbidimetric Measurements of Bacterial Population Densities

For comparison of chlorine effectiveness in the several chlorination experiments to be valid, it was necessary that cells inoculated into sewage be in the same growth phase at the beginning of each experiment. A rapid technique for estimating cell densities was required, one which could be monitored easily during the incubation of broth cultures that served as inocula for the sewage. A turbidimetric procedure was chosen.

The turbidimetric procedure required that turbidity measurements of the broth cultures be made at intervals during the growth period (24-48 hr.) and correlated with actual cell counts at the same time by plating methods previously described. Turbidity was measured with a Bausch and Lomb Spectronic 20 spectrophotometer set at 430 millimicrons. Graphs were made relating cell counts to optical density, and in this manner, the log phase of growth for each organism could be identified. Data derived from analyses of duplicate cultures were used to construct the curves, and periodically the accuracy of each curve was confirmed.

Routine Bacteriological Procedures

Approximately 24 hr. prior to beginning a chlorination experiment, separate 200 ml aliquots of nutrient broth were inoculated from agar slants with *E. coli* and *S. faecalis* and were incubated at 35.5° C. When the per cent transmittance readings were 80 and 72, the *E. coli* and *S. faecalis* cultures, respectively, were in the log growth phase. The volume of broth that contained enough cells to produce densities of 10^5 *E. coli* and 10^4 *S. faecalis* per milliliter of filtered sewage to be used in the chlorination experiments was withdrawn and centrifuged at 2400 revolutions per minute (rpm) for 20 minutes. (These volumes were 100 ml of the *E. coli* culture and 0.1 ml of the *S. faecalis* culture.) The packed cells were washed with a phosphate buffer solution (*Standard Methods*) and centrifuged again for 20 min. The washed cells then were resuspended and

thoroughly mixed in 8-10 liters of the filter-sterilized sewage to be treated with chlorine.

Samples of inoculated sewage, collected before and during chlorination, were diluted serially. Three dilutions were plated in triplicate on desoxycholate agar and on azide dextrose agar. All plates were fresh and contained approximately 20 ml of medium. Only those plates showing 30 to 300 colonies were counted.

On some occasions the filtered sewage was not completely sterilized and colonies other than E. coli colonies grew on the desoxycholate medium. In these instances, E. coli colonies were verified by microscopic examination and by gas production in lactose broth medium. S. faecalis colonies growing on the azide dextrose agar were verified periodically by microscopic examination.

Laboratory Treatment System

The coinvestigator (2) of this research was responsible for the system design. A continuous flow system approaching plug flow was used. The secondary sewage effluent and a hypochlorite solution were pumped through separate delivery tubes into a small (3 cm wide x 3 cm long x 2 cm deep) rapid-mixing chamber made from Plexiglas. The chlorinated sewage then flowed through 80 ft. of rubber tubing (inside diameter 0.79 cm) at a flow rate of 40 ml/min. Ports for removing samples after contact times of 10, 20, and 30 minutes were installed along the length of the tubing. A sampling port also was inserted in the system before the point of chlorination. Preliminary

studies of the system were conducted to determine how nearly the flow approximated plug flow.

The sewage was collected from the secondary clarifiers of the Blacksburg-VPI sewage treatment plant (a trickling filter plant located on Strouble's Creek) and filtered through sterile membrane filters (Millipore Corporation) varying in porosity from 5 microns (μ) to 0.45 μ. The filtered sewage was placed in a sterile, 12-liter (l) container. After filtration, an aliquot of the sterilized sewage was plated in triplicate on desoxycholate and azide dextrose plates to check for growth, and colonies, if present, were counted.

The effluent was inoculated to obtain 10^5 cells per ml of E. coli and 10^4 cells per ml of S. faecalis and was thoroughly mixed. Before and after each experiment, all tubing in the system was chlorinated with a strong hypochlorite solution and dechlorinated with sodium thiosulfate.

A hypochlorite solution (HTH) was used as the disinfectant. Dosages were varied prior to the inoculation of the sewage with test organisms to provide the desired residual after 30-min. contact. The dosages varied between 1.5 and 3.5 mg/l in all the experiments, but during any experiment, it was held relatively constant (within 0.05 mg/l). Residuals varied between 0.18 and 2.35 mg/l.

Regrowth Studies

Samples were collected at four sites within the system: before

chlorination and after 10-, 20-, and 30-min. contact with the chlorine. Samples were collected in sterilized dilution bottles containing 0.1 ml of a 10% solution of sterile sodium thiosulfate to immediately inactivate the chlorine residual. Because there was a necessary lapse of time to allow for plating, not all the samples were collected at the same time. The sampling sequence was as follows: before chlorination (Site 1, Sample 1-a), after 30-min. contact (Site 4), after 20-min. contact (Site 3), the Site 1 again (Sample 1-b) followed by Site 2 (10-min. contact). Two samples were taken from Site 1 to correct for any natural die-away of bacteria in the system because data were to be expressed as per cent kill of bacteria by specified chlorine residuals and contact times. Samples also were analyzed for chemical oxygen demand (COD), total Kjeldahl nitrogen (TKN), and ammonia nitrogen (NH_3-N).

Regrowth was followed by plating aliquots from each of the samples at daily intervals for a period up to but never exceeding one week. The samples were maintained at 20° C. during this period.

IV. RESULTS

Preliminary Studies

Selection of Organisms and Culturing Techniques

The first step in the preliminary work involved checking cultures of *E. coli*, *S. faecalis* and *S. epidermis* (pure and mixed, with and without sterilized sewage) for appearance and growth characteristics on the three types of media. Results are shown in Appendix Table A-1.

No problem was encountered in distinguishing *E. coli*. Growth occurred only on the desoxycholate agar. Desoxycholate agar inhibits organisms other than those of the enteric group. Coliform organisms are red in contrast to the colorless colonies produced by enteric organisms not capable of attacking lactose (43). *E. coli* colonies were large, red, and nucleated.

Confusion arose over distinguishing *S. faecalis* and *S. epidermis* colony types. *S. epidermis* grew on both the azide dextrose agar and the mannitol salt agar. In addition, colony type was similar to that of *S. faecalis*.

The research plan called for the use of plate counts as a procedure for evaluating the chlorination efficiency. Because colonies of *S. faecalis* and *S. epidermis* were indistinguishable, the data would have been confusing, so it was decided to eliminate *S. epidermis* from this study.

Enrichment Studies

The research plan called for determining the time in an enrichment broth that would allow recovery but not growth of bacterial cells damaged by chlorine in the treatment system. Cell counts after 5, 10, and 15 minutes in a nutrient broth medium series and in distilled water series were compared. The results are shown in Appendix Table A-2.

The 15-minute time period showed the greatest discrepancy in cell counts of E. coli between the nutrient broth dilution series and the distilled water dilution series. This is as would be expected as E. coli has an average generation time of 20 minutes under optimum conditions, and the time required for dilution and plating, added to the 15-min. contact period in the enrichment broth, is near the average regeneration time. Counts of S. faecalis remained relatively unchanged at all times used. An enrichment time of only 5-10 minutes was selected for this study to insure that growth in the enrichment broth did not occur.

Turbidimetric Measurements of Bacterial Population Densities

The growth curves for E. coli and S. faecalis developed by a turbidimetric estimation of microbial growth are shown in Figure 2. Viable organisms present, as determined by plate count, are shown in Figure 3. The figures were prepared from data presented in Appendix Tables A-3 through A-6. Appendix Tables A-3 and A-5 show data used in preparing Figure 2. Appendix Table A-4 and A-6 are the data used in preparing Figure 3.

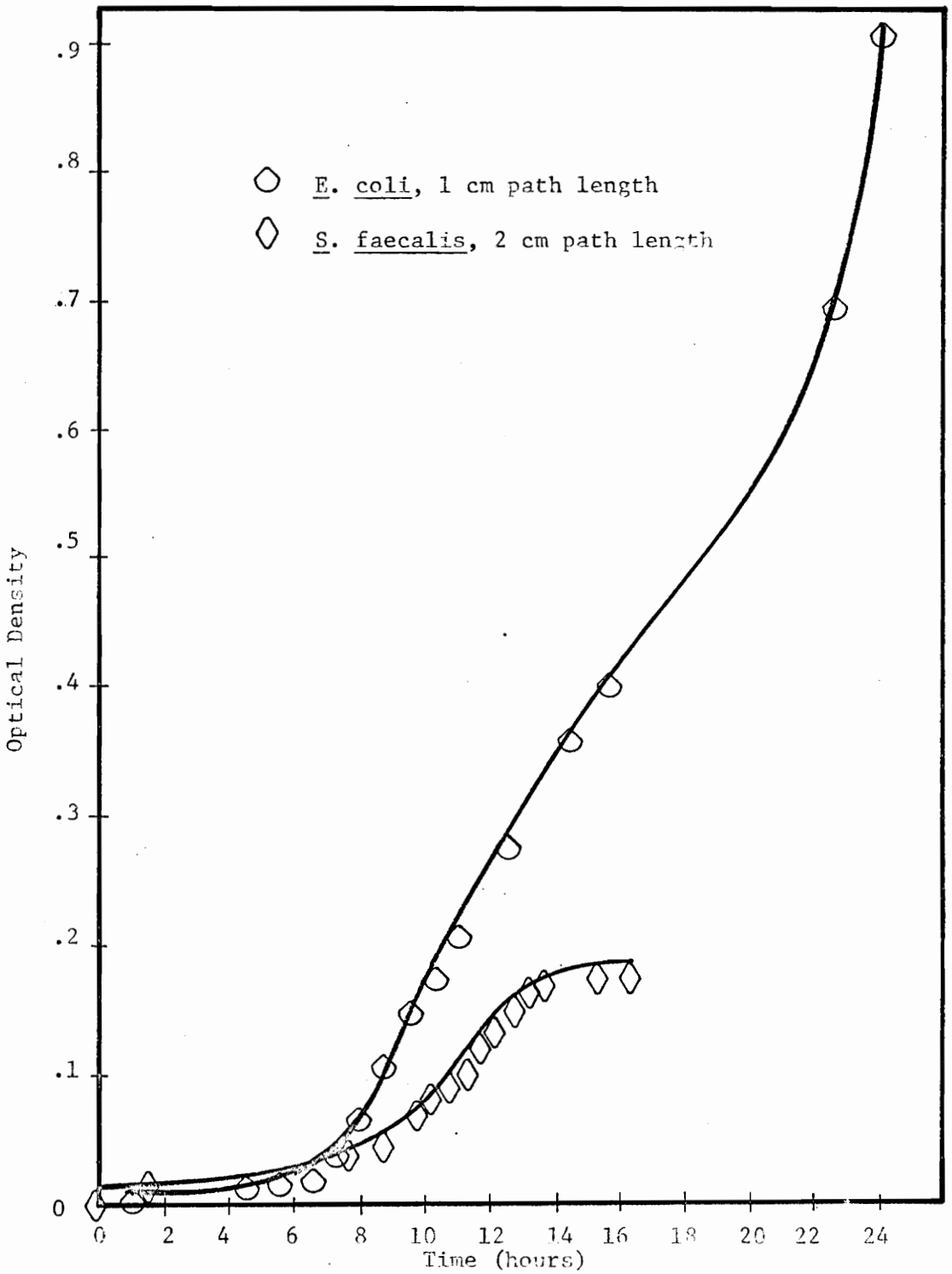

Figure 2. Growth Curves for E. coli and S. faecalis as developed by Turbidimetric Measurements of Nutrient Broth Cultures with a Bausch and Lomb Spectronic-20 Spectrophotometer.

