MICROBIOLOGICAL CAUSES OF TASTES AND ODORS IN LARAMIE'S DRINKING WATER

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Abstract

Blooms of algae tentatively identified as *Asterionella* and *Anabaena* occurred at times when tastes and odors developed in the surface water source of Laramie's drinking water during the summer of 1984. "Actinomycetes were found in Laramie's surface water source but did not appear to be numerous enough to be responsible for the tastes. Filamentous algae did not become numerous enough to present a problem at the wastewater treatment facility during the summer of 1984. Selected bacteria were enumerated through the surface water treatment plant during the summer of 1984. This plant reduced numbers of bacteria from raw to finished water by 99.99 percent. Fecal coliforms survived for three months in material taken from the sedimentation basins. In some cases R2A medium was the superior medium for determing the heterotrophic plant count.

Introduction

This project was developed from an inquiry to the PI from the City of Laramie Water Department asking for help concerning the identification of the cause of a taste and odor problem that recently seemed to be occurring each summer. Secondly, there was the potential for a problem of clogging the infiltration-percolation beds of the wastewater treatment facility by filamentous algae and help was requested with this problem.

The taste and odor problem could have been due to either algal growth or to the growth of actinomycetes. Actinomycetes are bacteria which may produce two chemical compounds which have been implicated in taste and odor problems of water. These are geosmin and 2 methyl-isoborneol. These two taste compounds are also produced by various algae. It was possible that the actinomycetes were growing in the treatment plant.

This project was undertaken primarily to determine what caused the tastes and odors and secondarily, to gain some information concerning the bacteriology of Laramie's drinking water.

Requirements of the Project (Objectives)

1. To determine the cause(s) of the tastes and odors in Laramie's surface water supply.

2. To determine if filamentous algae were growing in Laramie's sewage lagoons.

3. To enumerate actinomycetes through the various stages of the treatment plant.

4. To enumerate sulfate reducing bacteria through the various stages of the treatment plant.

5. To perform other preliminary bacteriological studies on Laramie's drinking water as time might allow.

Methodology

Studies concerned with algal growth were done by filtering various amounts of water through a Millipore HA gridded filter with a pore size of 0.45 micrometers. The filters were placed upon a glass slide and observed at 100X magnification. The number of algal cells per square of the grid was determined. In some cases total cells were counted while in other cases only the predominant organism was counted. Studies with the lagoons were only concerned with the enumeration of filamentous algae. At the water plant 100 mls of raw water were routinely filtered. Occasionally samples of Lake Sodergren were filtered. Five ml samples from the lagoons were filtered. Identification of algae was done using the 15th edition of Standard Methods for the Examination of Water and Wastewater.

Methodologies used for studying the bacteriology of Laramie's drinking water are detailed in the attached thesis.

Results

Presented in Table 1 are the results of approximately weekly samples taken to determine the presence of algae in the raw water. Very few algae were seen until the end of July. At the time of taste development the total algae were ignored and the predominant type counted. The first taste problem, which lasted for a short time, corresponded to an increase in the number of *Asterionella* up to eight colonies per square of filter. This organism is listed in Standard Methods as a taste causing organism. A slight taste then persisted through the bloom of an organism tentatively identified as *Ulothrix*. The real taste problem started with the appearance of an organism tentatively identified as *Anabaena*. The taste was present when as few as two filaments per square of filter were seen and got worse as the filaments increased in size and number. It was finally decided by the city to shut the plant down in early September. The numbers of this organism in the raw water reached 40-50 filaments per square of filter while they were as high as 80 per square in the surface water of Lake Sodergren.

Filamentous algae did not grow in the sewage lagoons to any extent during the summer of 1984, thus no problem was encountered and no data is available. The predominant algae in the sewage lagoons were identified as *Chlorella* and *Scenedesmus* with *Chlorella* being dominant in May and June while *Scenedesmus* was most numerous in July and August.

For the results of the bacteriological studies see the attached thesis.

Table 1. Algae in Laramie's Raw Water During the Summer of 1984

	Unidentified		Identified Organisms				
Date	Green or blue green algae	Diatoms	Asterionella	Ulothrix	Pandorina	Anabaena	Taste
5-22	1*	a+	a	a	a	a	No
6-6	a	a	a	a	a	a	No
6-13	a	a	a	a	a	a	No
6-20	2	a	a	a	a	a	No
6-26	a	a	a	a	a	a	No
7-5	a	a	a	a	a	a	No
7-12	1	a	a	a	a	a	No
7-19	2	a	a	a	a	a	No
7-25	-≠	-	8	a	1-2	a	Yes
7-26	-	-	1.4	a	1.6	a	Yes
8-6	many	-	a	80	a	a	Slight
8-21	-	-	a	10	1	2	Slight
8-22	-	-	a	10	1	2	Slight
8-23	-	-	a	10	1	4	Yes
8-24	-	-	a	-	a	4.4	Yes
8-25	-	-	a	Anabaena filaments were longer than previous days		4	Yes
8-30	-	-	a	-	-	35	Yes
9-5	Plant shut down very bad taste				-	47	Yes
9-11	-	-	-	-	-	40-50	Yes
9-13	-	-	-	-	-	40-50	Yes

* - number per square of gridded membrane filter
\+ - less than one per square of gridded membrane filter
≠ - not determined

Conclusions

1. Blooms of *Asterionella* and *Anabaena* caused tastes and odors in Laramie's surface water supply during the summer of 1984.

2. Actinomycetes were present in the raw water but were not responsible for the taste and odors.

3. Sulfate reducing bacteria may grow in the sedimentation basin.

4. R2A medium is a good medium to use to enumerate bacteria which may have been stressed by aluminum.

5. *Escherichia coli* can survive for extended time in the material in the bottom of the sediment basin.

Recommendations

1. The surface water source should be studied during the summer of 1985 to see if these organisms grow and cause a taste and odor again.

2. A method for early detection of the algal growth should be developed.

3. A study should be done to determine why the algae grow.

APPENDIX

MICROBIOLOGICAL STUDIES OF LARAMIE'S DRINKING WATER

M.S. Thesis

William M. Kiessling

December, 1985

Kiessling, William M., Microbiological Studies of Laramie's Drinking Water, M.S., Department of Microbiology and Biochemisty, December, 1985.

Selected bacterial populations were enumerated from raw and finished drinking water in Laramie, Wyoming. In certain cases a higher percentage of the total heterotrophic bacteria were recovered on R2A than on modified Henrici agar. The number of actinomycetes could not explain the appearance of tastes and odors. Sulfate reducing bacteria were enumerated. The predominant organisms varied with the site sampled.

MICROBIOLOGICAL STUDIES OF LARAMIE'S DRINKING WATER

by

William M. Kiessling

A Thesis

Submitted to the

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in Partial Fulfillment of the Requirements

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To my parents, William and Helen Kiessling, whose encouragement was also present, this thesis is dedicated.

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INTRODUCTION

Domestic drinking water, whether supplied from ground water sources such as springs and wells, or surface water sources from lakes and rivers, is usually treated in some manner to remove or kill microorganisms. The presence of total coliforms, fecal coliforms and fecal streptococci in potable water has been used widely as an indication of the general water quality and the efficiency of treatment. The heterotrophic bacterial population in drinking water however, often exceeds that of the coliform group. Previous work has shown that enumeration of both heterotrophic bacteria and the coliform group is required to adequately evaluate the bacteriological quality of water (20).

Monitoring the heterotrophic population is necessary because high plate counts may indicate the presence of non-fecal opportunistic pathogens (20). Also, high numbers of heterotrophic bacteria in water have been shown to interfere with the detection of coliform bacteria (20,34) thus resulting in the potential for coliforms in contaminated water remaining undetected by routine analysis. Some heterotrophic bacterial strains have been associated with the production of tastes and odors in water (63,64,70), and the deterioration of distribution systems as the result of formation of encrustations or tubercles on the interior of pipelines (1,37,40). These deposits provided habitats which protect bacteria from residual chlorine (70), cause internal corrosion (10,53), and reduce the water capacity of pipelines (70). Sulfate reducing

bacteria have been shown to be of particular importance in corrosion problems (10,40,53).

This study was initiated to determine: 1) if actinomycetes at some point in the treatment process were responsible for a taste and odor problem that was occurring in Laramie's drinking water. 2) isolate and identify bacteria from the distribution system and determine if particular organisms originated in the different water sources and were found at specific points in the distribution system. 3) determine the numbers of sulfate reducing and other types of bacteria at various points in the surface water treatment plant.

LITERATURE REVIEW

Interest in the quality of domestic drinking water supplies has grown in recent years because of the increased demands for water, the enactment of the Safe Drinking Water Act of 1974 and the waterborne epidemics which have occurred in the United States in past years. The goal of the Safe Drinking Water Act of 1974 was to enhance the quality of drinking water in the United States by imposing stricter water quality regulations (39).

In the period from 1971-1978, 224 waterborne outbreaks were reported in the United States (13,24). Contamination of the distribution system and treatment deficiencies were reported as the major causes of the outbreaks from municipalities (13). More outbreaks were reported from nonmunicipal sources than were reported from municipalities. However, most of the reported causes of illness were from municipal systems (14). A higher number of outbreaks occurred from municipal systems with ground water sources than from systems with surface water sources (14). McCabe et al. (47) reported numerous instances of poor quality water originating from ground water sources. The majority of these sources were unprotected or inadequately treated.

