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Growth of Heterotrophic Bacteria in Water Vending Machines

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by

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ABSTRACT OF THE THESIS

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The growth of bacteria in a water vending machine was examined using the heterotrophic plate count (HPC) method. Water samples were taken from the first 15 ml of the first gallon vended (after allowing the machine to sit idle overnight) and from the third gallon vended. Samples were also taken from the storage tank and standing water that collected in the tubing entering and exiting the ultraviolet (UV) disinfection unit. The efficiency of the UV disinfection process used in the vending machine was also evaluated.

Bacterial growth was found to occur in the storage tank and in the tubing entering and exiting the UV disinfection unit. Bacterial re-growth in the tubing downstream of the UV unit was found to cause higher HPC levels in the first gallon vended than in the third gallon vended. The UV disinfection process was found to achieve an average removal rate of about 82%. Though the removal efficiency was not complete, it was not low enough to indicate a failure of the disinfection process. It was concluded that preventing

bacterial growth in the tubing after the disinfection process would significantly improve the overall microbiological quality of vended water.

INTRODUCTION

Consumers have many choices when it comes to the source of their drinking water. Tap water, bottled water, and vended water are all available as reliable sources of high quality drinking water.

Tap water is the least expensive source with an average cost of less than 1 cent per gallon. However, the microbiological quality of tap water has recently come into question, mainly due to the 1993 outbreak of *Cryptosporidum* in Milwaukee's drinking water supply, which affected over 400,000 people (Fox and Lytle, 1996). *Cryptosporidium* is a protozoan parasite that can live in the intestines of humans and animals. If ingested, it can cause cryptosporidiosis, a gastrointestinal disorder with symptoms that include diarrhea, nausea, headache, and loss of appetite. Symptoms usually last for two weeks or less, but *Cryptosporidium* oocysts can be excreted in the person's feces for up to 60 days (Pontius, 1996). In 1994, an outbreak of *Cryptosporidium* occurred in Las Vegas, affecting HIV infected persons. A study by the Centers for Disease Control and Prevention concluded that persons with depressed immune systems had a much higher risk of contracting cryptosporidiosis if they drank the Las Vegas tap water versus bottled or filtered water (Roefer et al., 1996).

Bottled water is more expensive than tap water, ranging from $.69 to $1.20 per gallon when purchased from a store. Bottled water appeals to many consumers because they believe it is safer than tap water, and because it lacks the taste and odor associated with chlorine residuals found in tap water. However, the lack of a disinfectant residual makes bottled water susceptible to contamination by heterotrophic bacteria. One study

found that some bottled waters contained bacterial counts in the range of 1,000 – 100,000 colony forming units per milliliter (CFU/ml) which exceeded 500 bacteria per ml, a proposed limit by the Food and Drug Administration (Scarpino et al., 1987).

Vended water is another popular source of drinking water. One of the largest suppliers of water vending machines (WVMs) operates over 13,700 machines in 29 states and sold 240 million gallons of water in 1998 (Glacier Water Services, Inc.). Vended water appeals to some consumers because it is inexpensive compared to some bottled waters, ranging from $.25 to $.39 per gallon. Vended water also lacks a chlorine residual and is thus susceptible to bacterial contamination.

The Los Angeles County Agricultural Commissioner/Weights and Measures Department (ACWMD) recently conducted a pilot study on 279 of the 2,900 WVMs in Los Angeles County. The study included testing for heterotrophic bacteria in 270 samples of the vended water. ACWMD found that many vended water samples had bacterial counts exceeding 500 CFU/ml and thus concluded that the disinfection processes used in the machines were ineffective (Fiksdal and Shindy, 1998). However, the California Department of Health Services (CDHS) stated that a bacterial level of 500 CFU/ml in vended water has no public health significance and did not indicate that disinfection was ineffective (Richardson, 1998). The ACWMD study did not directly examine the efficiency of the disinfection processes used in the WVMs. Nor did it examine the possibility of re-growth after the disinfection process.

After the ACWMD report was released, the Food and Drug Branch (FDB) of CDHS conducted its own study on WVMs throughout the state. The FDB investigated

bacterial re-growth and found that it occurred in the tubing after the disinfection step, leading to high bacterial counts in the vended water. The study did not conclude that the disinfection processes used in the machines were ineffective (Lee et al., 1999).

The purpose of this report is to examine the growth of bacteria in a water vending machine. Bacterial levels in the vended water and re-growth in the tubing are evaluated. This report also expands on the ACWMD and FDB studies by examining the efficiency of the disinfection process used in the WVM. The impact on the disinfection of drinking water of other processes within the treatment train, i.e. carbon adsorption, membrane treatment, and ultraviolet disinfection, is also examined.

BACKGROUND INFORMATION

Heterotrophic Bacteria and HPC

Heterotrophic bacteria refers to a broad range of non-photosynthetic microorganisms. Heterotrophic bacteria exist in both natural and treated water (including tap water) and are measured using heterotrophic plate counts (HPC), with the results reported as colony forming units per milliliter (CFU/ml). Heterotrophic bacteria are generally not considered pathogenic and are not limited by any federal or state drinking water regulations. However, water containing high bacterial populations can increase the risk of human exposure to secondary pathogens. Infants and elderly individuals may be vulnerable to disease due to secondary pathogens (Geldreich, E. E. et al., 1975). The World Health Organization (WHO) has suggested an HPC standard for edible ice of less than 50,000 CFU/ml. For infants and people with suppressed immune systems, the WHO has suggested a limit of 3,000 CFU/ml in edible ice (Richardson, 1998).

In the drinking water industry, HPC is normally used as a general indicator of the microbiological quality of water. An HPC value greater than 500 CFU/ml may indicate water of poor microbiological quality (Scarpino et al., 1987). High HPC (greater than 10,000 CFU/ml) can mask total coliform counts. Total coliform counts are used as indicators of pathogens and are regulated by virtually every water quality agency.