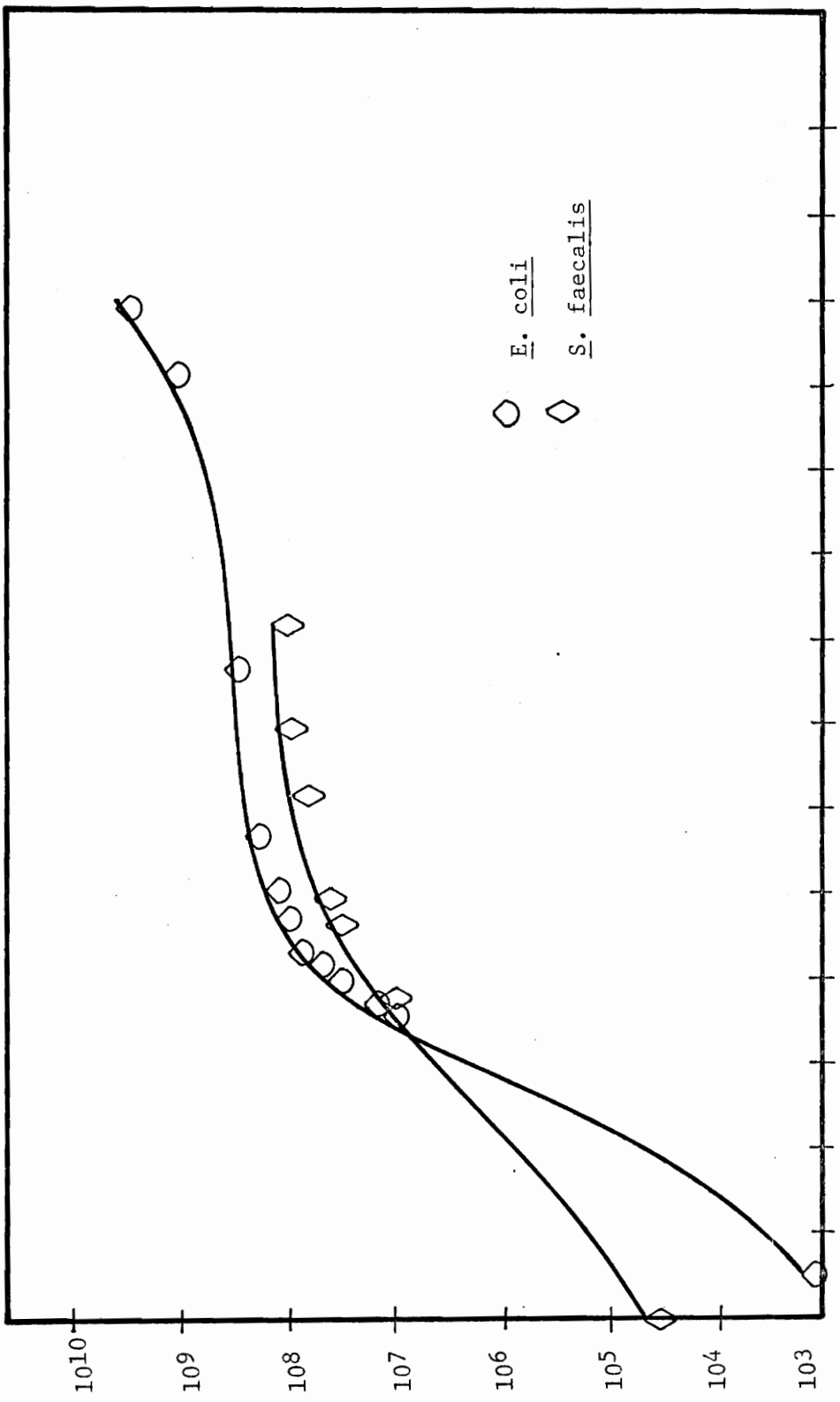

Figure 3. Viable Bacteria as Determined by Plate Counts of Nutrient Broth Cultures of E. coli and S. faecalis. Counts are averages of Duplicate Plates incubated at 35.5°C.

The turbidity-cell count relationships just presented were used to determine the time for harvesting broth cultures of E. coli and S. faecalis to be used as inoculum in each experiment. Table V shows the conditions for each inoculum.

Laboratory Treatment System

A dye study was performed on the continuous flow system to check for short circuiting. 1 ml of methylene blue was injected into the head of the rubber tubing. Samples were drawn from each site at different time intervals and read at 660 mu using the Spectronic 20 spectrophotometer. Figure 4 shows that minimal short circuiting occurred and the system did indeed approach plug flow.

Regrowth Studies

Chemical Parameters

Appendix Tables A-7 through A-11 show select chemical and physical characteristics of the sewage used in the disinfection studies. Only contact time and chlorine residual were controlled by the investigator.

The chlorine dosages for the five experiments varied from a low of 1.5 mg/1 to a high of 3.5 mg/1. The residual chlorine was in the form of monochloramine only and varied according to the chlorine demand of the sewage and contact time.

While pH and temperature showed little variation throughout the five experiments, the COD, TKN, and NH_3-N concentrations of the individual sewage samples varied greatly (COD: 16 to 96 mg/1, TKN: 6.7

TABLE V

Conditions of Inoculum in Each Experiment

Exp.	O.D. at harvest		Number cells/ml broth		Volume (ml) of Broth Culture for Harvesting Cells	
	E. coli	*S. faecalis*	*E. coli*	*S. faecalis*	*E. coli*	*S. faecalis*
1	.09	.145	5.9×10^7	7.5×10^7	100	3
2	.08	.145	4.1×10^7	7.5×10^7	100	3
3	.078	.143	4.1×10^7	7.5×10^7	100	3
4	.095	.115	6.0×10^7	6.5×10^7	100	5
5	.145	.140	1.24×10^8	7.3×10^7	60	3

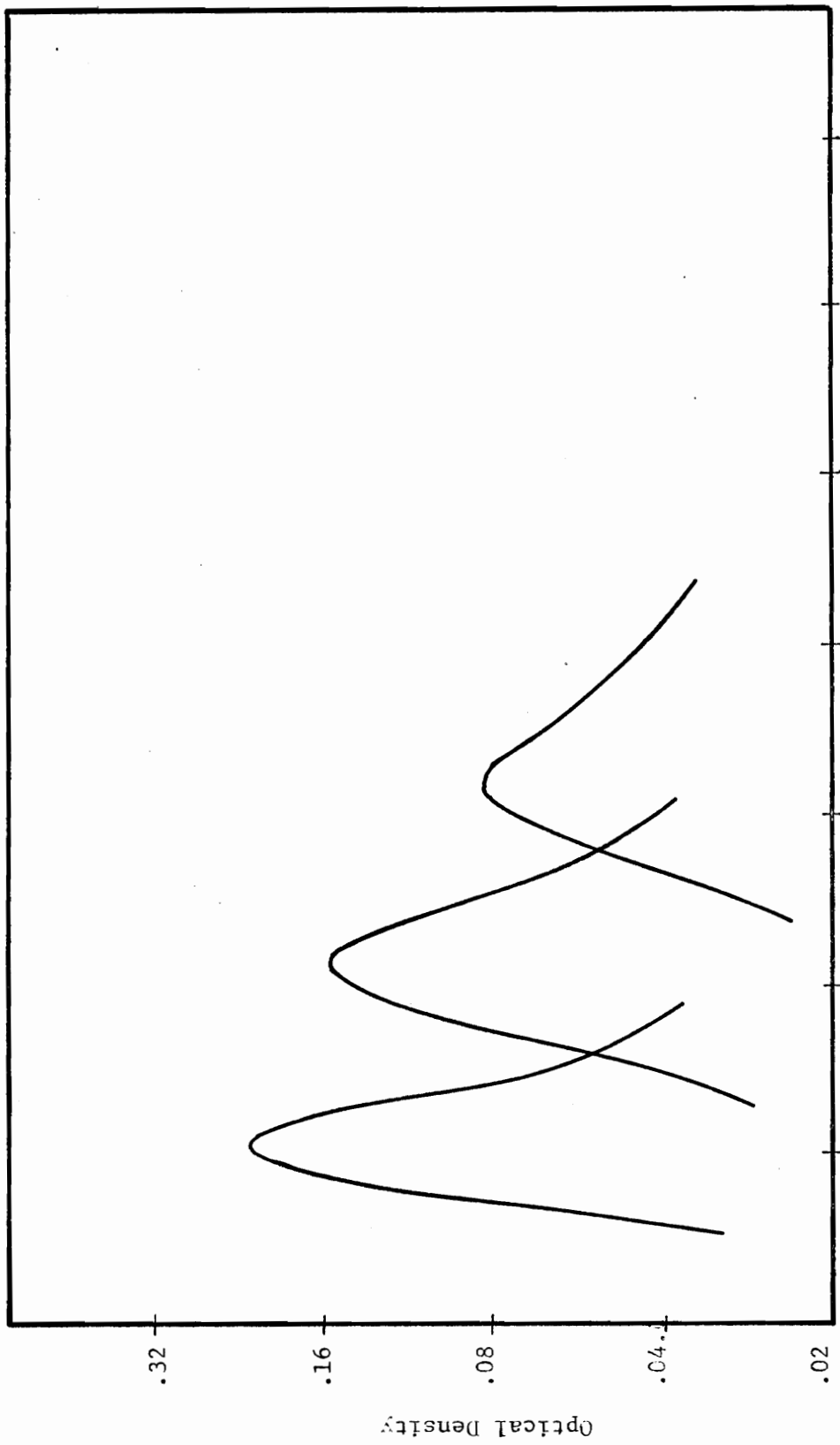

Figure 4. Results of Continuous Flow Study in the Pye Reactor. Peak Optical Density Values Occur at Actual Detention Times at Three Sampling Ports.

to 15.7 mg/1, NH_3-N: 4.8 to 7.3 mg/1).

Chlorine Lethality

The apparent bacteriocidal effects of chlorine at contact times of 10, 20, and 30-min. is shown in Table VI. Generally, an increase in chlorine residual and contact time meant an increase in the apparent bacterial kill. The best correlation occurred between contact time and apparent bacterial kill. The 30-min. contact time showed the least variation in the apparent bacterial kill as the chlorine residual changed.

The chlorine had a greater bacteriocidal effect upon E. coli cells than upon the cells of S. faecalis. This held true for all contact times and all chlorine residuals.

Bacteriological Results

Enrichment study. For two of the disinfection experiments, the plating following chlorination was carried out using a nutrient broth dilution series and a phosphate buffer water dilution series for 0, 10, and 30-min. contact times. Results indicate that the nutrient broth dilution series was of no benefit in the recovery of the chlorinated bacterial cells. Bacterial densities of S. faecalis were less when serial dilutions were prepared in nutrient broth. E. coli densities showed an inconsistent variation.

Regrowth. The bacteriological data collected in the regrowth studies were extensive and are summarized in Appendix Tables A-12

TABLE VI

Apparent Bacteriocidal Effects (Per cent) of Chlorine at Contact Times of 10, 20, and 30 minutes

10 Minutes			20 Minutes			30 Minutes		
Cl_2 Residual (mg/l)	E. coli	S. faecalis	Cl_2 Residual (mg/l)	E. coli	S. faecalis	Cl_2 Residual (mg/l)	E. coli	S. faecalis
2.35	86	31	2.20	94.6	63.7	1.84	99.96	98.71
1.25	26.7	*	1.47	74.4	78.2	1.12	99.73	97.4
.98	81.7	40.7	.96	97.3	91.4	.92	99.98	99.9
.76	31.9	22.8	.72	60	31.9	.70	94.3	87.8
.34	41.9	28.5	.30	85	34	.18	86	33.4

* insignificant

through A-17. Figures 5 through 14 show the variation in bacterial densities with time according to the chlorine contact time and the chlorine residual. The figures are in order of decreasing chlorine residual. Only one control (sample 1-b) is represented on the figures. Two controls were run initially to account for any change in the bacterial densities of the sewage as the experiment progressed. Sample 1-b was chosen as the control in the regrowth studies since the bacterial densities in sample 1-a always showed a faster die-off, due, perhaps, to the fact that this sample was the initial sample taken following inoculation of the filtered sewage. The greatest shock to the bacterial cells of a change in environment (from nutrient baths to filtered sewage) would be felt in this sample. For this reason, it was felt that sample 1-b was more representative of a true control.

Regrowth results should be examined with some caution for disinfection experiments 1 and 2. Improper sterilization of the sewage creates an overgrowth of other bacteria on the plates. This in turn affected the pattern of regrowth. Plating of sterilized sewage showed 1.5×10^3 cells/ml on the desoxycholate agar and 4.0×10^1 cells/ml on the mannitol salt agar for experiment 1. The sterilized sewage used in experiment 2 showed >300 cells/ml on the desoxycholate agar and 4.7×10^1 cells/ml on the mannitol salt agar.

E. coli regrew in all of the disinfection experiments regardless of the chlorine residual and contact time used. The bacterial

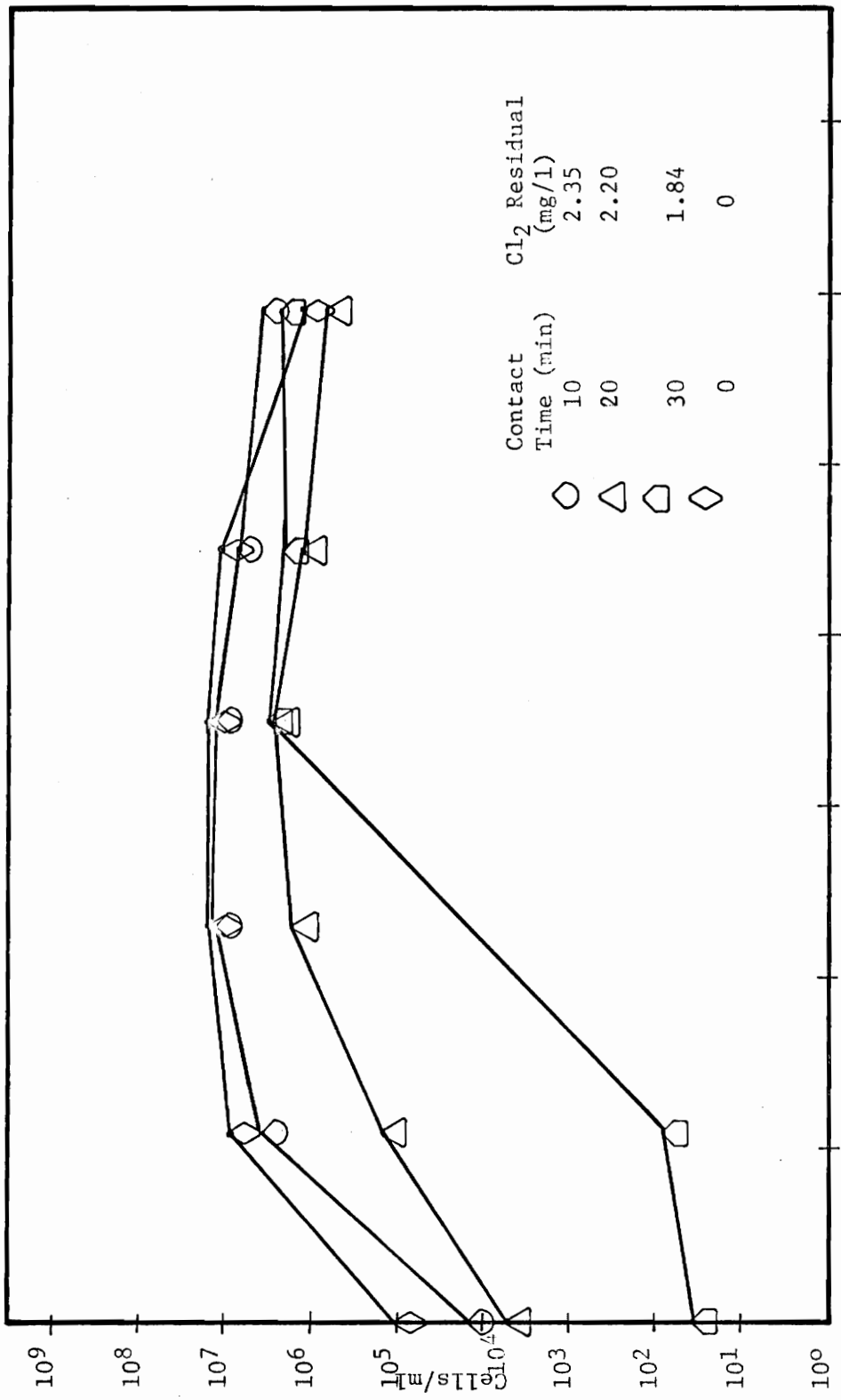

Figure 5. Regrowth of E. coli Exposed to Chlorine for Varying Chlorine Contact Times. Incubation Temperature 20°C.

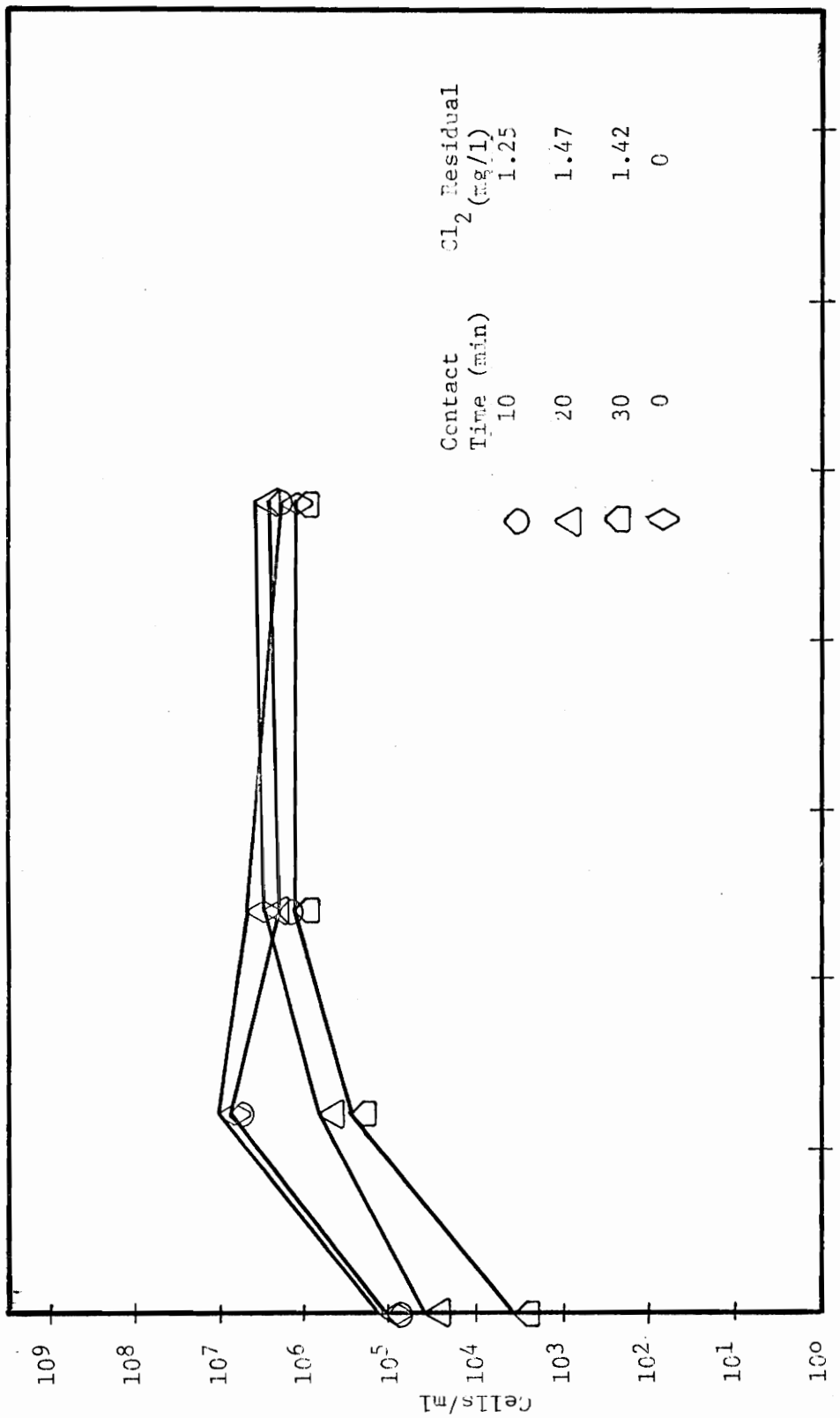

Figure 6. Regrowth of E. coli Exposed to Chlorine for Varying Chlorine Contact Times. Incubation Temperature 20°C.

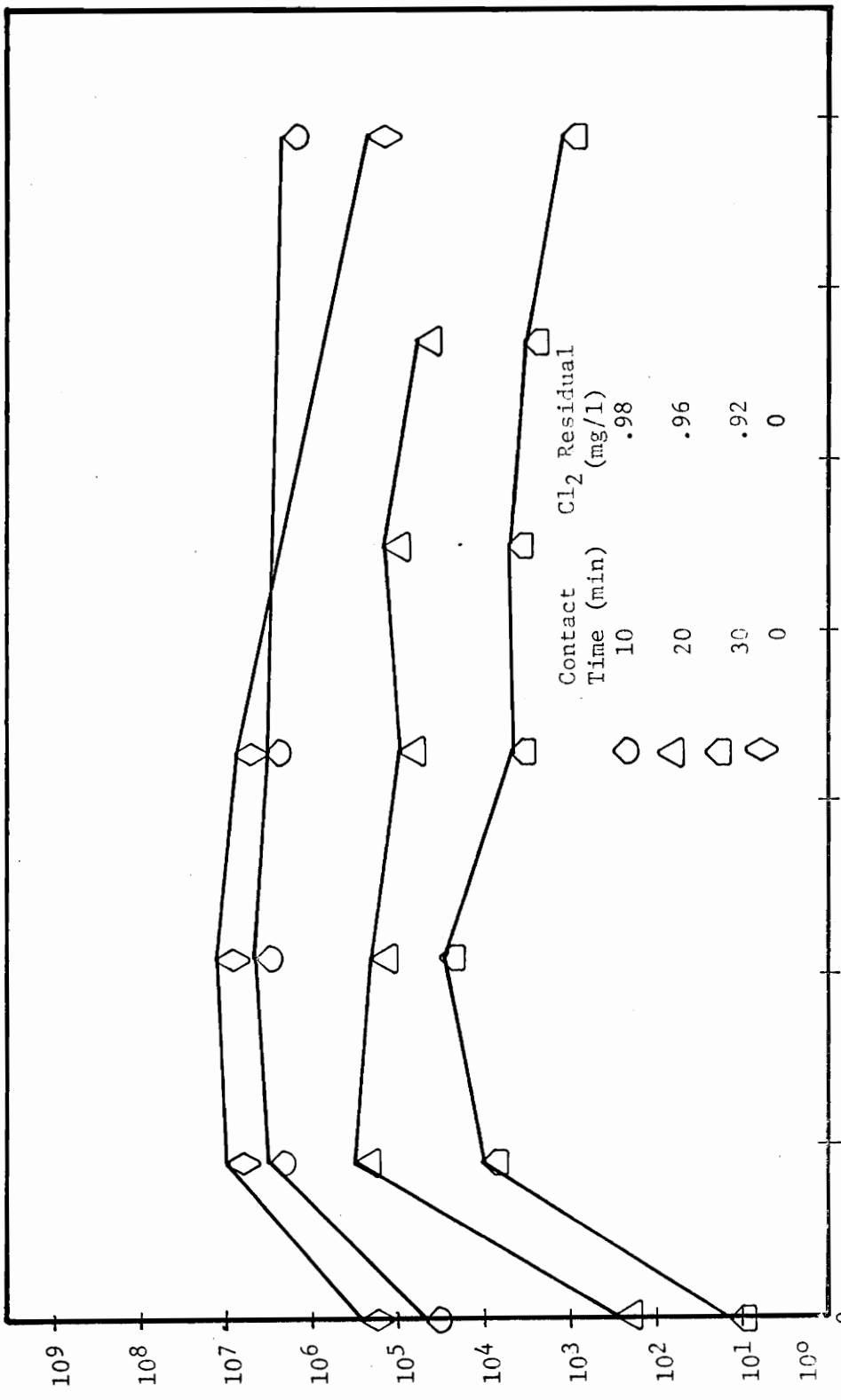

Figure 7. Regrowth of E. coli Exposed to Chlorine for Varying Chlorine Contact Times. Incubation Temperature 20°C.

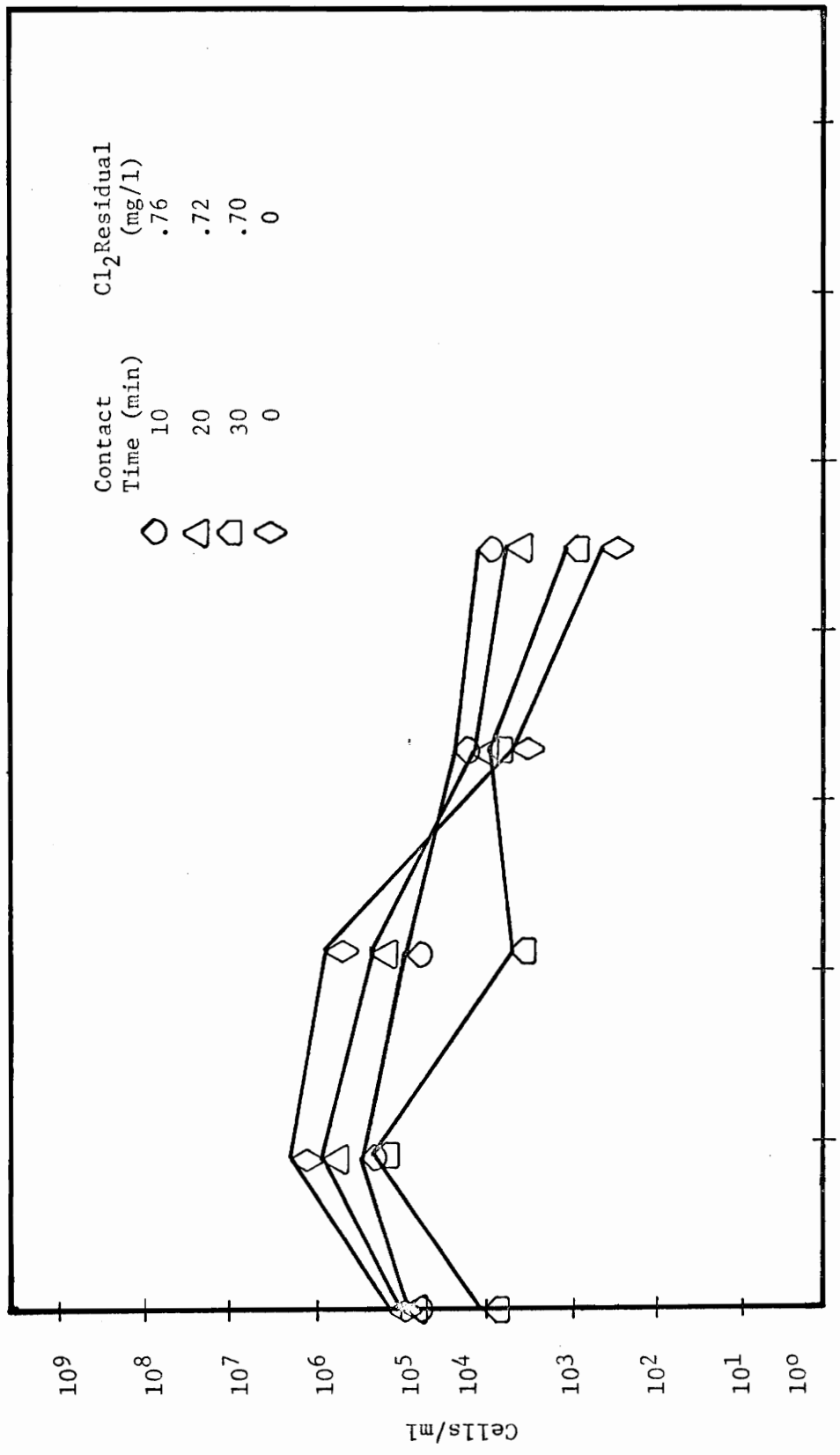

Figure 8. Regrowth of E. coli Exposed to Chlorine for Varying Chlorine Contact Times. Incubation Temperature 20°C.

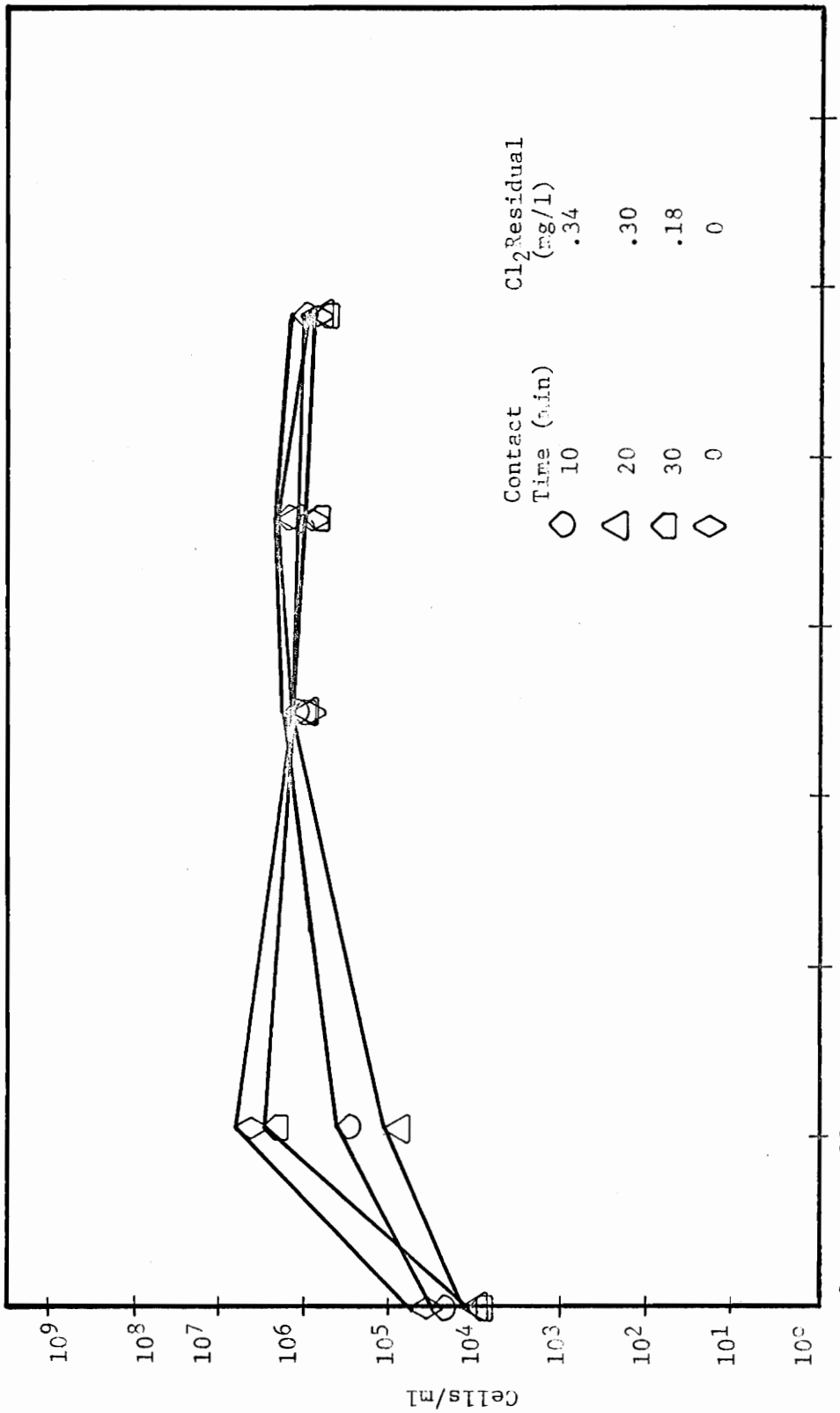

Figure 9. Regrowth of E. coli Exposed to Chlorine for Varying Chlorine Contact Times. Incubation Temperature 20°C.

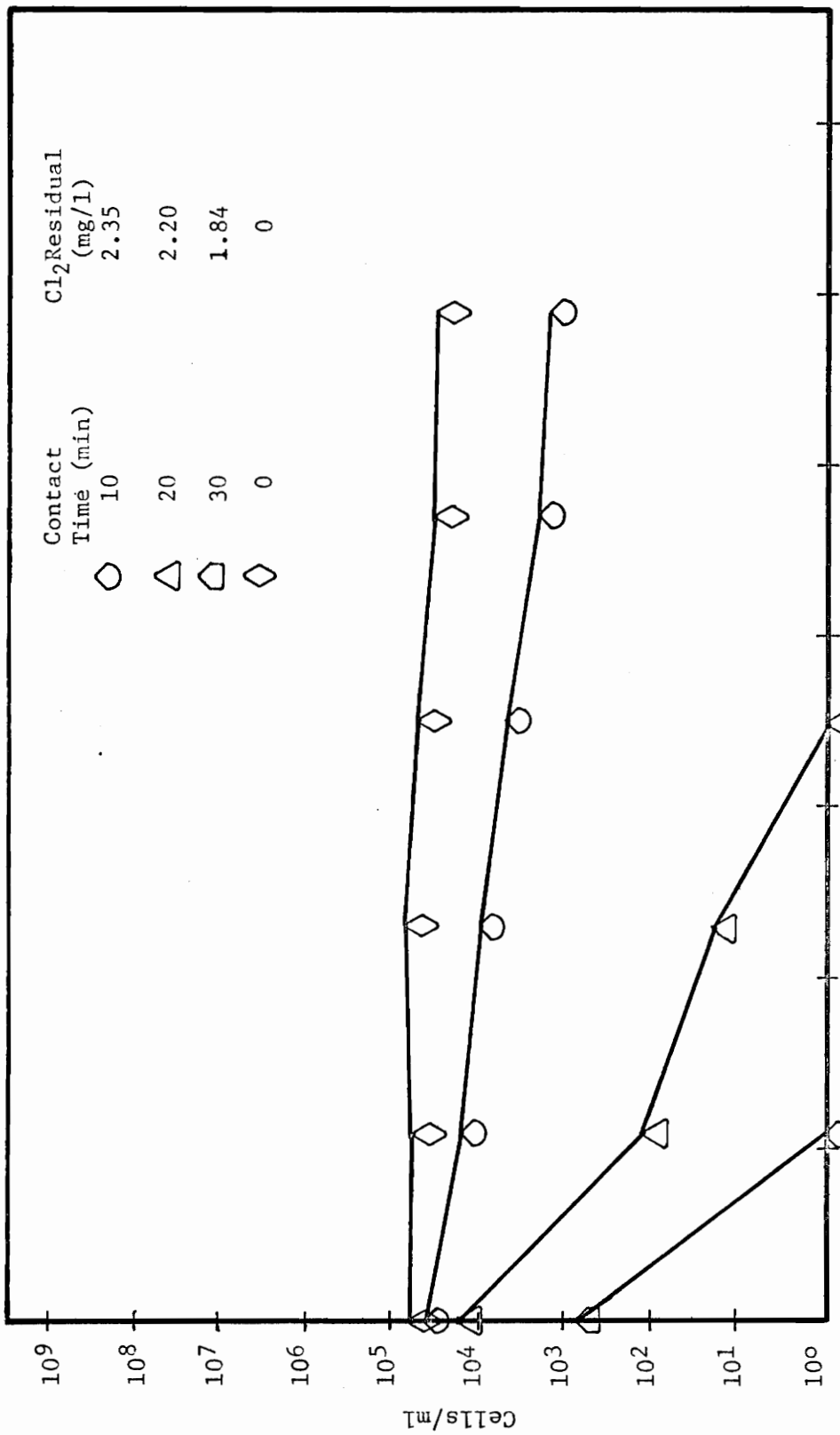

Figure 10. Regrowth of S. faecalis Exposed to Chlorine for Varying Chlorine Contact Times. Incubation Temperature 20°C.

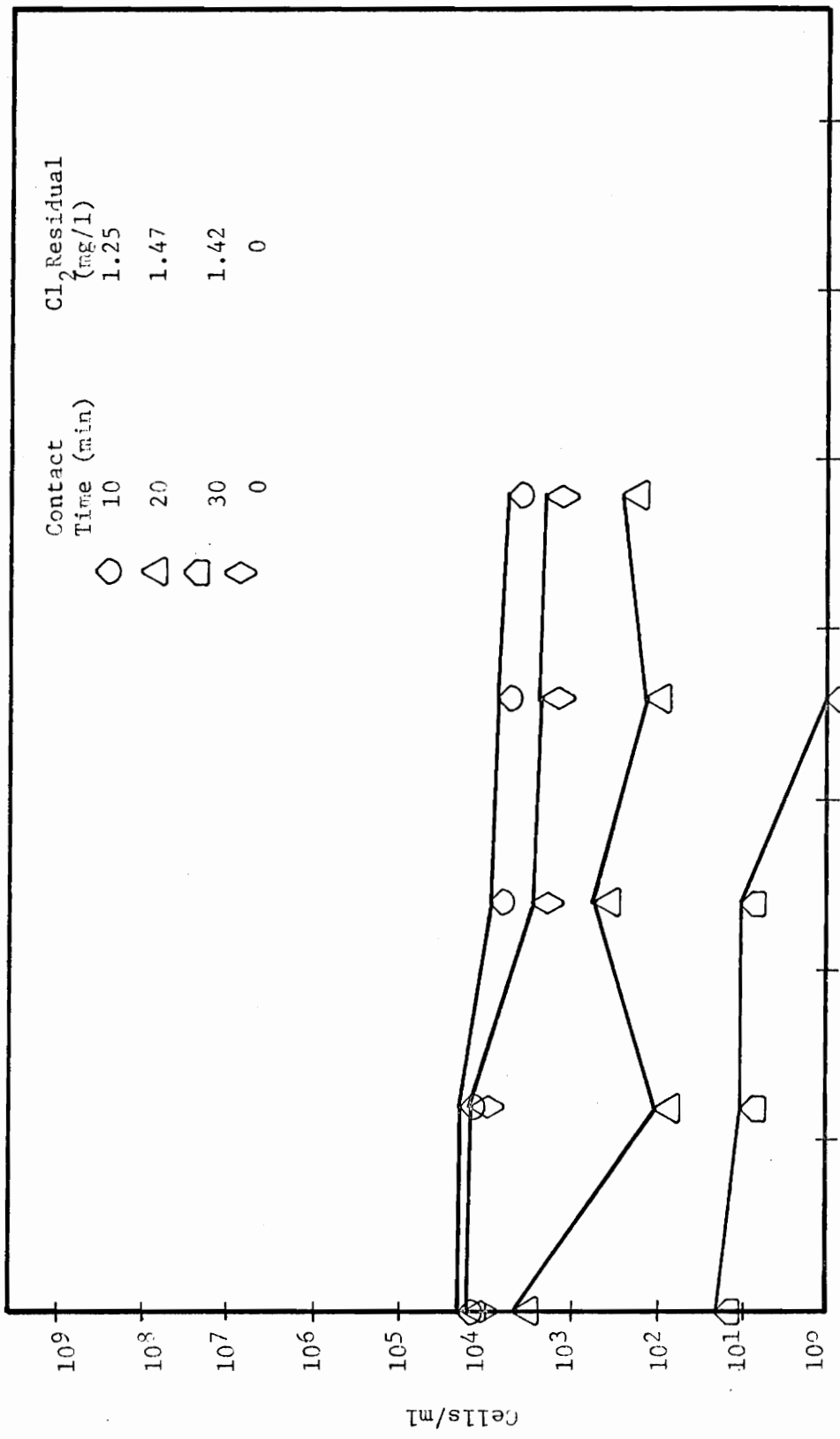

Figure 11. Regrowth of S. faecalis Exposed to Chlorine for Varying Chlorine Contact Times. Incubation Temperature 20°C.

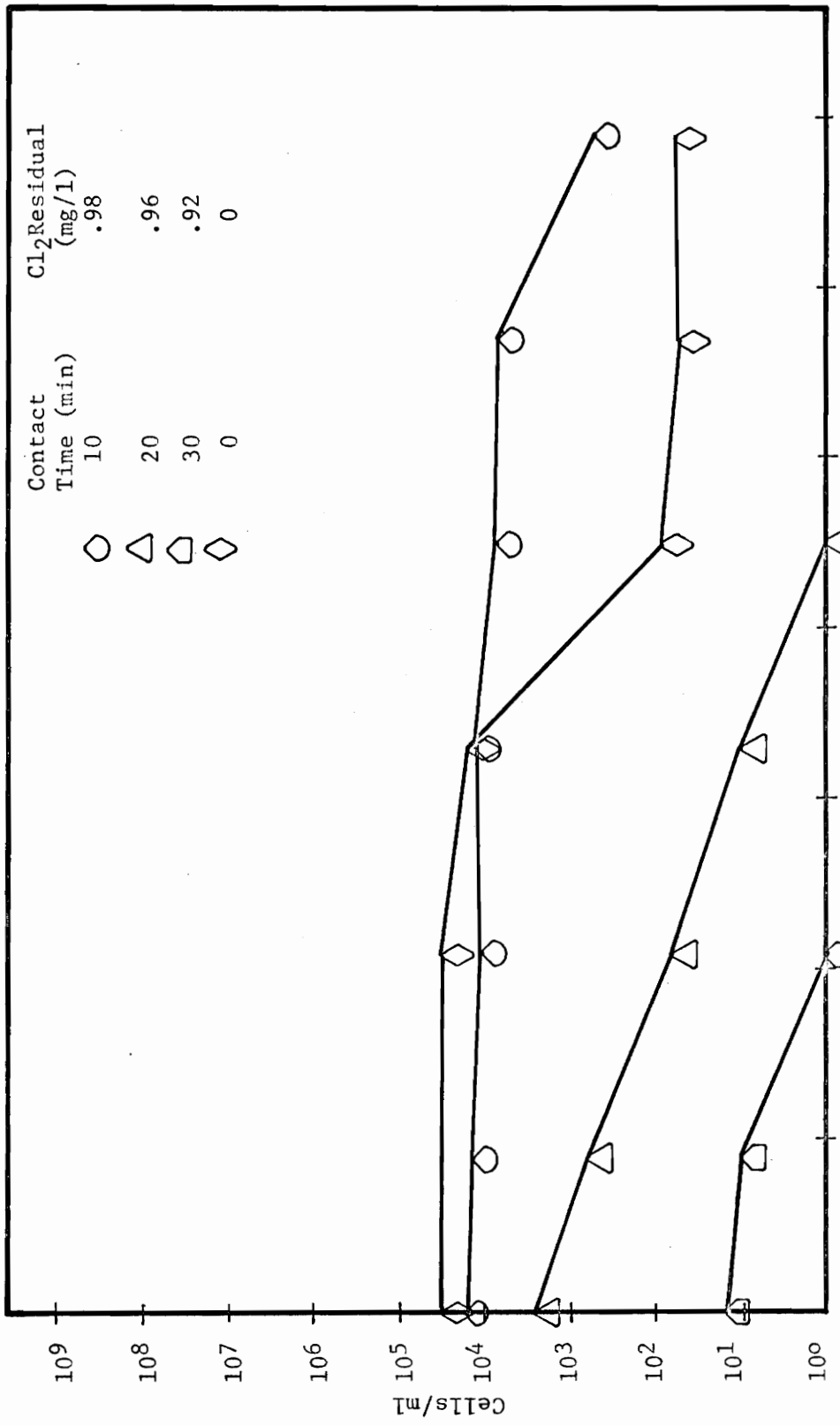

Figure 12. Regrowth of S. faecalis Exposed to Chlorine for Varying Chlorine Contact Times. Incubation Temperature 20°C.

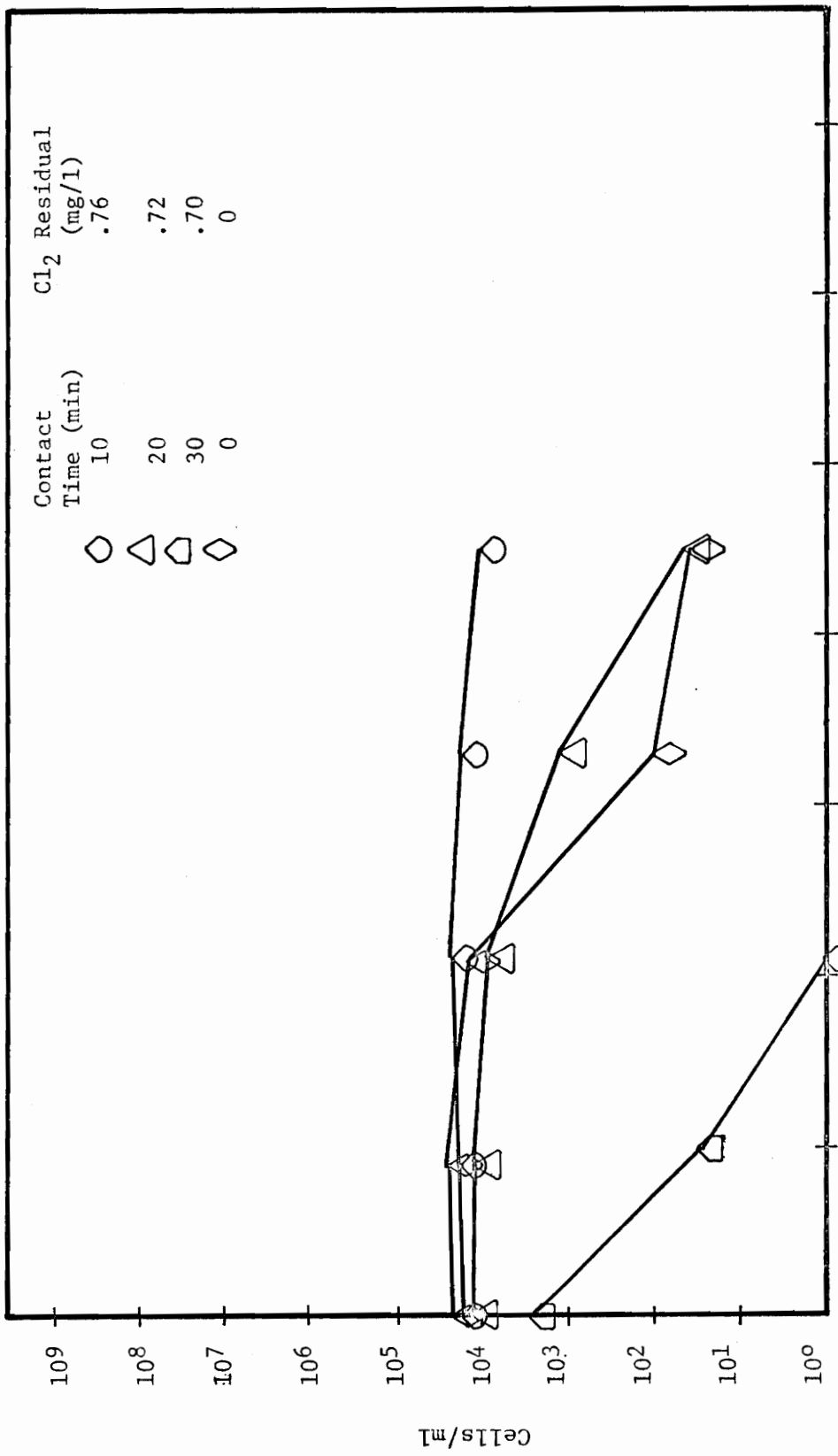

Figure 13. Regrowth of S. faecalis Exposed to Chlorine for Varying Chlorine Contact Times. Incubation Temperature 20°C.

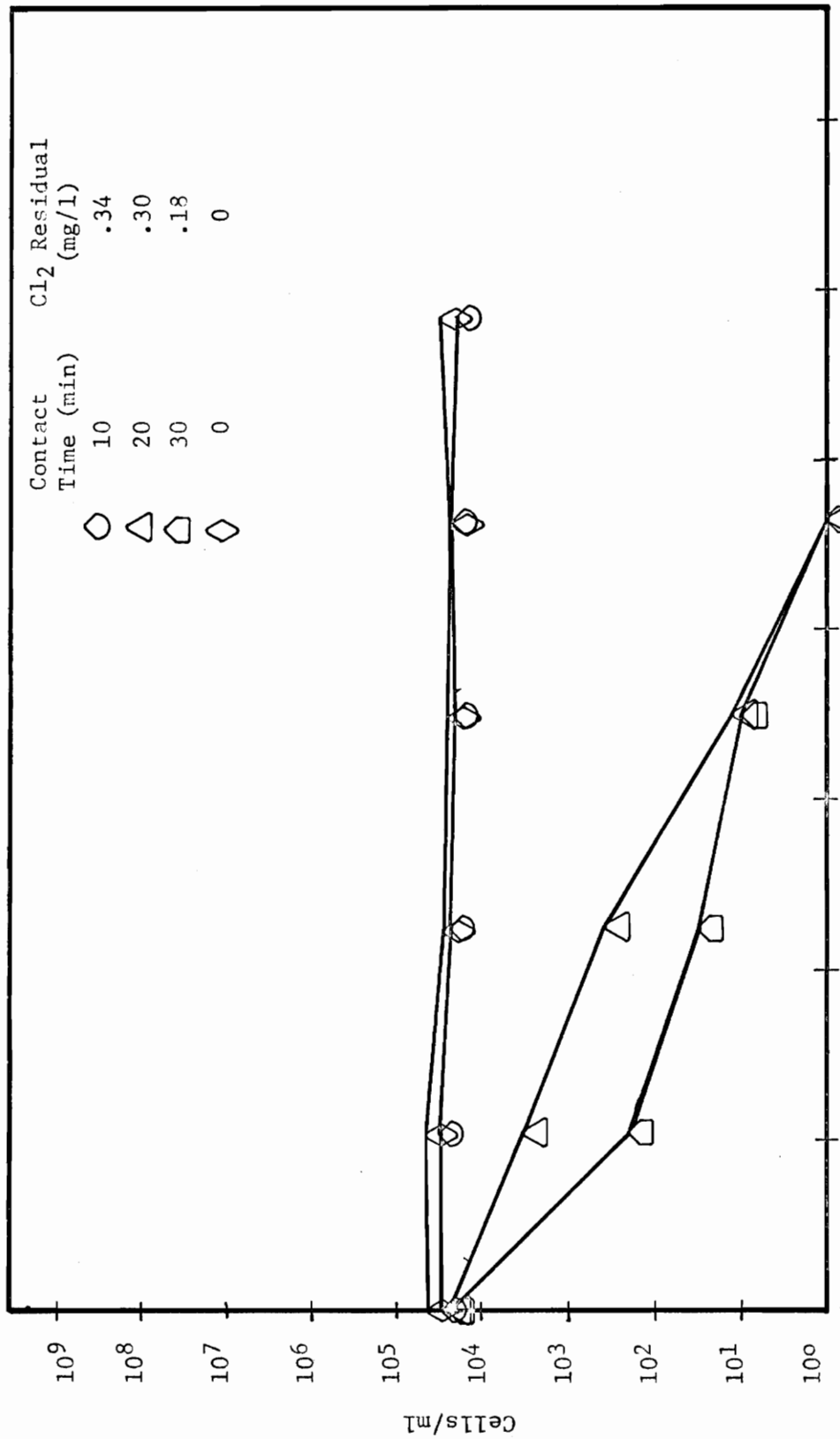

Figure 14. Regrowth of S. faecalis Exposed to Chlorine for Varying Chlorine Contact Times. Incubation Temperature 20°C.

densities reached their maximum within 20-40 hours after chlorination. Never did the population densities at the chlorination sites reach the maximum density obtained by the control populations. Table VII shows the per cent of the maximum control populations obtained by the chlorinated populations. No correlation was found between per cent of control attained by the chlorinated populations and chlorine residual or between per cent of control attained and contact time. A lack of correlation between per cent regrowth as compared to the control and COD also existed. Table VIII shows this clearly.

While E. coli regrew regardless of the chlorine residual and contact time, a difference in the regrowth pattern was noted. The reasons for such differences will be discussed in the following section.

S. faecalis, unlike E. coli, did not regrow in any of the disinfection experiments. This held true for the control populations and the chlorinated populations. There was a difference in the pattern of die-off for the five experiments, the higher the chlorine residual, and the longer the contact period, the faster the die-off rate.

TABLE VII

Per cent of Maximum Control Population (*E. coli*) Attained by the Maximum Chlorinated Populations (*E. coli*), Varying with Contact Time and Chlorine Residual

Experiment	30 min.	Cl_2 Residual (mg/l)	20 min.	Cl_2 Residual (mg/l)	10 min.	Cl_2 Residual (mg/l)
4	18.6	(1.84)	17.33	(2.20)	73.3	(2.35)
5	16.0	(1.42)	6.30	(1.47)	77.0	(1.25)
1	0.18	(0.92)	2.72	(0.96)	37.20	(0.98)
2	10.0	(0.70)	40.53	(0.72)	13.16	(0.76)
3	46.2	(0.18)	31.34	(0.30)	31.34	(0.34)

TABLE VIII

Per cent Regrowth (as compared to the Control Populations) of *E. coli* for Each Contact Time as COD Increases

COD (mg/l)	10 min.	20 min.	30 min.
16	77.0	63.0	16
27	31.34	31.34	46.2
50	73.3	17.33	18.6
60	13.16	40.53	10.0
96	37.2	2.72	0.18

V. DISCUSSION

Several distinct trends can be noted from the data collected during this research. E. coli and S. faecalis showed definite patterns and characteristics relating to the variables maintained throughout the research.

The regrowth of E. coli occurred in all of the disinfection experiments. The pattern of regrowth as well as the apparent per cent kill did show variation.

Generally, an increase in contact time and chlorine residual meant an increase in the apparent per cent kill. No correlation was found between maximum increase in the E. coli populations and contact time or between the maximum increase and chlorine residual. This held true for any correlation with COD. Such findings suggest that a number of variables, including competition, available substrate, etc., all play a role in determining the extent of regrowth. One controlling factor cannot be identified.

Figure 7 and 8 should be examined separately from the others due to the presence of bacteria in the filter-sterilized sewage. Such occurrence means an increase in competition for available substrate and growth factors. The data shown in Figure 8 was obtained from the experiment which had the highest population of contaminants in the sterilized sewage and this experiment showed the greatest die-away rate of E. coli for all five experiments. The importance of competition in determining the regrowth pattern cannot be overlooked.

Filter-sterilized sewage used in Experiments 3, 4, and 5 was

free of organisms until inoculation. Looking at these three experiments in order of decreasing chlorine residual, a definite pattern is noted. As the chlorine residual decreased, so did the rate of die-off until at the lower residual a stabilization of all populations occurred at the same level following the peak. The populations with a 30-minute contact time were most affected by the increase in chlorine residual. Such results show the importance of contact time and chlorine residual, together, in controlling bacterial populations.

The regrowth of E. coli is most probably due to the multiplication of the survivors following chlorination. Regrowth continued until all available substrate had been utilized. A continual die-off then followed.

Unlike E. coli, S. faecalis showed no tendency to regrow in any of the disinfection experiments. A variation in the rate of die-off did occur which can be correlated to chlorine residual and contact time.

The die-off of S. faecalis can be traced to its inability to live outside of its natural environment. Earlier investigations have shown the inability of S. faecalis to regrow. The absence of growth in the unchlorinated sites substantiates this.

In several of the experiments, the chlorinated sites (10 and/or 20 minute contact times) maintained their population level longer than unchlorinated sites. Enough of the bacterial population had been killed off by the chlorine to allow for more available sub-

strate for the survivors. The larger population in the unchlorinated sites meant faster utilization of available substrate.

As this research was a laboratory study, it is necessary to apply the results with some variation in interpretation to a lotic or lenthic environment. Physical conditions, such as stream depth, flow and bed characteristics will all have an influence over regrowth. The availability of food (which during this research was not maintained), competition, predators and chemical characteristics will also influence regrowth. Of utmost importance is to keep in mind that these factors act in combination to influence regrowth and it may be difficult to correlate regrowth with a single parameter.

VI. SUMMARY AND CONCLUSIONS

The purpose of this research was to examine the regrowth of *E. coli* and *S. faecalis* in chlorinated, secondary-treated sewage effluent using a continuous flow system. Several significant conclusions can be drawn:

(1) *E. coli* regrew in the chlorinated sewage regardless of the chlorine residual and contact time, even when an apparent 99% bacterial kill had been achieved. However, if kill is the only criterion to be considered, the Virginia state standard of 2 mg/l total chlorine residual after 30 minutes is a reasonable standard.

(2) No single parameter could be identified as the most important in controlling the extent of regrowth. The pattern of regrowth probably was influenced by a variety of factors such as competition, available substrate, chlorine residual and contact time acting in combination.

(3) *S. faecalis* was more difficult to kill with chlorine than *E. coli*. A residual of 1.84 mg/l after 30 minutes produced only 98.71% kill. However, *S. faecalis* did not regrow in the chlorinated sewage, although its pattern of die-off was influenced by the chlorine residual and contact time.

In summary, regrowth patterns of *E. coli* and *S. faecalis* were distinctly different. Such findings must be taken into consider-

ation when establishing standards and in considering their use as indicators. Of utmost importance is the need for a revaluation of the efficiency of current sewage chlorination practices.

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APPENDIX

APPENDIX TABLE A-1

Preliminary Appearance and Growth Characteristics of Selected Bacteria in Pure and Mixed Culture

Pure Cultures	E. coli	S. faecalis	S. epidermis
Desoxycholate	+	-	-
Azide Dextrose	-	+	+
Mannitol Salt	-	-	+
Pure Cultures w/sewage			
Desoxycholate	+	-	-
Azide Dextrose	-	+	+
Mannitol Salt	-	-	+
Mixed Cultures w/ and w/o sewage			
Desoxycholate	+	-	-
Azide Dextrose	-	*	*
Mannitol Salt	-	-	*

\+ - growth
\- - no growth
* - unable to distinguish colony types

E. coli - large, red, nucleated colonies on DES
S. faecalis - small, round, opaque colonies on AD

S. epidermis - small, round, opaque colonies on MS and AD

APPENDIX TABLE A-2

Population Densities* in Two Dilution Media

Time	Nutrient Broth		Distilled Water	
	E. coli	S. faecalis	E. coli	S. faecalis
5 minutes	1.3×10^6	1.9×10^7	1.4×10^6	1.6×10^7
10 minutes	1.4×10^6	1.6×10^7	1.2×10^6	1.3×10^7
15 minutes	2.7×10^6	1.7×10^7	1.6×10^6	1.7×10^7

* Cells/ml, 48-hr. incubation on differential media. Counts are averages of three replicates.

APPENDIX TABLE A-3

Optical Density of *E. coli* in Nutrient Broth using the Spec. 20 at 430 mu and a 1 cm path length

Time	Hour	Optical Density	
		Flask 1	Flask 2
1	2:30 p.m.	.005	.01
2	3:30 p.m.	.01*	.01
3	5:00 p.m.	.01	-
4	7:00 p.m.	.01	.02
5	8:00 p.m.	.015	.02
6	9:00 p.m.	-	.025
7	9:45 p.m.	-	.05*
8	10:00 p.m.	.045*	-
9	10:30 p.m.	-	.075*
10	11:00 p.m.	.09*	-
11	11:15 p.m.	-	.115*
12	12:00 p.m.	.145*	.155
13	12:45 a.m.	-	.185*
14	1:00 a.m.	.2	-
15	1:30 a.m.	-	.215
16	2:00 a.m.	.235	-
17	2:15 a.m.	-	.245
18	3:00 a.m.	.275	.285
19	3:45 a.m.	-	.3*
20	4:00 a.m.	.315	-
21	4:30 a.m.	.355	-
22	4:45 a.m.	-	.365
23	6:00 a.m.	.41	.41*
24	1:00 p.m.	.68*	.7
25	2:30 p.m.	.85	.925*

\- readings not recorded
* plate counts prepared

APPENDIX TABLE A-4

48-Hour Plate Counts of *E. coli* on Nutrient Agar
Counts are Averages of Duplicate Plates

Time	Counts cells/ml
2	1.5×10^3
7	1.3×10^7
8	1.8×10^7
9	4.1×10^7
10	6.0×10^7
11	8.8×10^7
12	1.2×10^8
13	1.5×10^8
16	2.2×10^8
19	2.5×10^8
23	3.3×10^8
24	6.8×10^8
25	2.8×10^9

APPENDIX TABLE A-5

Per cent Transmittance of S. faecalis in Nutrient Broth using the Spec. 20 at 430 mu and a 2 cm path length

Time	Hour	Optical Density Flask 1	Hour	Optical Density Flask 2
1	1:05 p.m.	.01*	1:05 p.m.	.01
2	2:30 p.m.	.025	2.40 p.m.	.02
3	8:30 p.m.	.045	8:45 p.m.	.045*
4	9:30 p.m.	.06	9:45 p.m.	.055
5	10:30 p.m.	.075*	10:45 p.m.	.08
6	11:30 p.m.	.09	11:15 p.m.	.09*
7	12:30 a.m.	.115	11:45 p.m.	.10
8	1:30 a.m.	.16*	12:15 a.m.	.11
9	2:30 a.m.	.18	12:45 a.m.	.13
10	3:30 a.m.	.19	1:15 a.m.	.145
11	4:30 a.m.	.185	1:45 a.m.	.16
12	5:30 a.m.	.185	2:15 a.m.	.175
13			2:45 a.m.	.18
14			3:15 a.m.	.185*
15			4:30 a.m.	.185
16			5:30 a.m.	.187*

* plate counts prepared

APPENDIX TABLE A-6

48-Hour Plate Counts of *S. faecalis* on Nutrient Agar - Counts are Averages of Duplicate Plates

Time	Counts Cell/ml
1	5.5×10^4
3	1.3×10^7
5	3.9×10^7
6	4.7×10^7
8	8.6×10^7
14	1.1×10^8
16	1.2×10^8

APPENDIX TABLE A-7

Chemical Data Derived from Disinfection Experiment #1

COD - 96 mg/l
TKN - 11.0 mg/l
NH_3-N - 6.5 mg/l

Chlorine Dosage - 2.60 mg/l

Site	Contact Time, Min.	pH	Temp. °C.	Residual Chlorine (monochloramine) mg/l
1 (Sample 1a)	0	7.1	18	-
1 (Sample 1b)	0	7.4	18	-
2	10	7.4	18	0.98
3	20	7.5	18	0.96
4	30	7.2	18	0.92

APPENDIX TABLE A-8

Chemical Data Derived from Disinfection Experiment #2

COD - 60 mg/l
TKN - 15.7 mg/l
NH_3-N - 7.3 mg/l

Chlorine Dosage - 2.10 mg/l

Site	Contact Time, Min.	pH	Temp. °C.	Residual Chlorine (Monochloramine) mg/l
1 (Sample 1a)	0	7.7	20	-
1 (Sample 1b)	0	7.7	20	-
2	10	7.7	20	0.76
3	20	7.7	20	0.72
4	30	7.6	20	0.70

APPENDIX TABLE A-9

Chemical Data Derived from Disinfection Experiment #3

COD - 27.3 mg/1
TKN - 10.2 mg/1
NH_3-N - 6.8 mg/1

Site	Contact Time, Min.	pH	Temp. °C.	Residual Chlorine (monochloramine) mg/1
1 (sample 1a)	0	7.1	18	-
1 (sample 1b)	0	7.6	18	-
2	10	7.7	18	0.34
3	20	7.6	18	0.30
4	30	7.6	18	0.18

APPENDIX TABLE A-10

Chemical Data Derived from Disinfection Experiment #4

COD - 50 mg/l
TKN - 9.2 mg/l
NH_3-N - 6.7 mg/l

Chlorine Dosage - 3.50 mg/l

Site	Contact Time, min.	pH	Temp.°C.	Residual Chlorine (monochloramine) mg/l
1 (sample 1a)	0	7.6	20	-
1 (sample 1b)	0	7.5	20	-
2	10	7.5	20	2.35
3	20	7.5	20	2.20
4	30	7.5	20	1.84

APPENDIX TABLE A-11

Chemical Data Derived from Disinfection Experiment #5

COD - 16.0 mg/l
TKN - 6.7 mg/l
NH_3-N - 4.8 mg/l

Chlorine Dosage - 2.8 mg/l

Site	Contact Time, Min.	pH	Temp. °C.	Residual Chlorine (monochloramine) mg/l
1 (sample 1a)	0	7.5	19	-
1 (sample 1b)	0	7.6	19	-
2	10	7.6	19	1.25
3	20	7.6	19	1.47
4	30	7.5	19	1.42

APPENDIX TABLE A-12

Change with Time in Population Densities in Dechlorinated Sewage Incubated at 20°C.
Numbers are Average cells/ml Determined from Triplicate Agar Plates Incubated 48 hours.

			Incubation Time (hrs.)					
Contact Time(min.)	Cl_2Residual(mg/l)	Bacteria	0	22	46	70	94	118
0,Sample 1a	0	E. coli	9.8×10^4	Error	3.4×10^7	1.1×10^6	5.1×10^5	2.5×10^5
		S. faecalis	5.5×10^4	6.6×10^4	3.6×10^4	0 at 10^{-2}	4.0×10^1	3.0
10	2.35	E. coli	1.4×10^4	3.5×10^6	1.1×10^7	1.1×10^7	6.4×10^6	3.6×10^6
		S. faecalis	4.3×10^4	1.6×10^4	1.0×10^4	5.1×10^3	2.3×10^3	1.7×10^3
20	2.20	E. coli	5.3×10^3	1.4×10^5	1.6×10^6	2.6×10^6	1.3×10^6	6.4×10^5
		S. faecalis	2.0×10^4	1.3×10^2	2.0×10^1	0	0	0
30	1.84	E. coli	3.7×10^1	8.3×10^1	>300 at 10^{-3}	2.8×10^6	2.0×10^6	2.3×10^6
		S. faecalis	7.1×10^2	0	0	0	0	0
0,Sample 1b	0	E. coli	1.0×10^5	9.2×10^6	1.5×10^7	1.4×10^7	9.8×10^6	1.2×10^6
		S. faecalis	6.2×10^4	5.9×10^4	6.9×10^4	5.4×10^4	3.7×10^4	3.8×10^4

APPENDIX TABLE A-13

Change with Time in Population Densities in Dechlorinated Sewage Incubated at 20°C. Numbers are Average cells/ml Determined from Triplicate Agar Plates Incubated 48 Hours

Contact Time (min.)	Cl_2 Residual (mg/l)	Bacteria	Incubation Time (hrs.) 0	24	48	72	96
0, Sample 1a	0	E. coli	1.6×10^5	9.9×10^6	5.2×10^6	1.9×10^6	9.9×10^5
		S. facealis	2.2×10^4	8.6×10^3	4.6×10^3	4.2×10^3	3.3×10^3
10	1.25	E. coli	1.1×10^5	7.7×10^6	2.0×10^6	>300at 10^{-3}	3.6×10^6
		S. faecalis	2.3×10^4	2.0×10^4	9.3×10^3	7.7×10^3	5.7×10^3
20	1.47	E. coli	4.1×10^4	6.3×10^6	2.8×10^6	>300at 10^{-3}	4.1×10^6
		S. faecalis	4.8×10^3	1.0×10^2	5.3×10^2	1.4×10^2	2.5×10^2
30	1.42	E. coli	3.7×10^3	2.8×10^5	1.5×10^6	>300at10^{-3}	1.6×10^6
		S. faecalis	2.0×10^1	1.0×10^1	1.0×10^1	0	0
0, Sample 1b	0	E. coli	1.5×10^5	1.0×10^7	5.0×10^6	Error	2.9×10^6
		S. faecalis	2.1×10^4	1.5×10^4	2.8×10^3	2.2×10^3	2.1×10^3

APPENDIX TABLE A-14

Change with Time in Population Densities in Dechlorinated Sewage Incubated at 20°C. Numbers are Average cells/ml Determined from Triplicate Agar Plates Incubated 48 Hours.

Contact Time (min.)	Cl_2 Residual (mg/l)	Bacteria	0	18	42	66	90	114	138
0, sample 1a	0	*E. coli* NB#	1.8×10^5	8.5×10^6	8.7×10^6	1.7×10^5	3.1×10^3*	4.4×10^4	6.7×10^3
		PO_4	1.1×10^5						
		S. faecalis NB	1.4×10^4	2.2×10^4	5.3×10^4	1.3×10^2	3.0×10^1	1.0×10^1	1.3×10^1
		PO_4	3.0×10^4						
10	0.98	*E. coli* NB	1.9×10^4	3.7×10^6	4.1×10^6	3.0×10^6	1.7×10^4*	300 at 10^{-3}	1.7×10^6
		PO_4	4.4×10^3						
		S. faecalis NB	9.0×10^3	1.4×10^4	1.2×10^4	1.3×10^4	6.8×10^3	2.6×10^3	4.8×10^2
		PO_4	1.9×10^4						
20	0.96	*E. coli*	3.0×10^2	3.0×10^5	1.0×10^5	9.3×10^4	1.3×10^5	5.4×10^4	0 at 10^{-3}
		S. faecalis	2.6×10^3	6.3×10^2	6.3×10^1	1.1×10^1	0	0	0
30	0.92	*E. coli* NB	6.0	1.0×10^4	2.0×10^4	4.4×10^3	5.6×10^3	2.8×10^3	1.0×10^3
		PO_4	1.3×10^1						
		S. faecalis NB	2.0×10^1	1.0×10^1	0	0	0	0	0
		PO_4	1.6×10^1						
0, sample 1b	0	*E. coli*	2.4×10^5	Error	1.1×10^7	6.8×10^6	1.0×10^4*	300 at 10^{-3}	1.8×10^5
		S. faecalis	3.2×10^4	300 at 10^{-3}	3.0×10^4	1.6×10^4	8.3×10^1	5.3×10^1	5.3×10^1

NB - Nutrient Broth Dilution Medium
+ PO_4 - Phosphate Buffer Dilution Water
* Dark Red Colonies *E. coli*

APPENDIX TABLE A-15

Change with Time in Population Densities in Dechlorinated Sewage Incubated at 20°C. Numbers are Average cells/ml Determined from Triplicate Agar Plates Incubated 48 Hours.

Contact Time (min.)	Cl_2 Residual (mg/l)	Bacteria	Incubation Time (hrs.) 0	18	42	66	90
0, sample 1a	0	E. coli N.B.*	2.5×10^5	3.3×10^6	1.0×10^5	1.2×10^4	1.5×10^2
		PO_4+	1.9×10^5				
		S. faecalis NB	8.1×10^3	1.8×10^4	8.4×10^3	4.3×10^1	1.0×10^1
		PO_4	2.2×10^4				
10	1.76	E. coli N.B.	8.6×10^4	2.5×10^5	7.3×10^4	2.2×10^4	1.1×10^4
		PO_4	7.5×10^4				
		S. faecalis NB	9.3×10^3	1.8×10^4	2.2×10^4	1.7×10^4	1.1×10^4
		PO_4	1.7×10^4				
20	0.72	E. coli	7.6×10^4	7.7×10^5	1.9×10^5	1.2×10^4	6.3×10^3
		S. faecalis	1.5×10^4	1.3×10^4	8.5×10^3	1.2×10^3	4.6×10^1
30	0.70	E. coli N.B.	7.4×10^3	1.9×10^5	5.0×10^3	1.0×10^4	1.1×10^3
		PO_4	1.1×10^4				
		S. faecalis NB	2.0×10^3	3.0×10^1	0	0	0
		PO_4	2.7×10^3				
0, sample 1b	0	E. coli	1.1×10^5	1.9×10^6	7.0×10^5	5.0×10^3	4.3×10^2
		S. faecalis	2.2×10^4	2.4×10^4	1.5×10^4	1.0×10^2	3.7×10^1

* N.B. - Nutrient Broth Dilution Medium
+ PO_4 - Phosphate Buffer Dilution Water

APPENDIX A-16

Change with Time in Population Densities in Dechlorinated Sewage Incubated at 20°C. Numbers are Average cells/ml Determined from Triplicate Agar Plates Incubated 48 Hours.

Contact Time (min.)	Cl_2 Residual (mg/l)	Bacteria	Incubation Time (hrs.) 0	21	45	70	93	117
0, sample 1a	0	E. coli	$1.0x10^5$	$1.4x10^6$	$2.3x10^6$	$2.0x10^6$	$1.5x10^6$	$1.6x10^6$
		S. faecalis	$3.0x10^4$	$4.1x10^4$	$2.7x10^4$	$2.3x10^4$	$2.2x10^4$	$1.5x10^4$
10	0.34	E. coli	$3.2x10^4$	$4.3x10^5$	$>300at10^{-3}$	$1.8x10^6$	$2.1x10^6$	$1.5x10^6$
		S. faecalis	$3.0x10^4$	$3.2x10^4$	$2.4x10^4$	$2.2x10^4$	$2.2x10^4$	$2.0x10^4$
20	0.30	E. coli	$1.5x10^4$	$1.2x10^5$	$>300at10^{-3}$	$1.5x10^6$	$2.1x10^6$	$1.0x10^6$
		S. faecalis	$2.6x10^4$	$3.6x10^3$	$3.9x10^2$	$1.3x10^1$	0	0
30	0.18	E. coli	$1.4x10^4$	$3.1x10^6$	$>300at10^{-3}$	$1.5x10^6$	$1.0x10^6$	$9.4x10^5$
		S. faecalis	$2.6x10^4$	$2.0x10^2$	$3.0x10^{-1}$	$1.0x10^1$	0	0
0, sample 1b	0	E. coli	$5.5x10^4$	$6.7x10^6$	$2.3x10^6$	$1.4x10^6$	$1.6x10^6$	$1.3x10^6$
		S. faecalis	$4.2x10^4$	$4.7x10^4$	$2.7x10^4$	$2.5x10^4$	$2.2x10^4$	$3.3x10^4$

X. VITA

Kathleen G. Saunders was born January 19, 1951 in Camden, New Jersey. She received her primary and secondary education in Pemberton, Ridgewood and Midland Park, New Jersey. Upon graduation from Midland Park Junior-Senior High School in June, 1969, she entered Virginia Polytechnic Institute, Blacksburg, Virginia, in September, 1969. The Bachelor of Science degree in Biology was awarded to her with distinction in June, 1973. In September, 1973, she entered the Master's program in Environmental Sciences and Engineering at Virginia Polytechnic Institute and State University. Since completing the requirements, she is employed by Bio-Analytical Laboratories, Inc., in Bristol, Tennessee.

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REGROWTH OF E. COLI AND S. FAECALIS IN TREATED SEWAGE AFTER CHLORINATION IN A CONTINUOUS-FLOW REACTOR

By

KATHLEEN G. SAUNDERS

(ABSTRACT)

The objective of this study was to examine the regrowth of E. coli and S. faecalis in chlorinated, secondary-treated sewage effluent using a continuous flow system. Regrowth was evaluated at varying chlorine contact times and chlorine residuals. In addition, chemical and physical characteristics of the sewage were evaluated in relation to regrowth.

The results showed that while a 99% kill of E. coli could be achieved, regrowth always occurred regardless of the chlorine residual and contact time. S. faecalis never regrew in the chlorinated sewage. No single parameter could be identified as the most important in controlling the extent of regrowth.