Locating a domestic source of water as free from contamination as possible is desirable, but people do not always settle in areas where water of this quality is available. Therefore, water must be treated to make it safe for human consumption. The basic treatment of water

involves the removal of organisms by filtration or by killing them with chlorine (18). Gainey and Lord (18) stated that the important treatment procedures were storage or sedimentation of water without coagulation, coagulation followed by sedimentation and rapid sand filtration, and chemical disinfection by chlorination. They reported that coagulation and sedimentation removed 50 to 99 percent of the bacteria in the raw water. The average removal was around 80 percent. For a coagulant to perform adequately it must form a water insoluble floc which traps bacteria and removes them from the water either by sedimentation or by filtration (18). Aluminum sulfate (alum) has been used widely for this purpose (18). The survival and possible release of organisms, including pathogens, from the floc is of recent public health concern (9).

Bulson et al. (9) studied the removal of bacteria from a lake treated with alum. Application of alum to Liberty Lake in Washington effectively removed 95 percent of the total culturable bacterial population. Ninety percent of the fecal coliforms and 70 percent of the fecal streptococci were removed from the water column and concentrated in the alum floc at the bottom of the lake. Laboratory experiments in this study showed that the concentration of *Escherichia coli* was reduced in the water column from 1.5×10^8 cells per 100 ml to 1.5×10^3 cells per 100 ml at 30 h after flocculation. *E. coli* cells were not released from the flocculated material to the water column and die-off was reported at a greater rate than was observed for unflocculated cells. The release of bacteria from the floc by use of a blender has been reported (48). Blending times from 2 to 18 seconds

resulted in no significant cell injury, however, blending exceeding 18 seconds was found to cause cell damage (9).

Rapid sand filtration has been shown to remove 60 to 90 percent of the bacteria applied to the filter with an average around 80 percent (18). Gainey and Lord (18) reported that some bacteria may pass through the filter and cautioned that all water processed by rapid sand filtration must be chlorinated to protect the consumer.

Chlorine is an effective disinfectant in water, however, the specific action of chlorine on bacteria remains unclear (18). Green and Stumf (23) reported that chlorine inhibits glucose oxidation by the cell. Knox et al. (32) indicated that chlorine affected the sulfhydryl groups of enzymes responsible for carbohydrate metabolism. Venkobachar et al. (72) examined the effects of chlorine on dehydrogenases and Benarde et al. (5) suggested that protein synthesis was the target upon which chlorine acted. Camper and McFeters (12) reported that chlorine affects the transport of extracellular nutrients across the cell membrane.

The ability of bacteria to repair chlorine injury has been debated. Some authors have indicated that the viable cells enumerated after chlorination resulted from the proliferation of the surviving population (19,28) while others have reported that actual repair of chlorine injured cells had occurred (12,46,50). The detection of these injured cells in water is important for an accurate assessment of water quality (46) because some might be pathogens which could recover in the human body.

There is much literature on detection and enumeration procedures for various types of bacteria in water and the efficiency of these methods (6,7,16,17,22,27,29,30,41,43,48,49,52,55,56,62,67,68). Reliable detection methods are necessary because high numbers of coliform bacteria can indicate the presence of pathogens (34). High non-coliform counts in water supplies have been shown to suppress the detection of coliform bacteria (20,34). Geldreich et al. (2) reported that the frequency of total and fecal coliform detection decreased as the heterotrophic plate count bacteria exceeded 1,000 per ml. Lamka et al. (34) reported similar results when heterotrophic plate counts increased above 500 per ml. Medium composition, incubation temperature and incubation time have a major influence on how successfully the bacterial population was enumerated from nonchlorinated and chlorinated water (17,48).

The standard plate count has been the method generally used to enumerate bacteria from water (56). The standard plate count procedure employs the pour plate technique using either plate count agar or tryptone glucose yeast extract agar incubated at 30°C for 48 hours (2). Many investigators have reported that the pour plate procedure yields lower counts than the streak or spread plate method (31,49,55,71). Means et al. (49) studied the recovery of bacteria obtained from unchlorinated water supplies to the city of Garden Grove and chlorinated water obtained from Irvine, California. Results from this study showed that the standard plate count procedure underestimated the heterotrophic bacterial population by 53 percent in the unchlorinated water system and 40 percent in the chlorinated system when compared to the spread plate

counts determined on R2A medium. Reasoner and Geldreich (56) have shown that the R2A medium was superior to plate count agar in enumerating heterotrophic bacteria from treated water. Modified Henrici agar and R2A agar contain diverse carbon and energy sources which may explain the increased recovery of bacteria as compared to standard plate count agar (56). R2A agar was the medium of choice to enhance the pigment production of heterotrophic bacteria (49,56). Reasoner and Geldrich (56) found that an incubation time of 7 days at 35°C resulted in R2A spread plate counts five-fold greater than counts obtained with plate count agar plates from finished water. Optimum counts were obtained when plates were incubated at 20°C for 7 days.

Studies have shown that slow-growing pigmented, water bacteria, which include species of Cytophaga, Aeromonas, Pseudomonas, and Flavobacterium, often survive chlorination and can be found in the distribution system (21,25). Herman (25) found that 78 percent of 150 drinking fountains studied had been contaminated with slow-growing pigmented water bacteria. Sink faucets, water baths and humidifying units were also found to be contaminated by these organisms. Matsen (44) reported that this group of organisms, with the exception of Pseudomonas aeruginosa, is seldom considered to be major pathogens and are usually only associated with hospital acquired infections. Healthy individuals did not appear to suffer from low-level contaminated water (21), however, the hazard to debilitated or compromised individuals is one which should not be ignored (25).

A major group of organisms found in fresh water is the fluorescent bacteria (26). Skinner et al. (65) have shown that stream systems

within a high mountain watershed in Wyoming contain around 1.0 x 10^4 fluorescent bacteria per 100 ml. One member of this group, P. aeruginosa, was a potential pathogen (15). Drake (15) has shown that the growth of P. aeruginosa is possible over a temperature range of 30°C and that the organism can tolerate wide pH values. The presence of this organism may also be evidence of recent fecal contamination (15,26).

The consumers basis for evaluating water quality includes clarity and the absence of any tastes and odors (42). Drinking water supplies may periodically develop taste and odor problems from various types of organisms (64). One group of microorganisms, the actinomycetes, have been linked to taste and odor problems in drinking water (51,63). Morris et al. (51) reported that the musty tastes and odors from Cedar River, Iowa, were caused by certain metabolites of actinomycetes. Romano and Safferman (58) found that soil forms were capable of producing musty tastes and odors in surface waters, thus indicating the potential for contamination caused by surface runoff. Silvey (64) made the observation that taste and odor problems in drinking water were widespread across the United States and suggested that problems due to actinomycetes could occur in any area of the nation when the average temperature is above 20°C for 3 months.

Microorganisms have not only been found to cause taste and odor problems but also have become linked with the deterioration of distribution pipelines (1,10,37,40,53,70). The deterioration was in the form of encrustations or tubercles on the interior of water mains. Diatoms, algae, actinomycetes and sulfate reducing bacteria have been

shown associated with these deposits (1,10,70). Tuovinen et al. (70) examined deposits from the Columbus, Ohio, distribution system and found that the interior of the tubercle provided a suitable habitat for anaerobic sulfate reducing bacteria. Butlin et al. (10) isolated approximately 1,000 sulfate reducing bacteria per gram from the interior of tubercles. Microorganisms within the tubercle were protected from the action of chlorine (70). Tuovinen et al. (70) reported that tubercles can become dislodged from the pipeline during periods of high flow. This indicated the possibility for very high heterotrophic plate counts from water within the pipe system when organisms were released in this manner (53). O'Connor et al. (53) found that the growth of sulfate reducing bacteria could produce areas of low pH at the pipe surface. The sloughing of groups of bacteria could allow oxygen to reach a portion of the interior pipe surface forming an electrode system which accelerates the corrosion process.

The use of granular activated carbon (GAC) filtration has been shown to reduce the level of organic substances responsible for tastes and odors in water (4,11,45,59). Lin (42) reported that the use of chemical oxidation or aeration was usually included with activated carbon treatment. Problems may develop, however, when carbon particles passed through the filters (57) or when bacteria were sloughed from the filter due to hydraulic forces (69). Bacteria could readily colonize carbon filters (8,35,73) and become highly resistant to chlorination (35). LeChevallier et al. (35) studied carbon particles by scanning electron microscopy and found that bacteria grew in cracks and crevices and became covered with a slime layer. An attachment period of 20

minutes was sufficient to protect Escherichia coli cells from the effects of chlorine. The short attachment period did not allow for extracellular polymer production therefore indicating that attachment alone was adequate in protecting cells from the affects of chlorine.

Coliforms may be detected by either the most probable number (MPN) procedure or the membrane filter method (2). Many investigators have reported better coliform recovery with the MPN technique than the membrane filter method (6,7,32,46,60,62). These observations can be explained by the nonselective environment the MPN procedure provides allowing stressed or injured cells to repair themselves before coming in contact with selective media (6,7,67). As an example, Shipe and Cameron (62) compared the efficiency of the membrane filter and MPN techniques from three sources of water. Water from the Watauga River in Tennessee, Nashville tap water and double distilled water were sterilized and seeded with two concentrations of Escherichia coli. The high concentration was approximately 6-10 x 10^5 cells per ml and the low concentration was approximately 6-10 x 10^1 cells per ml. The results showed a MPN recovery of 108.9 per cent from the 3 water sources while the membrane filter method yielded recoveries of 47.6 percent. The average recoveries of both methods from the river water, tap water and distilled water were 47.8, 91.0 and 86.1 percent, respectively. The tap water and distilled water percentages indicated that either procedure produced adequate recovery. This lends support to the concept that the particular water sampled affects the efficiency of specific coliform enumeration procedures (16).

MATERIALS AND METHODS

Types of Bacteria Enumerated

Heterotrophic plate count bacteria were enumerated on Henrici medium as modified by Stark and McCoy (66) and/or R2A agar (56) using the spread plate technique or the membrane filter method (2), depending upon the expected concentration of organisms. Actinomycetes were detected using Starch Casein agar and the procedure outlined in Standard Methods (2). Sulfate reducing bacteria were counted by the multiple tube procedure of Mara and Williams (43), using their modification of Iron Sulfite agar (Oxoid). All of the above media were incubated at 20°C for 7 days except sulfate reducing bacteria which were incubated at 20°C for 30 days.

Clostridium perfringens were detected on Perfringens/O.P.S.P. medium (Oxoid) following the procedure outlined in the Oxoid Manual (54). Plates were incubated in an anaerobic jar for 24 h at 37°C. Semi-anaerobic conditions were obtained by the use of a GasPak (BBL Microbiology Systems, Cockeysville, Md.).

Fluorescent colonies were identified on Starch Casein agar plates by exposing plates to long-wave ultraviolet light.

Total coliform populations were determined by the 5-tube most probable number (MPN) technique using Lauryl Tryptose broth (Difco Laboratories, Detroit, Mi.) in the presumptive test and Brilliant Green Bile broth (Difco) for confirmation (2).

Fecal coliforms and fecal streptococci were enumerated on Millipore HC membrane filters following the procedure outlined in Standard Methods (2). The media used were MFC (BBL) and KF (BBL), respectively. Fecal coliforms were also detected by transferring positive lauryl tryptose broth tubes to EC broth (BBL).

Water Distribution System

Drinking water to Laramie, Wyoming is supplied by ground water and surface water sources. The ground water sources originate from one spring and two well fields. The Laramie River serves as the surface water source and is stored in a lake until use. A surface water treatment plant processes water by flocculation/coagulation, sedimentation, filtration, fluoridation, and chlorination. Ground water receives fluoridation and chlorination only. Surface water is treated 20 miles west of Laramie and transported to town via gravity flow through a 20 and 24 inch main to the distribution system and storage reservoir. Mixing of the three water sources is achieved in the distribution system. One ground water source is treated within fifty yards of the reservoir. The second ground water source is treated three miles south of Laramie and can be sampled approximately 100 feet from the point of treatment. Finished surface water can be sampled directly at the treatment plant. The town's distribution system is supplied primarily from the storage reservoir or the transmission lines.

Water Plant Study

Water samples from four points within the surface water treatment plant were collected at weekly intervals from 14 May through 11 September, 1984. The sampling sites included the following: raw water

entering the plant, the effluent from the sedimentation basin, filtered water and finished water.

Samples were collected aseptically in one-liter, sterile polypropylene bottles. Finished water samples were dechlorinated at the time of collection with 100 mg/L sodium thiosulfate. Samples were packed in ice, transported to the laboratory, and processed within 2 to 4 h of collection.

Organisms enumerated in this study included heterotrophic plate count bacteria, actinomycetes and sulfate reducing bacteria. Samples of raw water and the effluent from the sedimentation basin were tested at certain times for the presence of *Clostridium perfringens* to determine if this organism was a significant component of the sulfate reducing bacterial population.

Filter Study

The filters at the surface water treatment plant were studied to determine the efficiency of filtration, and to see if any differences existed between filters.

Six filters, at various hours after backwash, were sampled at daily intervals from July 17 to 19. Two filters were sampled a second time at 2, 26, 50 and 74 hours after backwash from August 22-25, 1984. Samples were handled as previously described. Heterotrophic plate count and sulfate reducing bacteria were enumerated in this study.

Sedimentation Basin Study

The sedimentation basin was divided into two parallel chambers, each of which was 100 feet long. A walkway extended across the basin, allowing access to the chambers. Ten sites, 10 feet apart, on

alternating sides of the walkway, were marked for sampling, with site one being the closest to and site 10 being the most distant from the mixing basin.

Samples of the sedimented material were obtained with the aid of a sampling device which consisted of electrical conduit attached end-to-end with compression couplings. A casting reel, filled with braided nylon line, was attached to one end of the device. Fishing rod guides of decreasing size were attached in equal intervals from the reel to about one foot from the tip. The line was threaded through the guides and attached to a number 10 rubber stopper to seal a 500 ml polypropylene bottle. A sterile bottle was attached to the tip section with tape, the cap removed, and the stopper inserted. The bottle was lowered into the basin until it disappeared into the sediment. The line was then pulled to remove the stopper, thus filling the bottle with the desired sample. The bottle was retrieved and cap returned. Samples were packed in ice, transported to the laboratory, and processed within 4 to 7 h of collection.

The sedimentation basin, which had not been cleaned since May, 1984, was sampled a total of 3 times from mid-August through mid-September, 1984. The final sampling was taken after a trial addition of powdered activated carbon (PAC), used in an attempt to remove a taste and odor from the water.

The organisms enumerated in this study included heterotrohic plate count bacteria, actinomycetes, sulfate reducing bacteria, fluorescent bacteria, total coliforms, fecal coliforms and fecal streptococci.

Survival of Escherichia coli in Sediment Samples

A fecal coliform, identified by means of an Enterotube II (Roche Diagnostics, Division of Hoffman-LaRoche, Inc., Nutley, NJ) as *Escherichia coli,* isolated from the Laramie Lagoon system, was used for this and other parts of the study.

The survival of *E. coli* was studied in two sets of 4 sediment samples from the area of maximum deposition in the sedimentation basin. The second set of samples had received powdered activated carbon (PAC) in early September, 1984, in an endeavor to remove the aforementioned taste and odor.

Four sterile 8 oz prescription bottles and four Waring blender jars were used in this study. Each bottle was filled with a 100 ml aliquot of an unsterilized sample. The amount of sample was increased to 200 ml in blender jars, in order to cover the mixing blades with fluid. *E. coli* was grown in Nutrient Broth (Difco) at 37°C for 12 h. The bottles were inoculated with approximately 4.0×10^3 cells per ml whereas the blender jars received approximately 8.0×10^3 cells per ml. The containers were incubated at 15°C under stationary conditions and sampled at various intervals for approximately 3 mo. The prescription bottles were shaken 25 times and Waring jars blended 5 s on low speed prior to each plating.

The numbers of viable *E. coli* cells were determined by the membrane filtration procedure for fecal coliforms (2). Injured cells would not have been recovered by this methodology and hence would have been considered to be dead.

Polymer Effects on Escherichia coli

The Nalco 8102 polymer used in the coagulation process at the surface water treatment plant was studied to determine if it affected the growth of E. coli. The polymer was diluted to 0.22, 0.44, 0.66, 0.88 and 1.10 mg/L in Nephlo flasks (Bellco Glass, Inc., Vineland, Nj.) Each flask was inoculated with 0.1 ml of a 12-hour culture of E. coli grown in trypticase soy broth at 37°C. The flasks were incubated under stationary conditions. Growth was determined using a Klett-Summerson Photoelectric Colorimeter fitted with a red (number 66) filter.

Distribution System Study

Samples from 6 sites thought to be representative of the various parts of the distribution system were collected. These sites were believed to include water supplied mainly from the following: 1) ground water, 2) the surface water source and 3) that part of the system which received a mixture of surface and ground water. Sites 1 to 4 were private homes. Site 1 was located in the southern part of town, site 2 in the western, and sites 3 and 4 in the northern. Sites 5 and 6 were from Soldier Springs and Pope well field treatment facility. The sampling tap for this facility was located approximately 100 feet post-treatment. Site 5 was the designation used for those samples that were dechlorinated immediately while site 6 was the label for samples from the same tap that were dechlorinated 1 h after collection. A contact period of 1 h was thought to be a reasonable estimate of the elapsed time before the treated water from this source could be consumed.

Historically, sampling designs of distribution systems have been based upon samples from the line and not from samples obtained from the plumbing of the house (3). Sampling in this instance was designed to evaluate what microbes an individual might consume when obtaining a drink of water. Samples from the private homes were collected by individuals that worked in the Department of Microbiology and Veterinary Medicine at the University of Wyoming, Laramie, Wyoming. Instructions were to let the water run awhile before collecting the sample.

Heterotrophic bacterial plate counts and total coliforms were enumerated in this study.

Cup Sink Study

Five cup sinks in room 6035 of the Agriculture Building on the campus of the University of Wyoming, Laramie, Wyoming, were studied to determine the rate of repopulation by bacteria of a water line. The addition to the building opened in August, 1982, hence a relatively new water line was studied. The sinks had not been used for 22 days prior to this study. In an effort to obtain a sample from the water line as opposed to the spout, the first 100 ml of water from each tap was discarded. The next 500 ml of flow was collected for analysis, hereafter referred to as the before flush sample. After this collection each tap was allowed to flow at full force for 30 minutes and then sampled again. This will be referred to as the time zero sample. Flow through the line was allowed only when samples were obtained at 8, 24 and 48 hours post-flushing.

Chlorine residual of these samples were determined using a Hach DPD Chlorine Test Kit (Hach Co., Loveland, Co.). If a chlorine residual

was detected, the sample was dechlorinated with 100 mg/L of sterile sodium thiosulfate.

Identification of Organisms

Organisms were identified from the finished water at the surface water treatment plant, the distribution system part of this study and cup sink study. Colonies from Modified Henrici and R2A media were purified on R2A agar plates and pure cultures were maintained on R2A agar slants. All isolated cultures were placed into genera or groups following the procedure outlined by LeChevallier et al. (36). Pigmented bacterial isolates in this study which were otherwise identified as *Moraxella* or Group M Moraxella-like organisms were called pigmented *Moraxella* or pigmented Group M Moraxella-like organisms, respectively. No further identification of these isolates was done.

Statistical Analysis

Statistical analysis of the data was made at the University of Wyoming computer facility using the CYBER 835 computer. The statistical tests performed included: Analysis of Variance with completely randomized designs for 1 to 3 factors, Least-Squares Regression for data with unequal subclass numbers, and Duncan's New Multiple Range Test (33).

RESULTS

Surface Treatment Plant Study

The monthly averages of weekly samples taken at the surface water treatment plant are presented in Table 1. Total heterotrophic bacteria were enumerated using two methods; the spread plate procedure and the membrane filter technique using modified Henrici medium and/or R2A agar. When heterotrophic plate count bacteria were enumerated on Modified Henrici agar the number of viable cells in the raw water was significantly lower in August than any other month except June ($P < 0.05$). The highest number of cells leaving the sedimentation basin was seen in September ($P < 0.05$). The highest number of viable bacteria, in the finished water, 620 per 100 ml, was seen in June ($P < 0.05$). These differences were not observed when this group of bacteria was enumerated using R2A medium ($P < 0.05$). No significant differences in the number of actinomycetes on Starch Casein agar plates were seen between months within a sample site.

The numbers of sulfate reducing bacteria entering the plant each month were not significantly different; however, the highest number, 170 per 100 ml, passed the filter in June and the lowest number, 73 per 100 ml, were applied to the filter in August ($P < 0.05$). No sulfate reducing bacteria were detected in any sample after chlorination. To determine if these organisms were *Clostridium perfringens*, sixteen raw water and sedimentation basin effluent samples were plated on

Table 1. AVERAGE MONTHLY PLATE COUNTS OF SELECTED BACTERIAL POPULATIONS AT VARIOUS SITES IN THE SURFACE WATER TREATMENT PLANT

		Heterotrophic Plate Counts Per 100 ML														
		Modified Henrici Medium				R2A Medium				Actinomycetes Per 100ML				Sulfate Reducing Bacteria per 100ML		
Site	Month	Mean		Standard Deviation	Number of Samples	Mean		Standard Deviation	Number of Samples	Mean		Standard Deviation	Number of Samples	Mean		Standard Deviation	Number of Samples
	May	$1.4x10^6$	A##	$5.6x10^5$	3									$1.9x10^3$	A	$4.4x10^2$	3
	June	$6.7x10^5$	AB	$5.2x10^5$	4					$1.0x10^3$	A	$7.0x10^2$	4	$2.3x10^3$	A	$7.6x10^2$	4
RAW	July	$1.5x10^6$	A	$6.1x10^5$	4	$1.1x10^6$	A	0	1	$1.6x10^3$	A	$9.4x10^2$	4	$2.4x10^3$	A	$1.3x10^3$	4
WATER	Aug.	$4.5x10^5$	B	$2.5x10^5$	5	$7.4x10^5$	A	$3.8x10^5$	5	$9.2x10^2$	A	$1.8x10^2$	5	$2.3x10^3$	A	$3.0x10^3$	5
	Sept.	$1.6x10^6$	A	$9.7x10^5$	2	$5.7x10^5$	A	$2.0x10^5$	2	$8.5x10^2$	A	$2.1x10^2$	2	$7.8x10^2$	A	$5.9x10^2$	2
	May	$2.6x10^5$	B	$1.2x10^4$	3									$4.2x10^2$	A	$2.6x10^2$	3
EFFLUENT	June	$2.1x10^4$	C	$1.4x10^4$	4					$1.0x10^2$	A	0	1	$2.1x10^2$	AB	$8.3x10^1$	4
FROM SEDI	July	$2.9x10^5$	B	$2.5x10^5$	4	$3.1x10^5$	A	0	1	$1.5x10^2$	A	$1.7x10^2$	4	$3.5x10^2$	A	$2.5x10^2$	4
MENTATION	Aug.	$1.3x10^5$	BC	$6.2x10^4$	5	$1.8x10^5$	A	$7.7x10^4$	5	$6.0x10^1$	A	$5.5x10^1$	5	$7.3x10^1$	B	$5.4x10^1$	5
BASIN	Sept.	$6.1x10^5$	A	$1.3x10^5$	2	$3.8x10^5$	A	$2.8x10^5$	2	*	A	0	2	$1.5x10^2$	AB	$7.1x10^1$	2
	May	$4.8x10^4$	A	$2.5x10^4$	3									$1.1x10^1$	B	$1.9x10^1$	3
EFFLUENT	June	$3.1x10^4$	A	$3.4x10^4$	4					*	A	0	1	$1.7x10^2$	A	$1.5x10^2$	4
FROM	July	$9.6x10^4$	A	$1.6x10^5$	4	$1.5x10^5$	A	0	1	*	A	0	4	$6.7x10^1$	AB	$4.7x10^1$	4
FILTERS	Aug.	$2.7x10^5$	A	$2.7x10^5$	5	$1.1x10^5$	A	$7.4x10^4$	5	$2.0x10^1$	A	$4.5x10^1$	5	$6.6x10^0$	B	$1.5x10^1$	5
	Sept.	$2.4x10^5$	A	$3.0x10^5$	2	$1.1x10^5$	A	$2.5x10^4$	2	*	A	0	2	*	B	0	2
	May	$3.3x10^2$	AB	$5.8x10^2$	3									*	A	0	3
	June	$6.2x10^2$	A	$2.5x10^2$	4					*	A	0	1	*	A	0	4
FINISHED	July	$4.0x10^1$	B	$5.2x10^1$	4	$7.4x10^1$	A	$4.9x10^0$	1	$3.3x10^1$	A	$4.7x10^1$	4	*	A	0	4
WATER	Aug.	$6.1x10^0$	B	$4.0x10^0$	5	$6.7x10^0$	A	$5.9x10^0$	5	*	A	0	5	*	A	0	5
	Sept.	$1.6x10^1$	B	$7.8x10^0$	2	$3.2x10^0$	A	$3.5x10^{-1}$	2	*	A	0	2	*	A	0	2

Multiple tube determination
Identical letters in columns for each site are not significantly different at $P<0.05$
* None detected

Perfringens agar with only one colony found. Hence, it was concluded that C. perfringens did not make-up a very large part of the population of the sulfate reducing bacteria enumerated in this study.

When the plate counts on Modified Henrici agar were used to assess the efficiency of treatment it was found that coagulation and sedimentation removed an average of 76 percent of the incoming bacteria, filtration removed 47 percent of the organisms applied to the filters and chlorination killed 99.9 percent of the viable cells which passed the filters. Overall, 99.99 percent of the cells that entered the plant were either removed or killed. Furthermore, when analysis was made between sites, there were significant reductions in the number of actinomycetes and sulfate reducing bacteria due to coagulation and sedimentation ($P < 0.05$).

Filter Efficiency

The results of studying the efficiency of the filters are presented in Table 2. Filters 1 and 2 were not in operation and had been covered with finished water at the end of backwash. The effluent from both of these filters, had less heterotrophic bacteria than operating filters ($P < 0.05$). In the first study, (July 17 to 19), the number of heterotrophic bacteria passing the filter, as determined with R2A medium, was significantly higher as the run progressed while, in the second experiment, there were no significant differences in numbers at any time ($P<0.05$). In contrast, when the enumeration was made using Modified Henrici agar, in only one of four cases with active filters from the first study was there a significant increase in numbers of bacteria with time of use and, in the second experiment, the counts decreased with time of use ($P<0.05$).

Table 2. AVERAGE PLATE COUNTS OF SELECTED BACTERIAL POPULATIONS FROM FILTERS AT THE SURFACE WATER TREATMENT PLANT

		Heterotrophic Plate Counts Per 100 ML						Sulfate Reducing Bacteria per 100ML		
		Modified Henrici Medium			R2A Medium					
Filter	Hours of run after backwash	Mean	Standard Deviation	Number of Samples	Mean	Standard Deviation	Number of Samples	Mean	Standard Deviation	Number of Samples
					JULY 17-19, 1984					
	0	$5.0x10^0$ B#	$7.1x10^0$	2	$1.0x10^1$ B	0	2	* A	0	3
1	0	$5.0x10^0$ B	$7.1x10^0$	2	$2.0x10^1$ B	$1.4x10^1$	2	* A	0	3
	0	$1.0x10^2$ A	$2.1x10^1$	2	$1.0x10^2$ A	$7.1x10^0$	2	* A	0	3
	0	$8.0x10^1$ A	$1.1x10^2$	2	* A	0	2	* A	0	3
2	0	$5.0x10^0$ A	$7.1x10^0$	2	* A	0	2	* A	0	3
	0	$5.0x10^1$ A	$2.8x10^1$	2	$3.0x10^1$ B	$2.8x10^1$	2	* A	0	3
	23	$3.8x10^2$ A	$2.3x10^2$	2	$7.2x10^2$ B	$2.1x10^2$	2	$5.7x10^0$ A	$3.0x10^0$	3
3	47	$6.0x10^2$ A	$6.4x10^1$	2	$8.4x10^2$ B	$2.3x10^2$	2	$1.0x10^0$ B	$1.0x10^0$	3
	71	$9.8x10^2$ A	$3.1x10^2$	2	$1.7x10^3$ A	0	2	$2.3x10^0$ A	$1.5x10^0$	3
	23	$5.5x10^2$ A	$2.1x10^2$	2	$4.4x10^2$ B	$8.5x10^1$	2	$8.5x10^0$ A	$3.5x10^0$	3
4	47	$7.0x10^2$ A	$1.6x10^2$	2	$7.8x10^2$ B	$2.8x10^1$	2	$3.0x10^0$ AB	$1.0x10^0$	3
	71	$1.2x10^3$ A	$7.2x10^2$	2	$1.5x10^3$ A	$2.1x10^2$	2	$2.7x10^0$ B	$1.2x10^0$	3
	27	$4.3x10^2$ A	$9.9x10^1$	2	$5.8x10^2$ B	$2.8x10^1$	2	$6.7x10^{-1}$ A	$5.8x10^{-1}$	3
5	51	$1.2x10^2$ A	0	2	$7.3x10^2$ B	$5.6x10^1$	2	$1.0x10^0$ A	$1.7x10^0$	3
	75	$9.7x10^2$ A	$7.5x10^2$	2	$1.4x10^3$ A	$2.8x10^2$	2	$2.3x10^0$ A	$1.2x10^0$	3
	27	$3.0x10^2$ B	$2.8x10^1$	2	$5.7x10^2$ B	$1.4x10^1$	2	$2.7x10^0$ A	$5.8x10^{-1}$	3
6	51	$2.3x10^2$ B	$9.9x10^1$	2	$4.0x10^2$ B	$4.0x10^2$	2	$1.3x10^0$ A	$5.8x10^{-1}$	3
	75	$1.9x10^3$ A	$4.2x10^2$	2	$1.9x10^3$ A	$2.1x10^2$	2	$2.7x10^0$ A	$1.5x10^0$	3
					August 22-15, 1984					
	2	$7.4x10^3$ B	$3.0x10^3$	2	$3.4x10^3$ A	$5.0x10^2$	2	$6.7x10^{-1}$ A	$5.8x10^{-1}$	3
5	26	$1.7x10^4$ A	$7.1x10^2$	2	$8.3x10^3$ A	$9.7x10^3$	2	* A	0	3
	50	$1.8x10^3$ C	$6.4x10^2$	2	$1.8x10^3$ A	$3.5x10^2$	2	* A	0	3
	74	$2.5x10^3$ C	0	2	$1.5x10^3$ A	$1.1x10^3$	2	$3.3x10^{-1}$ A	$5.8x10^{-1}$	3
	2	$1.4x10^4$ B	$2.8x10^3$	2	$2.2x10^3$ A	$9.2x10^2$	2	$3.3x10^{-1}$ A	$5.8x10^{-1}$	3
6	26	$2.0x10^4$ A	$1.4x10^3$	2	$3.1x10^3$ A	$5.0x10^2$	2	* A	0	3
	50	$4.1x10^3$ C	$1.4x10^2$	2	$1.3x10^3$ A	$1.4x10^3$	2	* A	0	3
	74	$1.5x10^3$ C	$5.0x10^2$	2	$2.1x10^3$ A	$7.1x10^1$	2	* A	0	3

Multiple tube determination * None detected

Identical letters in columns for each filter are not significantly different at $p<0.05$

Sedimentation Basin Study

The averages of three samplings taken at the sedimentation basin are given in Table 3. The bulk of the bacteria appeared to settle within the first 30 linear feet of flow into the settling basins ($P<0.05$). The evidence for this is that the highest numbers for the heterotrophic plate count bacteria on Modified Henrici agar, the actinomycetes, the fluorescent bacteria, the pigmented bacteria, the fecal coliforms determined by the standard MF procedure and the fecal streptococci were found within this distance. In contrast, the heterotrophic plate count bacteria, as determined on R2A medium, and the total coliforms and fecal coliforms determined by MPN procedures, which can be considered resuscitation methods, showed no significant differences in number throughout the settling basin ($P<0.05$). Sulfate reducing bacteria appeared to settle equally throughout the sedimentation basin. This observation could also be explained if these organisms were actively growing in the sediment; however, no attempt was made to determine this.

Escherichia coli Survival Study

The survival of *E. coli* inoculated into sedimented material is presented in Fig. 1. The organism survived for a longer period of time in the material that did not have powdered activated carbon (PAC) than in the presence of PAC. The organism was also shown to survive longer in material which was not blended than in the sediments which were blended. Three percent of the cells remained viable in the unblended material after 120 d. of storage at 15°C. This could mean that bacterial cells, including those of pathogens, could survive for

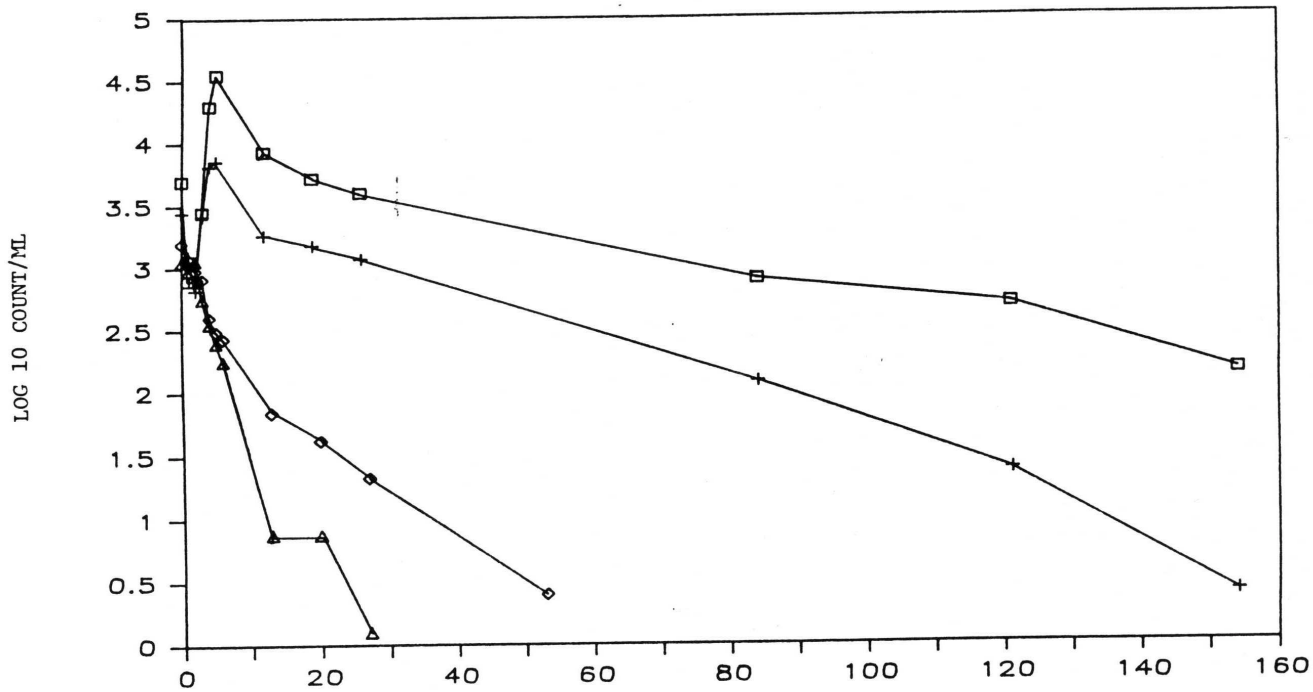

Fig. 1. Survival of Escherichia coli in flocculated material. Each datum point represents the average of duplicate platings of 4 samples. Symbols: □ bottled, + bottled with activated carbon, ◇ blended, Δ blended with activated carbon.

TABLE 3. AVERAGE PLATE COUNTS PER MILLILITER OF SELECTED BACTERIAL POPULATIONS IN SEDIMENT SAMPLES

Sedimentation Basin Sample Number	Heterotrophic Plate Counts: Modified Henrici Medium Mean	Modified Henrici Medium Standard Deviation	R2A Medium Mean	R2A Medium Standard Deviation	Actinomycetes Mean	Actinomycetes Standard Deviation	Sulfate Reducing Bacteria Mean	Sulfate Reducing Bacteria Standard Deviation
1$	$1.4x10^6$@B	$1.5x10^6$#	$2.5x10^6$A	$1.0x10^6$	$2.4x10^5$B	$2.2x10^5$	$3.2x10^4$B	$4.2x10^4$
2	$2.1x10^6$AB	$2.5x10^6$	$2.3x10^6$AB	$1.2x10^6$	$2.2x10^5$B	$2.1x10^5$	$6.8x10^4$A	$1.1x10^5$
3	$2.5x10^6$A	$2.7x10^6$	$1.4x10^6$B	$8.4x10^5$	$3.9x10^5$A	$5.2x10^5$	$2.8x10^4$B	$2.9x10^4$
4	$1.7x10^6$B	$1.0x10^6$	$2.3x10^6$AB	$2.0x10^6$	$1.7x10^5$C	$1.6x10^5$	$2.6x10^4$B	$2.2x10^4$
5	$1.5x10^6$B	$1.2x10^6$	$8.2x10^5$B	$3.9x10^5$	$3.3x10^4$E	$1.5x10^4$	$1.5x10^4$B	$1.9x10^4$
6	$1.5x10^6$B	$1.2x10^6$	$1.8x10^6$AB	$1.2x10^6$	$1.2x10^5$D	$1.6x10^5$	$3.7x10^4$AB	$6.1x10^4$
7	$3.6x10^6$C	$3.8x10^5$	$1.0x10^6$B	$6.9x10^5$	$7.0x10^4$DE	$4.4x10^4$	$1.4x10^4$B	$9.5x10^3$
8	$6.3x10^5$C	$7.4x10^5$	$1.3x10^6$B	$9.0x10^5$	$5.2x10^4$E	$5.9x10^4$	$1.3x10^4$B	$1.5x10^4$
9	$3.3x10^5$C	$9.6x10^4$	$1.4x10^6$B	$1.5x10^5$	$3.1x10^4$E	$2.6x10^4$	$9.3x10^3$B	$7.8x10^3$
10	$6.0x10^5$C	$3.7x10^5$	$1.6x10^6$B	$4.4x10^5$	$4.6x10^4$E	$4.7x10^4$	$6.4x10^3$B	$6.5x10^3$

	Fecal Coliforms Determined by Membrane Filtration Mean	Membrane Filtration Standard Deviation	Most Probable Number Mean	Most Probable Number Standard Deviation	Fecal Streptococci Mean	Fecal Streptococci Standard Deviation	Total Coliform Mean	Total Coliform Standard Deviation
1	$1.0x10^1$C	$1.7x10^1$	$3.2x10^1$A	$4.1x10^1$	$1.5x10^1$B	$1.0x10^1$	$6.3x10^1$A	$6.0x10^1$
2	$8.5x10^1$A	$1.4x10^2$	$3.4x10^2$A	$5.0x10^2$	$4.3x10^1$A	$6.6x10^1$	$4.0x10^2$A	$4.5x10^2$
3	$4.0x10^1$B	$5.4x10^1$	$8.2x10^1$A	$6.7x10^1$	$4.0x10^1$A	$6.1x10^1$	$7.8x10^1$A	$5.6x10^1$
4	$6.7x10^0$CD	$1.2x10^1$	$2.7x10^1$A	$2.3x10^1$	$6.7x10^0$B	$5.8x10^0$	$7.2x10^1$A	$8.7x10^1$
5	$3.3x10^0$CD	$5.8x10^0$	$2.6x10^1$A	$8.6x10^0$	$6.7x10^0$B	$2.9x10^0$	$1.5x10^2$A	$1.7x10^2$
6	*	0	$4.0x10^1$A	$4.6x10^1$	$8.3x10^0$B	$7.6x10^0$	$2.3x10^2$A	$2.4x10^2$
7	*	0	$5.9x10^0$A	$4.4x10^0$	*	0 B	$6.4x10^0$A	$4.1x10^0$
8	$5.0x10^0$CD	$5.0x10^0$	$1.2x10^1$A	$4.6x10^0$	*	0 B	$5.6x10^2$A	$9.0x10^2$
9	*	0	$2.8x10^0$A	$9.2x10^{-1}$	$8.3x10^0$B	$1.4x10^1$	$1.3x10^1$A	$7.8x10^0$
10	*	0	$9.6x10^0$A	$6.5x10^0$	$1.0x10^1$B	$1.3x10^1$	$1.5x10^1$A	$8.8x10^0$

	Pigmented Bacteria: Modified Henrici Medium Mean	Modified Henrici Medium Standard Deviation	R2A Medium Mean	R2A Medium Standard Deviation	Flourescent Bacteria Mean	Flourescent Bacteria Standard Deviation
1	$2.5x10^5$A	$3.1x10^5$	$1.9x10^5$A	$1.7x10^5$	$8.7x10^4$ABC	$4.9x10^4$
2	$1.7x10^5$B	$2.2x10^5$	$1.4x10^5$B	$2.0x10^5$	$1.3x10^5$A	$5.9x10^4$
3	$2.0x10^5$B	$2.6x10^5$	$1.2x10^5$B	$1.8x10^5$	$8.3x10^4$ABC	$9.4x10^4$
4	$1.2x10^5$C	$1.7x10^5$	$6.0x10^4$CD	$7.5x10^4$	$1.0x10^5$AB	$7.0x10^4$
5	$4.7x10^4$DE	$3.5x10^4$	$5.7x10^4$CD	$6.0x10^4$	$3.0x10^0$D	$5.2x10^3$
6	$8.7x10^4$CD	$9.5x10^4$	$8.0x10^4$C	$7.8x10^4$	$4.3x10^4$BCD	$2.9x10^4$
7	$7.7x10^3$E	$6.5x10^3$	$3.2x10^4$D	$2.8x10^4$	$2.9x10^4$C	$2.7x10^4$
8	$1.8x10^4$E	$1.9x10^4$	$4.3x10^4$D	$2.2x10^4$	$3.1x10^4$C	$2.5x10^4$
9	$6.0x10^3$E	$6.6x10^3$	$4.3x10^4$D	$3.0x10^4$	$1.5x10^4$D	$5.7x10^3$
10	$4.3x10^4$DE	$5.3x10^4$	$6.5x10^4$CD	$5.7x10^4$	$1.7x10^4$D	$5.8x10^3$

$ Closest to Mixing Basin * None detected @Values are the average of three samples

Identical letters in columns are not significantly different at $P < 0.05$

extended time in flocculated material and, as Bulson et al. (9) stated, the potential exists for disturbance and release of these organisms into the water column. Survival could also be affected by the type of sedimented material.

Polymer Effects on Escherichia Coli

The surface water treatment plant uses Nalco 8102 polymer to produce a water insoluble floc. The results of this study are shown in Fig. 2. In the laboratory, E. coli was grown in polymer concentrations that may reasonably be encountered within the treatment process. No observed effect was seen during the first 6 hours of growth between the control culture and those with polymer. Monitoring the growth of E. coli for up to 30 hours also yielded no observable effects.

Distribution System Study

Data from the distribution system study are given in Table 4. The heterotrophic plate counts in water from three homes were significantly higher than counts from water at the other home and the two differently dechlorinated ground water samples ($P<0.01$). Furthermore, two of the three homes had significantly higher numbers of bacteria in the drinking water during the fall of the year than in December ($P<0.01$). The heterotrophic plate count at one home was significantly higher from R2A medium than when Modified Henrici agar was used ($P<0.01$). Counts from the remaining samples on R2A medium, although not statistically significant, were equal to or higher than those on Modified Henrici agar. There was no apparent difference in numbers of bacteria in any particular part of town. Total coliforms were not detected from any sampling site.

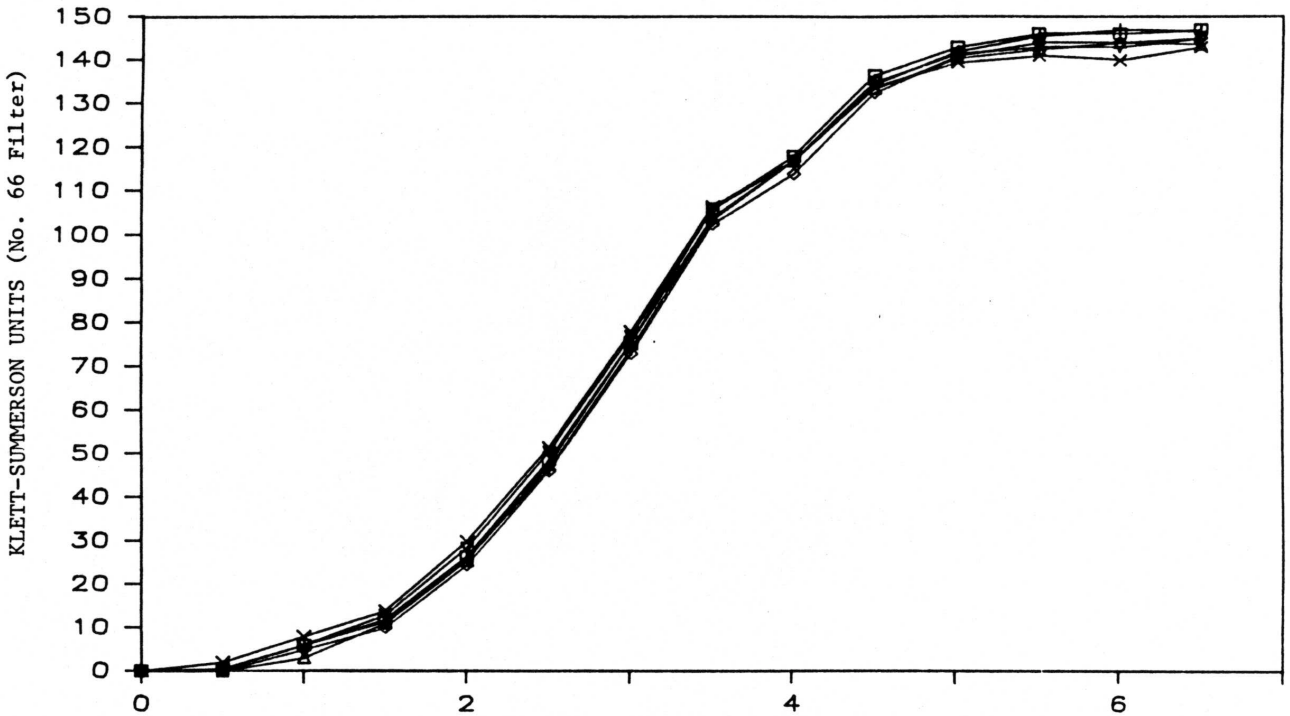

Fig. 2. Growth of Escherichia coli in the presence of NALCO 8102 polymer. Each datum point represents the average of duplicate experiments. Symbols indicate the concentration of polymer: □ 0, + 0.22 mg/l, ◇ 0.44 mg/l, △ 0.66 mg/l, X 0.88 mg/l, ▽ 1.10 mg/l.

TABLE 4. AVERAGE HETEROTROPHIC PLATE COUNTS PER 100 ML FROM THE DISTRIBUTION SYSTEM.

Site	Modified Henrici Medium			R2A Medium		
	Mean	Standard Deviation	Number of Samples	Mean	Standard Deviation	Number of Samples
			September			
1	$1.5x10^3$A##	$1.4x10^3$	5	$2.4x10^3$A	$1.1x10^3$	5
2	$7.3x10^2$B	$7.7x10^2$	6	$7.7x10^2$B	$6.8x10^2$	6
3	$1.0x10^3$AB	$9.5x10^2$	3	$1.0x10^3$B	$9.0x10^2$	3
4	$2.1x10^2$C	$3.3x10^2$	6	$2.0x10^2$C	$3.3x10^2$	6
5	$3.8x10^1$C	$5.4x10^1$	6	$3.9x10^1$C	$5.0x10^1$	6
6	$7.8x10^3$C	$1.2x10^1$	6	$8.7x10^0$C	$9.8x10^0$	6
			December			
1	$3.7x10^2$AB	$2.7x10^2$	5	$4.1x10^2$AB	$3.3x10^2$	5
2	$6.8x10^2$A	$1.2x10^2$	5	$7.4x10^2$A	$1.4x10^2$	5
3	2.6xlO^{2}AB	$1.8x10^2$	4	$2.9x10^2$AB	$2.0x10^2$	4
4	7.3xlO;B	$1.2x10^1$	5	$9.7x10^1$B	$1.6x10^2$	5
5	$2.2x10^1$B	$1.2x10^1$	3	$2.2x10^1$B	l.0xl0^l	3
6	$2.7x10^0$B	$1.2x10^0$	3	3.0xl0^{0}B	$2.0x10^0$	3

##Identical letters in columns for each sampling period are not significantly different at P lt. 0.05.

Cup Sink Study

Data concerned with the repopulation of five cup sink taps in room 6035 of the Agriculture Building are presented in Table 5. There was a significant difference in the results from the two experiments ($P<0.01$). The first time taps 4 and 5 were flushed the heterotrophic plate counts dropped from 120 to 75 and 720 to 420 cells per ml, respectively from the start until the end of flushing, and there was little change in numbers for the next 48 h. In contrast, there was at least a 90 percent reduction in numbers in the other taps and in all taps during the repeat experiment. The reason or this is unclear; however, taps 4 and 5 were rarely used before the first experiment. Generally, the flushing of the taps reduced the bacterial numbers by 90 to 99 percent, and there was gradual repopulation of the taps as the chlorine residual was depleted.

Bacterial Identified from Finished Water

The identification of the bacteria found in finished water are given in Table 6. All isolated cultures were placed into genera or groups following the procedure outlined by LeChevallier et al. (36). There did not appear to be any specific organism at any specific location in the distribution system; therefore all sites were lumped together. The two predominant bacteria were identified as nonpigmented as Group M Moraxella-like, which comprised 22 percent of the isolates, and pigmented *Moraxella* species which were 26 per cent of the total, The most frequently isolated bacterium from the finished surface water at the treatment plant was identified as pigmented Group M Moraxella-like bacteria whereas *Micrococcus roseus/M. varians* and actinomycetes were each isolated 23 percent of the time from the

TABLE 5. AVERAGE HETEROTROPHIC PLATE COUNTS OF SAMPLES FROM CUP SINKS

TIME	CHLORINE RESIDUAL	SINK TAP NUMBER 1 MEAN	1 STANDARD DEVIATION	2 MEAN	2 STANDARD DEVIATION	3 MEAN	3 STANDARD DEVIATION	4 MEAN	4 STANDARD DEVIATION	5 MEAN	5 STANDARD DEVIATION
		27 November									
Before Flush	ND	$8.8x10^{3}$@A	$2.0x10^{2}$##	$8.0x10^{3}$A	$9.2x10^{1}$	$1.5x10^{3}$A	$3.5x10^{2}$	$1.2x10^{2}$A	$9.2x10^{1}$	$7.2x10^{2}$A	$2.1x10^{1}$
Time Zero	0.1	$4.5x10^{1}$C	$3.5x10^{1}$	$6.0x10^{1}$D	$1.4x10^{1}$	$1.2x10^{2}$B	$2.1x10^{1}$	$7.5x10^{1}$A	$5.0x10^{1}$	$4.2x10^{2}$B	$1.9x10^{2}$
8h	0	$1.2x10^{2}$C	$7.1x10^{0}$	$2.9x10^{2}$C	$1.4x10^{1}$	$1.6x10^{2}$B	$7.8x10^{1}$	$1.0x10^{2}$A	$5.6x10^{1}$	$1.0x10^{2}$C	$2.1x10^{1}$
24h	ND	$1.3x10^{2}$C	$1.4x10^{1}$	$4.1x10^{2}$C	$3.5x10^{1}$	$3.2x10^{2}$B	$2.1x10^{1}$	$1.2x10^{2}$A	$2.1x10^{1}$	$8.0x10^{1}$C	$2.8x10^{1}$
48h	ND	$4.6x10^{2}$B	$1.4x10^{2}$	$1.9x10^{3}$B	$7.1x10^{0}$	$2.5x10^{2}$B	$1.4x10^{1}$	$5.0x10^{1}$A	$1.4x10^{1}$	$3.0x10^{1}$C	$1.4x10^{1}$
		12 December									
Before Flush	ND	$3.8x10^{3}$	$2.3x10^{3}$A	$6.4x10^{3}$	$2.5x10^{2}$	$4.4x10^{3}$A	$4.0x10^{2}$	$4.9x10^{3}$A	$4.2x10^{2}$	$5.6x10^{3}$A	$3.2x10^{2}$
Time Zero	0.2	$6.3x10^{0}$C	$1.4x10^{-1}$	$1.3x10^{1}$C	$1.1x10^{0}$	$1.9x10^{1}$C	$2.8x10^{0}$	$1.4x10^{1}$	$1.1x10^{0}$	$2.9x10^{1}$	$2.3x10^{0}$
8h	0	$6.5x10^{1}$C	$7.1x10^{0}$	$1.0x10^{2}$C	$2.8x10^{1}$	$1.7x10^{2}$C	$1.4x10^{1}$	$1.1x10^{2}$	$4.2x10^{1}$	$7.0x10^{1}$	$1.4x10^{1}$
24h	0	$2.3x10^{2}$C	$1.8x10^{2}$	$1.7x10^{2}$C	$4.2x10^{1}$	$3.4x10^{2}$C	$5.6x10^{1}$	$4.0x10^{2}$	$6.4x10^{1}$	$3.9x10^{2}$	$1.4x10^{1}$
48h	ND	$6.7x10^{2}$C	$9.9x10^{1}$	$9.6x10^{2}$B	$5.0x10^{1}$	$1.5x10^{3}$B	$7.1x10^{0}$	$7.8x10^{2}$	$2.1x10^{1}$	$1.8x10^{3}$	$3.8x10^{2}$

@ Values presented are the average of duplicate plates, and are on a per ml basis
Identical letters in columns for each filter are not significantly different at $p<0.05$

TABLE 6. IDENTIFICATION OF HETEROTROPHIC PLATE COUNT BACTERIA

Organism	Finished Surface Water		Finished Ground Water		Distribution Water	
	Total	% of Total	Total	% of Total	Total	% of Total
Acinetobacter spp.	0	0	2	6.7	9	7.3
Actinomycete	0	0	7	23.3	2	1.6
Alcaligenes spp.	1	3.1	0	0	0	0
Arthrobacter spp.	5	15.6	1	3.3	11	8.9
Bacillus spp.	1	3.1	4	13.3	5	4.0
Corynebacterium spp.	0	0	1	3.3	0	0
Group M (nonpigmented)	1	3.1	2	6.7	27	21.8
Group M (pigmented)	9	28.1	3	10.0	13	10.5
Micrococcus luteus	4	12.5	1	3.3	1	0.8
Micrococcus roseus/M. varians	4	12.5	7	23.3	9	7.3
Moraxella sp. (nonpigmented)	0	0	1	3.3	12	9.7
Moraxella sp. (pigmented)	2	6.2	0	0	32	25.8
Unidentified	5	15.6	1	3.3	3	2.4
Total	32	99.8	30	99.8	124	100.1

Group M (Pigmented) Bacteria and Moraxella spp. (Pigmented) may have been Flavobacterium spp. And Pseudomonas spp. Respectively, However inadequate tests were performed to determine this.

finished ground water. Based upon the limited number of isolates in this study there did not appear to be a seasonal effect. Members of the *Acinetobacter*, *Corynebacterium*, nonpigmented *Moraxella* and the actinomycete group were not found in the finished surface water but were isolated from finished ground water.

DISCUSSION

One objective of this study was to determine if the presence and growth of actinomycetes were responsible for the tastes and odors that developed in Laramie's drinking water during the warm summer months. Surface water was identified as the potential source of the problem because tastes and odors quickly subsided when the surface water treatment plant closed for the season in September. The number of actinomycetes detected from water in various stages of treatment was not significantly different before or after the appearance of the taste and odor problem. Although this does not prove that actinomycetes were not partly responsible for the aesthetic problem, it does indicate that investigations should be expanded to include other groups of aquatic organisms, such as algae, to adequately identify the causative agent.

The number of heterotrophic bacteria in the raw water at the surface water treatment plant remained reasonably stable except during the August enumeration with Modified Henrici agar. No significant decrease in counts was detected with R2A medium during this month. The reason for this is unclear.

In general, the heterotrophic plate counts from the effluent of the sedimentation basin as determined by Modified Henrici agar changed significantly with each month of sampling while counts on R2A medium

showed no difference. R2A medium enumerated a higher percentage of the heterotrophic bacterial population in July and August than did Modified Henrici agar; however, counts during September indicated that Modified Henrici medium was superior. The treatment plant closed for the season in mid-September and a limited number of samples were collected. Perhaps the trend noticed in July and August would have been seen during September if more samples had been available,

When comparisons were made by lumping the counts from the effluent of active and inactive filters it was found that Modified Henrici agar enumerated a significantly greater portion of the heterotrophic bacterial population than did R2A medium; however, when only active filters were analyzed R2A medium produced significantly higher counts. Cell injury during filters operation could explain this observation (8). During the filtering process cells trapped in the filter may become injured, and as filter study 1 indicated, the number of organisms passing the filter increased with time of operation. This suggests that a portion of the injured cells are released to the water column. The carbon and energy sources in R2A medium may more closely meet the nutritional requirements of these stressed cells.

Significantly higher numbers of actinomycetes, fluorescent bacteria, pigmented bacteria and fecal streptococci were seen to settle within the first 30 linear feet of flow into the sedimentation basin. This may suggest that bacteria did not settle equally within the basin but settled in specific locations i.e. near the influent of the basin. Heterotrophic bacteria enumerated with Modified Henrici medium and fecal coliforms detected with the standard MF procedure additionally showed

this trend of early settling. Enumeration of total and fecal coliforms with resuscitation procedures, and heterotrophic bacteria with R2A medium indicated that settling in the basin was equally distributed. Resuscitation techniques have often been reported to detect higher numbers of physiologically injured total and fecal coliforms than the standard MF procedure (7,62,67). Flocculated material has previously been shown to cause injury to Escherichia coli (9). This suggests that other groups of bacteria could also be susceptible to injury from the flocculated material. Data from the present study indicates that R2A medium may recover a higher percentage of the injured heterotrophic bacterial population from flocculated material than Modified Henrici agar.

E. coli was found to remain viable for up to 5 months at 15°C in flocculated material from the sedimentation basin. The addition of powdered activated carbon (PAC) appeared to accelerate the die-off of this organism. The literature indicated that the surface of activated carbon particles provide an ideal habitat for bacterial colonization (4,8,11,73). LeChevallier et al. (35) have noticed that bacteria accumulate in cracks and crevices on the carbon particle, become covered with a slime layer, and are well protected from the effects of chlorine. E. coli in the present study could be expected to attach to the PAC surface in the same manner, remain undetected and give the impression that rapid inactivation is occurring. Investigations by scanning electron microscopy would reveal if colonization was actually taking place.

In addition, E. coli seemed to be inactivated at a faster rate when samples were blended for 5 s. as opposed to shaken. In a similar study, Bulson et al. (9) found that blending for up to 18 seconds did not result in significant injury to E. coli. Other than blending, the only difference between the two samples was the nature of the container each was incubated in. Shaken samples were held in glass bottles while blended samples were in stainless steel containers. Studies with glass blender jars would be useful in determining if blending, the type of container, or both were responsible for the higher die-off of E. coli.

The coagulation and flocculation techniques practiced at the surface water treatment plant are much like those used to remove bacteria from the water column of lakes. Bulson et al. (9) recommended that flocculation of lakes for the removal of bacteria be performed at times when recreational use had subsided for the season to reduce the chance of individuals coming in contact with the floc. The survival of indicator bacteria and pathogens within the flocculated material from sedimentation basins may closely approximate the survival rates of bacteria removed from the water column of lakes by the same method. Sedimentation basin survival studies may prove useful in predicting the die-off of pathogens in the flocculated material and the potential health hazard before the actual treatment of lakes is initiated.

Past studies have linked sulfate reducing bacteria with accelerated corrosion and tubercle formation on the interior of pipelines (10,37,53,70). Deterioration of distribution systems in this manner can greatly affect the maintenance costs and longevity of water mains. Tuovinen et al. (70) indicated that when preventive measures are not

practiced, extensive distribution system breakdown can result. The number of sulfate reducing bacteria entering the surface water treatment plant during May through September were considerable; however no bacteria from this group were ever detected in this finished water leaving the plant. Sulfate reducing bacteria may grow once in the distribution system, and monitoring this group of organisms should be continued to determine if the distribution network is susceptible to tuberculation.

The classification scheme outlined by LeChevallier et al. (36) allowed quick and easy identification-of waterborne bacteria to the genus or group level. None of the species of Moraxella produce any pigment (38). For this reason a distinction was made between pigmented and nonpigmented isolates identified as Moraxella species or Group M Moraxella-like organisms. These organisms were suspected of belonging to the Pseudomonas and Flavobacterium genera, respectively. Further biochemical tests would have had to have been included to adequately identify these pigmented organisms if time had allowed. These tests would have included the following: oxidation of maltose and lactose, hydrogen sulfide production, arginine dihydrolase synthesis, and the absence of lysine decarboxylase for suspected Pseudomonas isolates, and ONPG for ß- galactosidase production, oxidase synthesis, and the oxidation of mannitol for isolates thought to be Flavobacterium (38,61).

Heterotrophic plate count bacteria in drinking water may establish themselves in niches within the distribution system (1,53,70). The predominant organisms identified from the surface water treatment plant (pigmented Group M Moraxella-like) and ground water sources (actinomycetes and Micrococcus roseus/M. varians) differed from the

predominant organisms found in distribution water (pigmented Moraxella sp. and nonpigmented Group M Moraxella-like). This may indicate that the environment of the distribution system is selective for certain types of bacteria.

LeChevallier et al. (36) used the same identification scheme for bacteria isolated from raw and finished drinking water supplied to an Oregon coastal town. The predominant strains identified from the raw water source included Acinetobacter spp., Aeromonas spp. and Enterobacter agglomerans, actinomycetes, Corynebacterium spp. and Aeromonas spp. constituted the largest portion of organisms from distribution water. This could be an indication of the bacterial habitats within the distribution system selecting for certain types of organisms (17).

Data from the distribution study show that great variations can exist between the heterotrophic plate counts from private homes. The differences that were seen between sampling sites might well be due to differences in how various people took the samples since the home with the lowest number of bacteria and the treatment facility were sampled by the same person.

LITERATURE CITED

1. Allen, M.J., R.H. Taylor, and E.E. Geldrich. 1980. The occurrence of microorganisms in water main encrustations. J. Am. Water Works Assoc. 72:614-625.

2. America Public Health Association. 1980. Standard methods for the examination of water and wastewater, 15th ed. American Public Health Association, Inc., Washington, D.C.

3. American Public Health Association. 1971. Standard methods for the examination of water and wastewater, 13th ed. American Public Health Association, Inc., New York, New York.

4. AWWA Research and Technical Practice Committee on Organic Contaminants. 1981. An assessment of microbial activity on GAC. J. Am. Water Works Assoc. 73:447-454.

5. Benarde, M., W.B. Snow, V.P. Olivieri, and B. Davidson. 1967. Kinetics and mechanism of bacterial disinfection by chlorine dioxide. Appl. Microbiol. 15:257-265.

6. Bissonnette, G.K., J.J. Jezeski, G.A. McFeters, and D.G. Stuart. 1975. Influence of environmental stress on enumeration of indicator bacteria from natural waters. Appl. Microbiol. 29:186-194.

7. Bissonnette, G.K., J.J. Jezeski, G.A. McFeters, and D.G. Stuart. 1977. Evaluation of recovery methods to detect coliforms in water. Appl. Environ. Microbiol. 33:590-595.

8. Brewer, W.S., and W.W. Carmichael. 1979. Microbial characterization of granular activated carbon filter systems. J. Am. Water Works Assoc. 71:738-740.

9. Bulson, P.C., D.L. Johnstone, H.L. Gibbons, and W.H. Funk. 1984. Removal and inactivation of bacteria during alum treatment of a lake. Appl. Environ, Microbiol. 48:425-430.

10. Butlin, K.R., M.E. Adams, and M. Thomas. 1955. Sulfate-reducing bacteria and internal corrosion of ferrous pipe conveying water. Nature 163:26-27.

11. Cairo, P.R., J. McElhaney, and I.H. Suffet. 1979. Pilot plant testing of activated carbon adsorption systems. J. Am. Water Works Assoc. 71:660-673.

12. Camper, A.K., and G.A. McFeters. 1979. Chlorine injury and the enumeration of waterborne coliform bacteria. Appl. Environ. Microbiol. 37:633-641.

13. Craun, G.F. 1980. Disease outbreaks caused by drinking water. J. Water Pollut. Control Fed. 52:1833-1839.

14. Craun, G.F. 1981. Disease outbreaks caused by drinking water. J. Water Pollut. Control Fed. 53:1134-1138.

15. Drake, C.H. 1966. Evaluation of culture media for the isolation and enumeration of Pseudomonas aeruginosa. Health Lab. Sci. 3:10-19.

16. