KINETICS OF CHLORINE DEPLETION AND MICROBIAL GROWTH IN HOUSEHOLD PLUMBING SYSTEMS

ABSTRACT

Studies of the depletion of chlorine and growth of bacteria in household and building plumbing systems were conducted in Columbia and Jefferson City, Missouri to distinguish between regrowth and aftergrowth.

Measurements were made of the effect of water temperature on the rate or chlorine reduction due to aqueous reducing agents alone. Bacterial growth was observed through measurements of total bacterial populations and heterotrophic plate count organisms.

Separate, controlled studies were made to distinguish between regrowth and aftergrowth in a building plumbing.

Detailed data from the Columbia, Missouri water utility were utilized to make estimates of the total length, surface area and volume of water distribution piping. In addition, estimates were made of the volumes of water contained in municipal storage reservoirs, building plumbing, toilets and hot water storage tanks.

The Microbial Ecology in Water Distribution Systems

Microbial ecology is the science that explores the relationships between microorganisms and their environment. A study of microbial ecology involves an assessment of the changes in the *total* and *individual* members of the microbial community. In drinking water distribution systems, the microbial ecology would be influenced by the influx of organisms, the surface colonization of distribution mains, the invasion of distribution systems by organisms from external sources, variations in flow, the chemical composition of the distributed water and the effective concentration of residual disinfectant. In addition, seasonal water temperature changes would be expected to markedly affect total microbial populations by influencing the kinetics of organism inactivation and the rate of disinfectant dissipation as well as organism growth rates.

Recent evidence indicates that the microbial ecology in water distribution systems is extremely dynamic and diverse (Brazos and O'Connor, 1984). This is surprising in light of the low nutrient, low temperature, chemically hostile conditions which prevail in distribution systems. In studies involving numerous water distribution systems in Missouri, the total populations of planktonic organisms were enumerated, providing comprehensive data on the total number of bacteria present in distributed drinking waters (O'Connor et al., 1984, 1985). These studies indicated that most of the organisms found in the distribution systems were routinely discharged through the water treatment plants, particularly during periods of low water temperature. From these studies it is evident that large numbers (10^7 –10^{10} bacterial cells/l) of micrometer sized bacteria enter the water distribution systems through the filtration plant or well. Since the adverse effects of microorganisms on distributed drinking water quality due to regrowth can only be controlled at the treatment plant, it is necessary to distinguish between organisms and particles entering the distribution system from the plant from those which are recruited from pipe surfaces during distribution (aftergrowth).

Planktonic and Periphytic Organisms

A distribution system can be envisioned as an elongated, continuously diminishing bottle in which the surface area to volume ratio increases from large mains to small household plumbing. The contribution of the periphytic community to the total microbial biomass is unknown. It is essential that quantitative measures of the total number of planktonic and periphytic organisms be made in a variety of distribution systems under different temperature, nutrient and disinfectant conditions. The effect of main flushing on the periphytic population must also be assessed to evaluate the effectiveness of this technique in controlling biologically–mediated corrosion and water quality deterioration.

The method used for enumerating the total number of bacteria in the present study is a slight modification of the method described by Hobbie et al. (1977). The acridine orange direct count (AODC) method involves staining a suspension of bacteria with the fluorochrome, acridine orange. The suspension is subsequently membrane–filtered on irgalan black–stained 0.2 µm polycarbonate membrane filters. The bacteria are then counted while still moist under epifluorescent (reflected ultraviolet) illumination.

In addition to quantifying the total population, the direct count can serve to provide *activity indices* when used in conjunction with other microbial measurements (6). These indices are formed with the total population as the denominator, and any measure of bacterial activity, metabolic state or biomass as the numerator.

The heterotrophic plate count (HPC) is a conditional measurement of organisms capable of growing on either rich or dilute nutrient media at either 35 °C or 20 °C. In conjunction with the total population, the HPC can provide one index of distribution system organism activity. The ratio of HPC to the total bacterial count, which can theoretically range from zero to one, has been observed to markedly increase following the dissipation of disinfectant residuals in distribution systems (O'Connor et al., 1984; 1985). The HPC percent of the total, as presented in this report, is an example of an activity index.

Living, Injured and Dead Bacterial Cells

Early evaluations of the *"viable plate count," "total plate count," "total bacterial count"* and *"standard plate count"* created the impression among some users of the data that all living organisms were enumerated by the procedure. This is far from correct since autotrophic organisms, heat–sensitive organisms, anaerobic organisms and many disinfectant–injured cells, although alive, cannot survive and reproduce under plate count culture conditions.

The direct microscopic count, on the other hand, permits the enumeration of all organisms, living, injured and dead. Whereas the living cells are presumably capable of activity and replication, injured cells may be undergoing metabolic repair. However, the dead cells require further description.

The definition of a dead microorganism is still a matter of controversy among microbiologists. For example, a dead microorganism may be defined as a microorganism which is unable to reproduce. If this is due to inactivation of its enzyme or reproductive system, the cell may still have living DNA or RNA inside its cell membrane. Ultimately, the cell wall lyses and disappears, decreasing the observed direct microscopic count. Replication of the direct count at different times can therefore provide useful information on the fraction of the total planktonic organisms present which are *"dead"*, even though their DNA or RNA is still intact. If the direct count of the number of organisms entering a distribution system was observed to markedly decline during travel in the mains, many of the organisms may have been, by the previous definition, *"dead"* at the time they entered the system. This phenomena has been occasionally observed in Missouri distribution systems where the water is heavily chlorinated. More often, however, direct counts are not found to decrease during time of travel in the distribution system (O'Connor, et al., 1985), but increase when chlorine residuals are depleted.

Effect of Organisms on Chlorine Demand

"Chlorine Demand" is not an intrinsic or specific property of water. It is a *conditional* measurement which varies, even for the same water, with temperature, pH, time and, particularly, with the external environment (surface) with which the water is in contact. A wide range of aqueous reducing agents, both inorganic (Fe^{2+}, Mn^{2+}, H_2S, NH_4^+) and organic (proteins, etc.), react at varying rates with chlorine.

The single most important factor influencing chlorine demand and bacterial growth in a water distribution system is the *surface area* of the distribution piping. Since the pipe surface area to water volume ratio increases exponentially as pipe size decreases, the effects of pipe surface reactions with chlorine and the growth of bacteria would be expected to be most noticeable in households and buildings beyond the service connection. In addition, warmer household temperatures accelerate chlorine reduction reactions and accelerate organism growth rates. As a result, distribution system sampling programs which sample distribution main rather than household water offer an incomplete and inaccurate assessment of organism population, ecology, pipe corrosion and water quality deterioration. Contrary to the frequent complaints of *"bad"* distribution samples due to inadequate faucet flushing, the *"bad"* samples may have been appropriate for assessing drinking water quality at the tap.

In order of decreasing magnitude, the chlorine demand in a distribution system is the sum of:

1. the chlorine demand due to the reactions of chlorine at the pipe surface with iron, copper, brass or attached organic reducing agents,

2. the chlorine demand due to reducing substances in aqueous solution, and

3. the chlorine demand due to reactions with the particulate matter in suspension.

The regulation of turbidity as a primary (microbiological) drinking water standard has previously been justified, in part, because the removal of raw water turbidity was believed to have a major influence on the removal of chlorine–demanding substances. In household systems in Missouri, the chlorine demand due to reactions within the household and building system piping has been found to be, by far, the greatest portion of the chlorine demand. As described in Standard Methods (APHA, 1985) chlorine demand measurements made on tap water collected and stored in clean glass bottles, where surface reactions are minimized, have little relevance to conditions in the distribution system.

Distribution Systems Studies

With these considerations in mind, a number of studies were conducted in the distribution systems of Columbia and Jefferson City, Missouri. These studies included repeated evaluations of chlorine depletion and organism growth in the plumbing of private households, public and commercial buildings.

The overall survey was conducted in four parts. First, an inventory of distribution system piping in Columbia, Missouri, was made to obtain an estimate of the total surface area in the distribution system as a function of pipe size. In particular, the extent of the system beyond the service connection, which is not generally considered in assessing changes in drinking water quality, was quantified.

Second, a study of City of Columbia water was made to determine the chlorine demand due to aqueous reducing agents. This was contrasted with the chlorine depletions observed in water stored in building and household plumbing which included losses due to reactions at the pipe surfaces.

Third, reduction of chlorine was observed as a function of tap water temperature.

Fourth, repetitive studies were conducted in numerous unoccupied households in both cities where water lines were flushed and bacterial growth was observed as a function of time, of storage and seasonal temperature change.

Together, these studies provide a unique view of the kinetics of chlorine depletion and microbial growth in the distribution system beyond the service connection.

Surface Area of Distribution System Piping

A comprehensive inventory of the size and length of distribution system piping for the City of Columbia, Missouri (Population: 62,000) was compiled so that estimates could be made of the total pipe surface area and volume of water stored in the distribution system piping as a function of pipe diameter (Table 1, Figure 1). The household plumbing and residential service connections, comprised of 0.5 and 0.75 inch internal diameter piping, provided 82 percent of the total pipe length and an estimated 24 percent of the total surface area in the system. However, it contained only 1.6 percent of the 8 million gallon storage volume in the mains (Figure 2). The total distribution system volume (16 million gallons) provides an average of 2 days retention at average flow (8 mgd). For each pipe diameter classification, the ratio of pipe surface to volume of water stored was calculated.

For example, 0.5 inch diameter household plumbing places approximately 315 cm^2 of surface area in contact with every liter of water. The ratio of surface area to volume is plotted as a function of pipe diameter in Figure 3. These estimates simply confirm the importance of the piping beyond the service connection with respect to its potential for accommodating the periphytic organism populations in water distribution systems and promoting chlorine reduction reactions with accumulations on the pipe surface. The large surface area to volume ratios combined with the longest travel time for chlorine depletion, highly intermittent flows and increased water temperatures due to heating of residences and commercial buildings would be expected to provide the greatest opportunity for organism colonization and growth.

Chlorine Demand in Household Plumbing

Since the household plumbing system provides the greatest opportunity for chemical reactions at pipe surfaces, a study of chlorine depletion in household and building system plumbing was conducted to determine the effect of contact with pipe surface alone on the loss of chlorine residuals.

Figures 4 and 5 provide comparisons of the chlorine demand primarily due to aqueous reducing agents alone versus the total demand exerted when water is stored in distribution pipes. The aqueous fraction of the chlorine demand was measured using water flushed from household or building plumbing and stored thereafter in chlorine–demand–free 50 ml screw cap glass tubes. Under such conditions, a chlorine residual persists for days and weeks. All chlorine residuals were measured by the DPD–ferrous ammonium sulfate method (APHA, 1985). On the other hand, the water which was allowed to remain in contact with the plumbing system was depleted to near–exhaustion within days, or, in some cases, hours. Numerous replications of this experiment in households in Columbia, Missouri and in a University laboratory building demonstrated that such chlorine depletions are commonplace. The total organic carbon concentration in the Columbia water distribution system averaged 1.2 g C/m^3 whereas the University water system contained an average of 0.2 g C/m^3 TOC.

Specific kinetic equations cannot be written for the observed chlorine depletions. The aqueous and attached reducing agents which react with chlorine are varied in composition and the chlorine species present are not well–defined. However, the chlorine depletions shown in Figures 4 and 5, appear to be pseudo–first order with respect to chlorine. The kinetics of the chlorine depletion in pipes is thought to be related to the condition of the interior pipe surface with respect to the reducing substances present.

If the chlorine demand exerted in small diameter piping (household plumbing) is not accounted for in distribution system management, the principal factor contributing to microbial growth and drinking water quality deterioration beyond the service connection will be overlooked.

Chlorine Profile in Household Plumbing and Service Connections

An extensive series of simple, but revealing, experiments were conducted using unoccupied private homes in Columbia and Jefferson City, Missouri. These were done to determine the chlorine residuals in water drawn from the kitchen tap through the service connection to the main in the street. To initiate each experiment, each tap was flushed for fifteen minutes, then closed. The taps remained unused for a period of one, three or seven days. On the sampling day, 250 ml aliquots of water were collected sequentially at a rate of one liter per minute until the chlorine residual remained constant, representing the chlorine concentration present in the distribution main.

The results of a typical experiment are shown in Figure 6. The water at the tap, which is immediately within the house, is virtually devoid of chlorine. The chlorine in water from the service connection, at ground temperature, is depleted to 0.3 g Cl/m^3 whereas water from the main contains 1.2 g Cl/m^3.

From these results, the question arises, *"What is the chlorine residual at the household tap?"* Obviously, any value from zero to the distribution main total residual can be obtained, depending on the volume of water drawn before sampling. Where the household plumbing is flushed for three to five minutes before sampling, as recommended by the AWWA Committee on Bacteriological Sampling Frequency in Distribution Systems (1985), it is the distribution system main, and not the consumer's tap, which is being sampled.

Effect of Temperature on Depletion of Chlorine Residuals in Bulk Water

Seasonal temperature changes affect the depletion of chlorine residuals during water distribution. For that reason, applied chlorine dosages are sometimes increased during periods of warm water temperatures to offset losses of chlorine residuals.

Within heated households and buildings, warm water temperatures prevail around the year. Still higher water temperatures are maintained in hot water heaters and piping. The effect of these temperature increases on chlorine residuals are evident from Figure 7 which shows the kinetics of chlorine depletion in Columbia, Missouri water stored in four liter, chlorine–demand–free brown glass bottles and incubated at 1.5 , 18.5 and 34 °C, respectively.

In these studies, chlorine depletions were not related to reactions at pipe surfaces, but were observed in water flushed from the distribution system and confined in clean glass vessels. Contact with reducing agents on pipe surfaces would have further accelerated the observed rates of depletion. In addition, the water used in these chlorine depletion studies was relatively free of organic reducing agents. Derived from a ground water supply, the organic carbon concentration in Columbia, Missouri treated water is only 1.2 g C/m^3. As a result, these rates of chlorine depletion may be slower than those observed in waters with greater quantities of organic reducing agents.

In building plumbing systems the accelerated chemical reduction of chlorine at high temperature may be related to the oxidation of pipe material, particularly iron, copper and brass. Corrosion and chlorine depletion are observed to proceed rapidly in recirculating hot water systems, for example.

Bacterial Growth in Household Plumbing Systems

The fourth part of the present study was conducted in households where the plumbing system could be flushed and the water allowed to remain unused for periods from one to seven days. After the preselected period of storage, each increment of water was slowly collected from the kitchen tap, until temperature, chlorine residual, heterotrophic plate count, and total bacterial direct count were nearly constant. This indicated that the water was being sampled from the distribution main. Water was drawn at a rate of one liter per minute until a total of 80 sequential 250 ml samples were collected. Each aliquot was split so that chemical and microbial analysis could be performed on parallel samples. The microbial sample bottle contained sterile sodium thiosulfate. Both microbial and chlorine samples were stored on ice and transported to the laboratory in ice chests where analyses were initiated and the direct count was determined by a slight modification of the method of Hobbie et al. (1977). Finally, the line was flushed for 15 minutes to obtain a distribution main sample.

Figure 8 shows that water temperature was a constant 25 °C throughout this particular summer sampling sequence. As was frequently observed, the chlorine in the household plumbing had been completely dissipated during storage. In those same samples, both HPC and the total bacterial direct count had increased dramatically over the levels observed in the distribution system mains. HPC increased by over two orders of magnitude to 4×10^4 CFU/ml while total bacteria had increased by more than an order of magnitude to 9×10^4 cells/ml. The ratio of HPC/AODC had increased from 5 percent in the distribution system to 41 percent in the household plumbing. Similar growth was observed in numerous households supplied by different water sources, but only after the chlorine residual had virtually disappeared.

Evaluation of the data on microbial growth and chlorine depletion gives extraordinary insight into the changes occurring in household plumbing. To begin with, the rate and extent of chlorine depletion can be quantified. Then, the temperature profile can be observed as a function of volume of water withdrawn from the tap.

Data on the total bacterial and heterotrophic plate colony counts indicate specific regions in which bacterial growth takes place. Although bacterial regrowth and aftergrowth was generally greatest at the household tap, in some households, there were more remote regions where very high microbial populations were observed (Figure 9). Replications of the sequential tap sampling survey generally resulted in remarkably similar results. These replicate studies confirmed the utility of the analytical techniques and the reproducibility of the overall survey technique (Figure 10).

The data presented in Table 2 allows a comparison of the bacterial populations observed at the tap and in the distribution main. In most of the households studied, chlorine was absent at the tap after the indicated period of storage. Where a residual concentration of chlorine was found, total bacterial count and HPC values were low.

Distinguishing Between Regrowth and Aftergrowth

To clarify the distinction between regrowth and aftergrowth, an annotated representation of bacterial activity in a distribution system is given in Figure 11. This figure also indicates how it might be possible to discriminate between the two phenomena. Aftergrowth, for example, can only be observed within the distribution system piping.

Preliminary studies were conducted in an effort to distinguish between regrowth and aftergrowth. To make this differentiation, three conditions were observed. A portion of chlorinated water was obtained by flushing the building tap for one hour. A one liter flask of the flushed water was split into two sets of chlorine–demand–free 50 ml screw cap test tubes with Teflon-lined caps to minimize the effect of surface reactions on chlorine depletion. One set was used to monitor the chlorine residual while the other was used for microbiological analyses. Another portion of the same water was dechlorinated with the stoichiometric requirement of sodium thiosulfate. Aliquots of this portion were also stored in clean glass tubes. In this set, bacterial regrowth in the absence of chlorine was observed with time of storage. Finally, water was allowed to remain in the building distribution piping where chlorine residuals disappeared in seven hours. The effect of both regrowth and aftergrowth on the direct count and HPC was observed on samples carefully withdrawn from the tap.

The results of three separate studies in different buildings are shown in Table 3. Both the total bacterial direct count and heterotrophic plate count increased rapidly in the absence of chlorine. In the building distribution piping, where periphytic organisms presumably populated the pipe surface, the increase was generally greatest. By subtracting the direct count observed in dechlorinated tap water stored in clean glass tubes from the values observed in the distribution system piping, estimates were made of the aftergrowth. Regrowth was estimated by subtracting the initial direct count (t = 0 days) from the direct count measured in the dechlorinated tap water stored in clean glass tubes.

Of special interest is the change in the ratio of HPC to the total direct count with time. Where the chlorine residual was absent, the ratio increased markedly. In most instances, the percentage of HPC to the total direct count was greatest after seven days.

The chlorine residual in chlorinated tap water stored in clean glass bottles persisted for two to three weeks and effectively retarded regrowth. When the residual had virtually disappeared, large increases in the direct count, but not the HPC, were observed. These results would suggest that the non–heterotrophic plate count organisms can replicate in the presence of low concentrations of chlorine whereas the HPC organisms cannot.

Figure 12, a plot of the three separate studies from which regrowth and aftergrowth were estimated indicates that regrowth contributed more than aftergrowth to the observed increases in the total (planktonic) bacterial direct count. The variation in the estimation of aftergrowth appears to confirm the expectation that the sampling technique (flushing rate) may have a great influence on the measured value. The results show, however, that aftergrowth may contribute a significant portion of the total organism population observed at the tap. Knowledge of the extent of this contribution will be important to an assessment of remedial actions taken at the treatment plant to control regrowth. Subsequent studies of this phenomena should be conducted in a flowing system so that the effect of velocity on the contribution to aftergrowth can be observed.

Summary

The present studies demonstrate the profound chemical and microbiological changes that occur in household and building plumbing systems due to microbial activity. The rapid depletion of chlorine in small diameter piping indicates that reactions occur at pipe surfaces. Chlorine residuals can be dissipated overnight, particularly at elevated temperatures which accelerate the reduction of chlorine.

The dissipation of chlorine is rapidly followed by extensive microbial growth as measured by both the total bacterial direct count and the heterotrophic place colony count. While the total population of bacteria at the tap may increase two orders of magnitude over that found in the distribution main, the HPC often increases by over three orders of magnitude. Bacterial growth at the tap is not inevitable, however. In those households or buildings where a measurable concentration of chlorine persists, bacterial growth is inhibited.

The major chemical and microbiological changes which occur at the consumer's tap indicate that serious research efforts are necessary to gain an understanding of the microbial ecology of water distribution systems. It may be possible to make treated water and household plumbing systems sufficiently stable so that the total depletion of chlorine and extensive bacterial growth does not occur. Less reactive internal household plumbing surfaces and improved treatment for reducing the number of organisms contributing to regrowth should significantly reduce total bacteria and HPC at the consumer's tap.