Water Vending Machine Overview

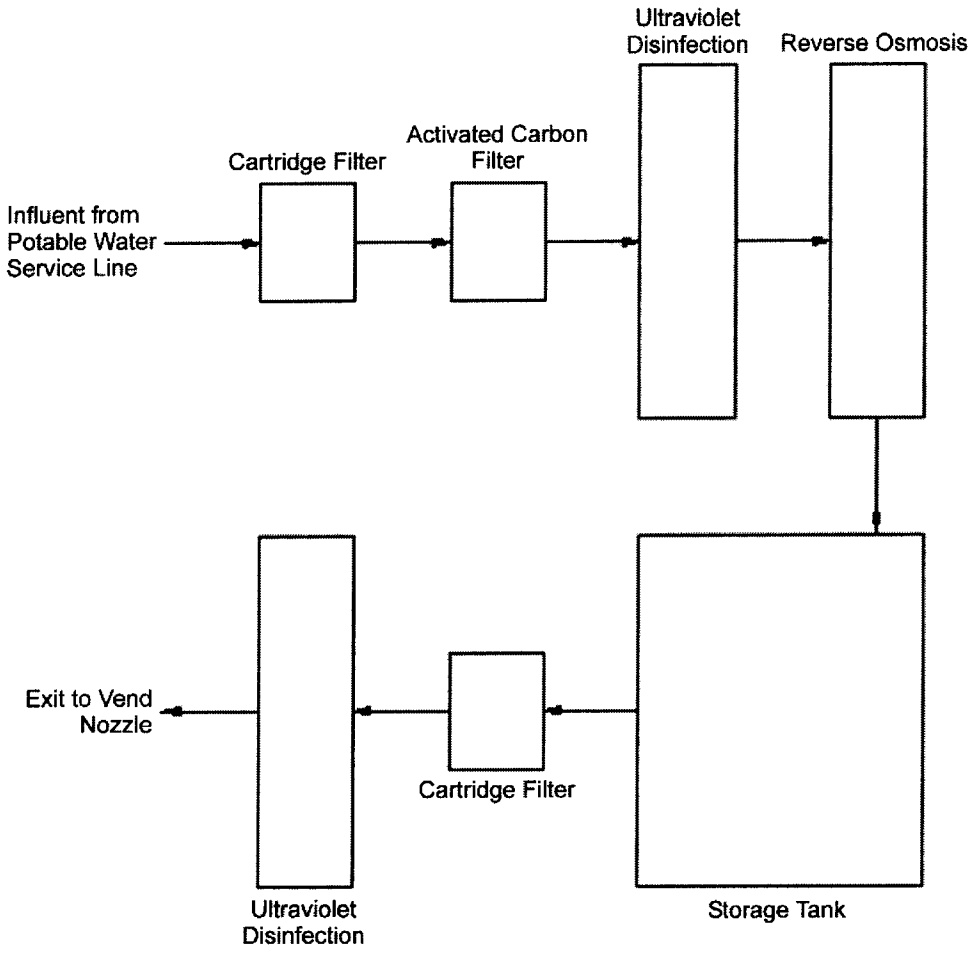

Figure 1. Water vending machine process diagram

The water vending machine used in this study is identical to the machines installed for public use. Influent water is taken from a potable water service line. The process train consists of several unit operations. The water goes through a cartridge filter, an activated carbon filter, a high-pressure reverse osmosis (RO) unit, and makes one pass through an ultraviolet (UV) disinfection unit before entering a storage tank. When the water is vended, it is drawn from the storage tank, goes through another

cartridge filter, and makes two passes through the UV unit before exiting through the vend nozzle (See Figure 1). The machine used in this study is fitted with a timer that causes it to vend a gallon every twelve minutes during the course of the day to simulate typical daily usage.

The machine used in this study was not cleaned regularly, allowing bacterial growth to occur over time. Machines installed for public use are cleaned and inspected on a regular basis. The vend nozzle is disinfected weekly with a 50% solution of household bleach. The filters, RO membrane, and UV lamp are inspected weekly and changed if necessary. The storage tank is disinfected by adding10 ml of household bleach into the tank once a month. These procedures reduce bacterial growth within the various units and in the storage tank.

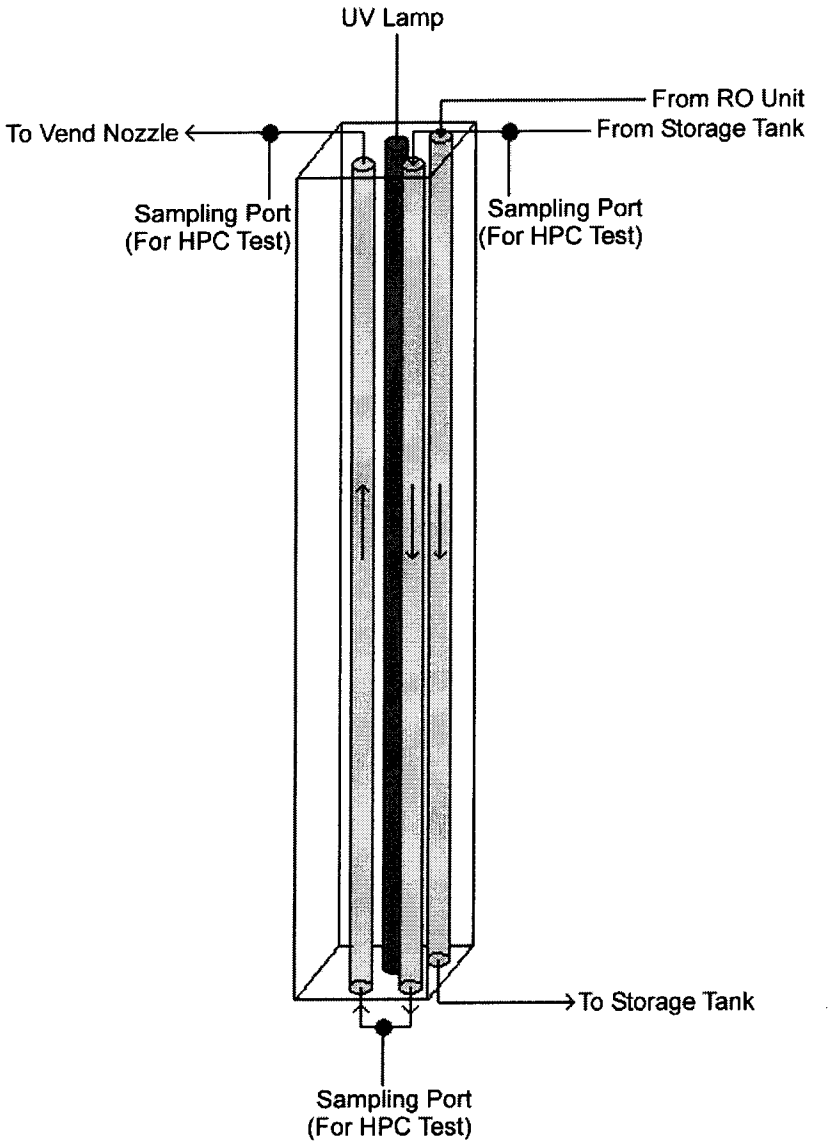

Figure 2. Ultraviolet Disinfection Unit

Influent water passes through the cartridge filter first to remove rust and particulate matter from the water. This is useful for preventing clogging of pumps and for reducing fouling of the reverse osmosis membrane. The activated carbon filter

removes the chlorine residual, disinfection by-products (some of which are suspected carcinogens), and other organic compounds present such as haloforms or humics.

The high-pressure RO unit removes inorganic and/or ionic dissolved solids such as hardness (Ca^{2+}, Mg^{2+}, salts) and colloidal material. RO produces very low turbidity water, which is necessary for the UV disinfection process to be effective. The RO process also filters out pathogens, including viruses, and thus is an important "barrier" in the treatment system.

The UV disinfection unit contains a 27-inch long UV lamp running through the center. The lamp intensity is 95 $\mu W/cm^2$ at one meter. Three one-inch diameter glass pipes are mounted around the lamp. Water passes through one of the pipes in the back of the unit before entering the storage tank. The other two pipes are connected with a short length of tubing, allowing water to make two passes through the unit before being vended (Figure 2).

LITERATURE REVIEW

Carbon Adsorption

Granular activated carbon (GAC) is useful for removing disinfectant residuals and any organic pollutants present, including disinfection by-products, from water. GAC also removes some bacteria from water. A reduction of approximately one order of magnitude has been achieved with a six-foot GAC filter bed (Brewer and Carmichael, 1979). However, a pilot plant study shows that the bacterial populations in water passed through a GAC column usually increase. The results of the study indicate that bacterial growth occurs inside the GAC column (Wilcox et al., 1983).

The surface of the GAC can provide many ideal sites for bacterial colonization. Oxygen and organic carbon sorbed to the GAC provide the necessary ingredients for microbial metabolism. Fissures and pores in the carbon grains can shield bacteria from fluid shear forces. Electron microscopy shows that both bacteria and protozoans can attach to GAC (Weber et al., 1978). Heterotrophic bacteria found in GAC columns and in GAC effluents are generally the same bacteria found in the GAC influents. Pretreatment of the influent water with chlorine or ozone does not appear to affect the type of heterotrophic bacteria in the GAC column or GAC effluent (Burlingame et al., 1986).

Combining ozonation of water with GAC treatment creates a process known as biological activated carbon (BAC). Ozone treatment may or may not improve the removal of some organic compounds by GAC. Ozonation can increase the organic

biodegradability in some waters by reducing the competition for adsorption sites. However, in other waters, ozonation may generate compounds that adsorb less readily to GAC, leading to more rapid breakthrough (Maloney et al., 1985). A study on ozone-GAC used after a conventional treatment process shows that preozonation of the GAC influent water increases the biodegradability of dissolved organic carbon (DOC), but reduces the activated carbon's capacity to absorb volatile halogenated organics (VHOs) (Maloney et al., 1985). Long term biological removal of total organic carbon (TOC) from water was believed to be enhanced by GAC, due to the carbon's adsorptive properties and to bacteria on the carbon surface. Bacteria can convert biodegradable organics to biomass, carbon dioxide, and other products (Bancroft et al., 1983). A pilot plant study shows that TOC removal by GAC is not significantly better than TOC removal through a sand filter, thus GAC does not appear to enhance biological TOC removal (Maloney et al., 1984).

Gram-negative enteric bacteria have been isolated from both ozonated and untreated waters that were filtered through a GAC column. While these bacteria are not pathogenic, there is a possibility that pathogenic bacteria of similar physiology could colonize the GAC (Brewer and Carmichael, 1979). Three enteric pathogens, *Yersinia enterocolitica, Salmonella typhimurium,* and *Escherichia coli* are known to readily colonize sterile GAC, maintaining populations of $10^5 - 10^7$ colony forming units per gram (CFU/g) for up to 14 days. The presence of heterotrophic bacterial colonies on the GAC can cause attached pathogens to decline at a rate of 0.10 – 0.22 log/day. An indigenous heterotrophic bacteria population of $10^6 - 10^8$ CFU/g is believed to be

sufficient to protect the filter from pathogens. However, some evidence exists showing that pathogens can colonize mature GAC filters, though their levels are usually low. It is therefore important that fresh GAC filters be fed pre-disinfected water to reduce the possibility of pathogenic colonization (Camper et al., 1985).

Water treated with GAC often carries carbon fines out of the filter column. The carbon fines can be colonized with heterotrophic or coliform bacteria. It is believed that release of fines from a GAC column is a random event and is not related to filter operation (Camper et al., 1986). A Metropolitan Water District of Southern California pilot plant study shows carbon particle levels of 10 – 62 particles/liter in GAC filter effluent. Also, a steady state heterotrophic bacteria level of 10^4 CFU/ml can be found in GAC filter effluent (Stewart et al., 1990).

Bacteria, when attached to activated carbon, are believed to be more resistant to disinfection by chlorine (free or combined). Carbon is a reducing agent and chemically reduces chlorine (Suidan et al., 1980). Heterotrophic bacteria attached to carbon particles from a GAC column are shown to be resistant to disinfection with 1.5 mg/l of chlorine or chloramine at 40 minutes of contact time and at a particle concentration of 25 g/l (wet particle weight) (Stewart et al., 1990). However, heterotrophic bacteria attached to carbon fines are not resistant to disinfection with 2 mg/l of chlorine at 2 minutes of contact time and at a particle concentration of less than 0.018 mg/l. Increasing the particle concentration to 0.18 mg/l or greater does result in a decrease in the disinfection efficiency (Stringfellow et al., 1993). A more recent study shows that a 0.7 – 2 log reduction of heterotrophic bacteria attached to carbon fines can be achieved with chlorine

disinfection. The study used particle concentrations of 1, 10 and 100 mg/l, chlorine concentrations of 2 and 2.5 mg/l, and contact times of 5, 15, and 30 minutes. At a particle concentration of 1000 mg/l, a chlorine concentration of 2 mg/l, and a contact time of 1 hour, no significant reduction in heterotrophic bacteria is observed (Pernitsky et al., 1997).

GAC is shown to be susceptible to colonization by heterotrophic bacteria. The attached heterotrophic bacteria can protect the GAC from colonization by pathogenic bacteria. However, water filtered through GAC can slough bacteria out of the filter. Water filtered through GAC may also contain carbon fines with attached bacteria. These are potential problems that must be considered when using GAC in a drinking water treatment system. A disinfectant used after a GAC process is clearly needed to ensure that the water has a low bacteria count.

Membrane Filtration

Membrane technology is very useful in the production of clear, disinfected drinking water without the use of chemical disinfectants. Microfiltration (MF), ultrafiltration (UF), nanofiltration (NF), and reverse osmosis (RO) are membrane processes commonly used to treat water. Table 1 shows pore sizes and applied pressures for various membrane processes.

Table 1: Pore size and applied pressure for membrane processes (Madaeni, 1999)

	RO	NF	UF	MF
Pore Size	Have no detectable pore size	2-3 nm	5-20 nm	20 nm-1 μm
Applied Pressure	30-150 atm	5-20 atm	2-7 atm	1-3 atm

When using membranes for water disinfection, pore size is an important parameter to consider. Membranes can remove biological colloids by sieve retention or by adsorption sequestration. In sieve retention, colloids are retained on the surface of the membrane. In adsorption sequestration, colloids are captured within the membrane matrix. These two methods of rejection allow membranes to remove bacteria that are either larger or smaller than the membrane pores (Madaeni, 1999). However, bacteria which are larger than the nominal pore size can pass through a membrane. This phenomenon can be due to imperfections in the membrane or the availability of pores larger than the nominal pore size (Jacangelo et al., 1989). A proposed theory is that bacteria deform to fit through smaller pores (Pall et al., 1980). However, bacterial cell walls are non-deformable, which suggests that it is much more likely that the bacteria pass through openings in the membrane that are larger than the nominal pore size. Another possibility is that the bacteria change size. Since bacteria divide faster than they grow, they may pass through the membrane before they have grown to full size (Madaeni, 1999). The material that the membrane is made may also affect its ability to retain bacteria. Celluose, polypropylene, and polysolfone membranes retain bacteria better than nylon and PVDF membranes do (Simonetti and Schroeder, 1984).

In drinking water, a limited number of non-pathogenic bacteria are allowed to be present. Thus, membranes are very capable of treating water to drinking water standards. Ultrafiltration membranes can yield a 4 log reduction of heterotrophic bacteria and complete removal of coliforms (Jacangelo et al., 1989).

Membrane fouling is a problem that is caused by an irreversible deposition of material onto or into the membrane. Fouling can cause a flux decline or an increased pressure drop across the membrane (Bicknel et al., 1985). Fouling can also reduce the rejection efficiency, increase the operating pressure, and shorten the life of the membrane. The development of a biofilm on the membrane is a major cause of membrane fouling. Microorganisms that are captured by the membrane can grow and form a biofilm, which clogs the pores. Techniques to control fouling include pretreatment of feed water to reduce particulate density, adjusting operating conditions such as pressure, crossflow, backwashing, etc., and membrane regeneration - washing with chemicals such as sodium hydroxide, detergents, or disinfectants (Madaeni, 1999).

Current research shows that membranes are effective in removing biological contaminants from water. Membranes can consistently deliver high quality water even with variable influent quality. Fouling is major problem in the operation of membranes. Many techniques are available to minimize fouling, but this problem still requires much work to solve (Madaeni, 1999).

Ultraviolet Disinfection

Ultraviolet (UV) disinfection is another means of reducing heterotrophic bacteria in drinking water. Unlike chlorine disinfection, UV does not form trihalomethanes (THMs), which are suspected carcinogens. Due to the light reflecting properties of colloids, UV disinfection is generally only effective in waters with low turbidity (<5 NTU). Also, UV is generally not effective at biofilm control in water distribution systems. Furthermore, some microorganisms exposed to sub lethal doses of UV have the ability to repair damage through photoreactivation or dark repair. Photoreactivation occurs in light with wavelengths of 310-420 nm, while dark repair occurs in the absence of light (Shaban et al., 1997).

Inactivation of bacteria by UV light occurs through the process of dimerization, pyrmidine base pair formation (Harris et al., 1987). Radiation absorbed by the bacterial DNA results in dimerization of thymine bases in the DNA strand. The double helix of the DNA is distorted, which interferes with normal DNA replication. Bacterial enzymes work continuously to repair this type of damage. However, if the UV dose is high enough, so many dimers are formed that the enzymes cannot repair the DNA. This results in the inability of the bacteria to reproduce, leading to their inactivation (von Sonntag, 1986).

Exposing the damaged bacterial cells to visible light can result in a phenomenon known as photoreactivation, where the damaged DNA is repaired. A study on *E. coli* and *S. faecalis,* two indicator microorganisms used to evaluate disinfection efficiency, shows that both strains of bacteria are capable of photoreactivation. Up to 3.4 logs of

reactivation for the *E. coli*, and up to 2.4 logs of reactivation for the *S. faecalis* can be observed. Thus, the UV dose to attain 4 log reduction in these bacteria is twice the dose normally used when photoreactivation is not taken into account (Harris et al., 1987). It was previously believed that the degree of photoreactivation is independent of the applied UV dose. However, Lindenauer and Darby (1994) show that increasing the applied UV can reduce the degree of photoreactivation. The maximum survival of bacteria after photoreactivation decreases exponentially in proportion to the UV dose (Kashimada et al., 1996).

Humic matter in water can incorporate oxidizing reagents such as hydroxyl radicals when exposed to UV radiation. The hydroxyl radicals can inactivate bacteria by damaging the bacterial membranes, or by breaking DNA strands (von Sonntag, 1986). The UV irradiated humic water is then capable of inactivating some bacteria, and inhibiting the metabolism of others. Approximately 60% of heterotrophic bacteria can be inactivated after one hour of contact time with freshly UV irradiated humic water (Lung and Hongve, 1994).

The efficiency of UV disinfection depends on the lamp intensity and the transmittance of the water. For single lamp UV disinfection units, the lamp intensity has a greater impact on the disinfection than the transmittance of the water. However, for multiple lamp systems, lamp intensity and transmittance appear to have equal effects on the disinfection efficiency of the system (Sommer et al., 1997).

UV disinfection offers a good alternative to chlorine disinfection for low turbidity waters. It is effective at inactivating pathogens and does not form THMs. However,

photoreactivation of bacteria is a concern, as it increases the UV dose required to reach 4 logs of bacterial inactivation. The ability of some UV irradiated humic waters to inactivate heterotrophic bacteria is an interesting effect but humic substances are generally not desirable in drinking water. Humic matter would likely be removed before the disinfection process by activated carbon and reverse osmosis. Thus this phenomenon would not be useful in the production of disinfected of drinking water.

MATERIALS AND METHODS

Bacteriological Methods

The bacteria used in this study were indigenous heterotrophic bacteria found in water. Pure strains of bacteria were not used. Bacteria were not artificially introduced into the machine. Bacteria were allowed to grow naturally, as they would under actual field conditions. Samples were examined using the heterotrophic plate count method as described in *Standard Methods for the Examination of Water and Wastewater, 17th Ed.* The spread plate method was employed with an incubation time of 48 hours at 37°C.

Heterotrophic Plate Count

The majority of the samples were tested with no dilution. Samples that were expected or known (from a previous test) to yield a too numerous to count result were diluted to 10^{-2} or higher with sterile phosphate dilution water. A too numerous to count result occurs when the bacterial density on the plate is too high, making it difficult to distinguish the individual colonies. Diluting the sample reduces the bacterial density on the plate, allowing an accurate count to be obtained. The plates were made with approximately 15 ml of plate count agar as the growth medium and inoculated with either 0.1 or 0.2 ml of sample. To ensure that the dilution water and plates were not contaminated, control plates were made each time a test was performed. The control plates were made by inoculating two plates with sterile phosphate dilution water and

leaving one plate blank (no sample). The plates were then incubated for 48 hours at 37°C.

Sampling

For this study, several sets of samples were taken. The first set of samples was taken from the water dispensed out the vend nozzle. After allowing the machine to sit idle overnight, a gallon was vended and the first 15 ml of the vended water was captured for testing. A second gallon was then vended and allowed to drain to waste. A third gallon was vended and the first 15 ml of the vended water was captured for testing. This procedure was most important for the sample taken from the first gallon vended, as it allowed capture of the standing water in the tubing, where bacterial growth was believed to be occurring. The second set of samples were taken from the influent water (after the activated carbon and cartridge filters), the storage tank and the standing water that collected in the tubing entering and exiting the UV disinfection unit. The third set of samples was taken to test the efficiency of the UV unit. Sampling ports were installed in the tubing entering and exiting the UV unit. To allow sampling at half the contact time, a sampling port was installed in the tubing connecting two of the internal pipes in the unit (see Figure 2 for sampling port locations). The contact time in the UV unit was increased by placing a clamp on the exit tubing and forcing more water to flow out of the port on the entrance tubing. Samples were taken at contact times of 5, 10 and 14 seconds (the contact time with no ports installed was 7 seconds).

RESULTS AND DISCUSSION

The HPC results showed a certain amount of scatter. However, this is expected when performing microbiological examinations of water. Bacteria are generally not randomly distributed in water. To give an accurate picture of the microbiological quality of the water, the highest, lowest, and average counts are graphed or reported for each sample. Raw HPC data are reported in Appendix A.

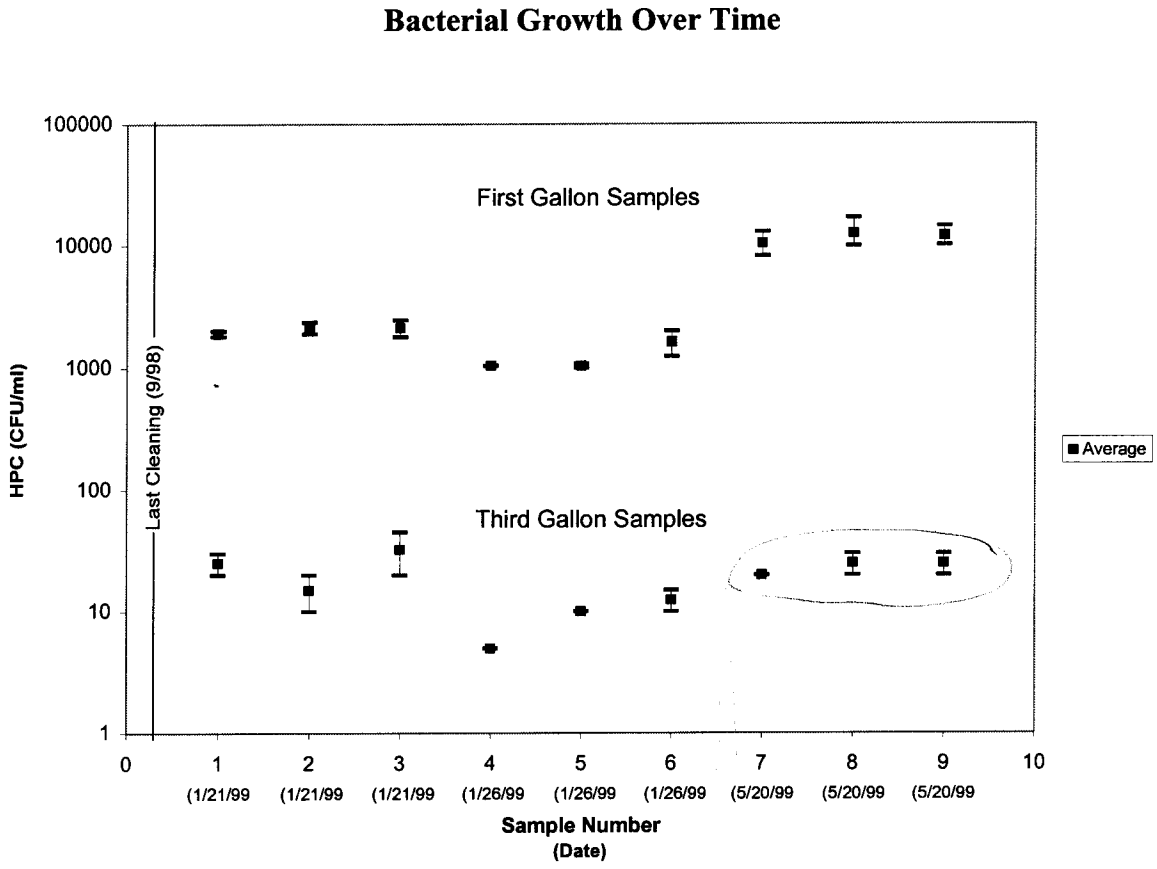

Figure 3. HPC growth over time - high, low and average HPC values are shown for water samples from the first and third gallons vended

Figure 3 shows HPC values for water samples from the first and third gallons vended. The tests shown in Figure 3 began approximately four months after the machine was last

cleaned, allowing some bacterial growth to occur inside the machine. The first gallon vended contained more HPC than the third gallon vended, which was consistent with the California Department of Health Services study. Bacteria were most likely growing in the standing water in the tubing downstream of the UV disinfection unit. The first gallon vended cleared the bacteria out of the tubing, causing subsequent vends to have lower HPC values.

Table 2: HPC in the influent, storage tank and UV tubing

		HPC (CFU/ml)		
Date	**Sample**	**High**	**Low**	**Average**
2/2/99	020299-I (Influent)	<5	<5	<5
2/4/99	020499-I (Influent)	<5	<5	<5
2/2/99	020299-T (Tank)	880	550	715
2/4/99	020499-T (Tank)	680	680	680
5/12/99	051299-UV1 (Pre-UV tubing)	20000	6700	11763
5/12/99	051299-UV2 (Pre-UV tubing)	16000	7000	12438
5/18/99	051899-UV1 (Post-UV tubing)	27500	7350	16588
5/18/99	051899-UV2 (Post-UV tubing)	35000	8150	20913

Table 2 shows HPC results for water samples taken from the influent, the storage tank, and the standing water in the UV tubing. This set of samples was taken to assess the growth of bacteria in the machine. The results from this set of samples showed that the influent water was not bringing large amounts of bacteria into the system. However, there was bacterial growth in the storage tank. Machines installed for public use generally do not have problems with growth in the tank because they are disinfected monthly with a small amount of household bleach. The results from the standing water in the UV unit tubing were the critical issue of the study. Bacterial growth was observed in the tubing around the UV unit. The UV lamp heated part of the tubing near the unit to

about 32 °C. The elevated temperature in the tubing provided an ideal environment for bacterial re-growth to occur.

Ultraviolet Disinfection Efficiency

Table 3: UV disinfection efficiency

		Percent Removal (%)		
Contact Time (s)	Dose ($\mu W \cdot s/cm^2$)	High	Low	Average
5	475	98.6	53.8	79.1
10	950	98.5	56.5	86.1
14	1330	98.7	85.7	94.6

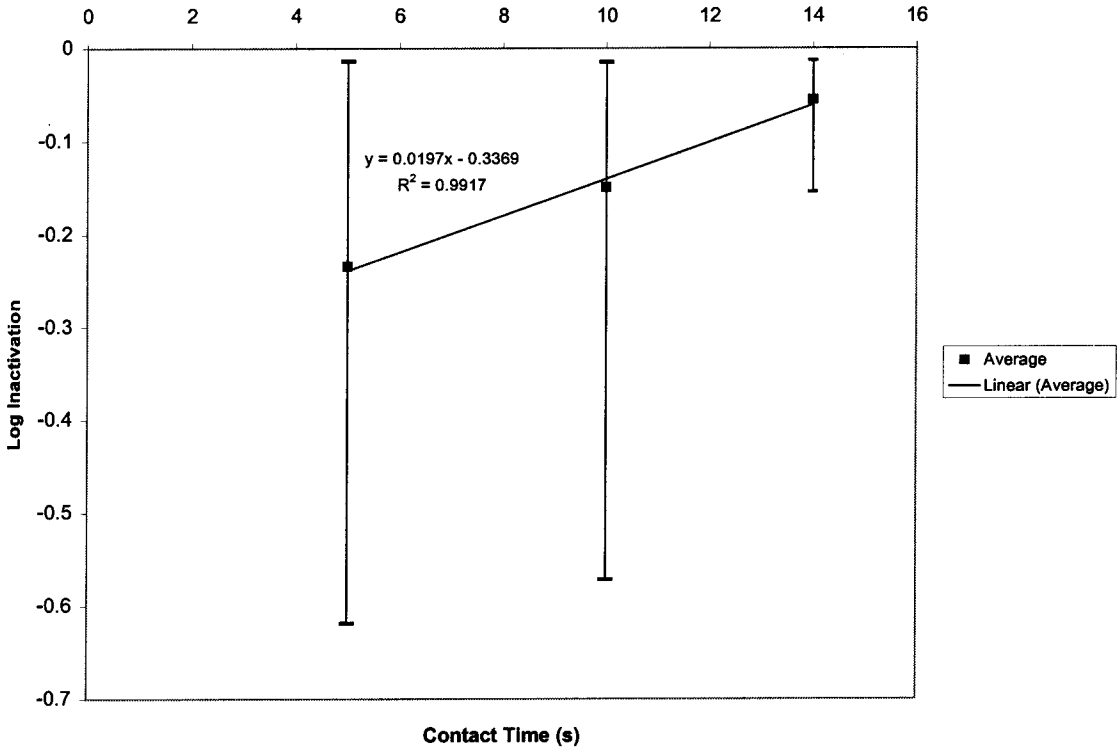

Figure 4. Natural logarithm of average removal fraction versus contact time

Table 3 shows bacterial removal fractions achieved by the UV disinfection unit.

The results from this set of samples showed some interesting results. It was observed that the highest removal efficiency at each contact time was about the same. The average values showed that, as expected, removal efficiency increased with increasing contact time (dose). Figure 4 shows a plot of the natural logarithm of the average fraction removed versus contact time. A linear regression yielded an R^2 value of 0.9917, indicating a fairly good linear fit. The linear nature of this data indicated that the UV disinfection process followed Chick's law of disinfection (Reynolds and Richards, 1996), given by the pseudo-first order reaction

$$-\frac{dN}{dt} = kN$$

where dN/dt = rate of cell destruction, number/time

k = rate constant

N = number of living cells remaining at time t

The linearized form of Chick's law is

$$\ln(N/N_0) = kt$$

where N_0 = the initial number of living cells

The linear regression gave a rate constant of 0.0197 s^{-1}. Based on the regression equation, during normal operation of the machine (no ports installed – contact time of 7 seconds), the UV unit should achieve an average removal efficiency of 81.95%. Removal efficiency in a UV disinfection unit is dependent on the applied dose, which is a function of the contact time. UV dose is normally given as lamp intensity ($\mu W/cm^2$) multiplied by contact time (s) (Chang et al., 1985). The UV lamp in this unit has an

intensity of 95 $\mu W/cm^2$. Thus, during normal operation, at a contact time of 7 seconds, the dose is given by

$$95\ \mu W/cm^2 \times 7\ s = 665\ \mu W \cdot s/cm^2$$

UV disinfection has been shown to be capable of achieving 99.99% removal of HPC bacteria at a dose of 35,000 $\mu W \cdot s/cm^2$ (Chang et al., 1985). Typical commercial UV disinfection units are designed with doses ranging from 25,000 to 35,000 $\mu W \cdot s/cm^2$ (Wolfe, 1990). The UV disinfection unit in this machine delivers a fairly low UV dose compared to commercial units, which would explain why the unit is not able to achieve as high a removal efficiency. Though the UV unit in this machine does not achieve complete removal, it demonstrates that the disinfection process has a positive effect.

Recommendations

Bacterial re-growth in the tubing downstream of the UV disinfection unit is the main problem that needs to be resolved. Increasing the contact time or lamp intensity (thus increasing the dose) in the UV unit would reduce the average HPC levels in the vended water, but would likely not prevent re-growth in the tubing. The California Department of Health Services suggested locating the UV unit closer to the vend nozzle. This would reduce the length of tubing, thereby reducing the surface area available for bacterial growth to occur. However, since the UV lamp heats the tubing near the UV unit to a temperature that is very conductive to bacterial growth, cooling the tubing could be a better solution. Thermoelectric cooling of the tubing is one possibility. This would be very effective (cool temperatures can inhibit bacterial growth) but electricity would have

to be supplied continuously, which could make the costs prohibitive. Another solution would be to periodically flush the tubing with UV disinfected water. A timer could be used to vend water every once and awhile to clear the tubing of bacteria in the standing water. The water could either be wasted or redirected into the storage tank. This would be a fairly simple solution to implement, but could potentially interfere with normal service. Whatever method is used, the results of this study suggest that preventing bacterial re-growth in the tubing would do much to lower the HPC level and thus improve the overall microbiological quality of the vended water.

CONCLUSIONS

Based on the results of this study it can be concluded that:

- Bacterial re-growth in the tubing downstream of the UV disinfection unit negatively impacts the microbiological quality of the vended water
- Elevated temperatures in the tubing near the UV unit provide an ideal environment for bacterial re-growth to occur
- Reducing the temperature of the tubing or periodically flushing the tubing would help to prevent re-growth from occurring
- Prevention of re-growth in the tubing would greatly improve the microbiological quality of the water dispensed by the water vending machine

APPENDIX A
Raw HPC Data

Table A1: Vended water samples (first gallon)

Date	Sample	HPC (CFU/ml)
1/21/99	012199-000B1	1800
1/21/99		2000
1/21/99	012199-000B2	2345
1/21/99		1880
1/21/99	012199-000B3	1780
1/21/99		2440
1/26/99	012699-000B1	1040
1/26/99		<1000
1/26/99	012699-000B2	1080
1/26/99		1000
1/26/99	012699-000B3	1240
1/26/99		2000
5/20/99	052099-000B1	10000
5/20/99		8200
5/20/99		13000
5/20/99	052099-000B2	9900
5/20/99		10750
5/20/99		17000
5/20/99	052099-000B3	11500
5/20/99		10050
5/20/99		14500

Table A2: Vended water samples (third gallon)

Date	Sample	HPC (CFU/ml)
1/21/99	012199-002B1	30
1/21/99		20
1/21/99	012199-002B2	10
1/21/99		20
1/21/99	012199-002B3	45
1/21/99		20
1/26/99	012699-002B1	5
1/26/99		<10
1/26/99	012699-002B2	10
1/26/99		<10
1/26/99	012699-002B3	15
1/26/99		10
5/20/99	052099-002B1	20
5/20/99		20
5/20/99	052099-002B2	20
5/20/99		30
5/20/99	052099-002B3	30
5/20/99		20

Table A3: Storage tank and influent water samples

Date	Sample	HPC (CFU/ml)
2/2/99	020299-T	555
2/2/99	(Storage Tank)	880
2/4/99	020499-T	TNTC
2/4/99	(Storage Tank)	680
2/2/99	020299-I	<5
2/2/99	(Influent)	<5
2/4/99	020499-I	<5
2/4/99	(Influent)	<5

Table A4: UV tubing standing water samples

Date	Sample	HPC (CFU/ml)
5/12/99	051299-UV1	6700
5/12/99	(Pre-UV)	9350
5/12/99		11000
5/12/99		20000
5/12/99	051299-UV2	7000
5/12/99	(Pre-UV)	11350
5/12/99		15400
5/12/99		16000
5/18/99	051899-UV1	7500
5/18/99	(Post-UV)	7350
5/18/99		27500
5/18/99		24000
5/18/99	051899-UV2	8150
5/18/99	(Post-UV)	11000
5/18/99		29500
5/18/99		35000

Table A5: UV disinfection efficiency (contact time = 5 s)

Date	Sample	HPC (CFU/ml)
4/16/99	041699-T1	400
4/16/99	(In)	580
4/16/99		<1000
4/16/99	041699-T2	530
4/16/99	(In)	580
4/16/99		<1000
4/16/99	041699-UV1	110
4/16/99	(Out)	140
4/23/99	042399-T1	1050
4/23/99	(In)	900
4/23/99		<1000
4/23/99	042399-T2	620
4/23/99	(In)	520
4/23/99		1000
4/23/99	042399-UV1	150
4/23/99	(Out)	240
4/23/99	042399-UV2	135
4/23/99	(Out)	100
4/28/99	042899-T1	170
4/28/99	(In)	<1000
4/28/99	042899-T2	160
4/28/99	(In)	170
4/28/99		<1000
4/28/99	042899-UV1	<5
4/28/99	(Out)	30
4/28/99	042899-UV2	15
4/28/99	(Out)	20

Table A6: UV disinfection efficiency (contact time = 10 s)

Date	Sample	HPC (CFU/ml)
3/4/99	030499-UV1	500
3/4/99	(In)	750
3/4/99		1000
3/4/99	030499-UV3	675
3/4/99	(In)	970
3/4/99		2000
3/4/99	030499-UV2	30
3/4/99	(Out)	50
3/4/99	030499-UV4	60
3/4/99	(Out)	150
3/11/99	031199-UV1	405
3/11/99	(In)	500
3/11/99		1000
3/11/99	031199-UV3	325
3/11/99	(In)	380
3/11/99		<1000
3/11/99	031199-UV2	50
3/11/99	(Out)	40
3/11/99	031199-UV4	95
3/11/99	(Out)	100
3/16/99	031699-UV1	310
3/16/99	(In)	450
3/16/99		2000
3/16/99	031699-UV3	670
3/16/99	(In)	600
3/16/99		2000
3/16/99	031699-UV2	65
3/16/99	(Out)	70
3/16/99	031699-UV4	135
3/16/99	(Out)	110

Table A7: UV disinfection efficiency (contact time = 14 s)

Date	Sample	HPC (CFU/ml)
3/23/99	032399-T1	550
3/23/99	(In)	540
3/23/99		<1000
3/23/99	032399-T1	350
3/23/99	(In)	660
3/23/99		1000
3/23/99	032399-UV1	25
3/23/99	(Out)	30
3/23/99	032399-UV2	25
3/23/99	(Out)	50
3/24/99	032499-T1	420
3/24/99	(In)	640
3/24/99		<1000
3/24/99	032499-T1	400
3/24/99	(In)	410
3/24/99		<1000
3/24/99	032499-UV1	30
3/24/99	(Out)	<10
3/24/99	032499-UV2	30
3/24/99	(Out)	40
3/30/99	033099-T1	790
3/30/99	(In)	1500
3/30/99		1000
3/30/99	033099-T1	1100
3/30/99	(In)	1120
3/30/99		1000
3/30/99	033099-UV1	20
3/30/99	(Out)	50
3/30/99	033099-UV2	30
3/30/99	(Out)	70

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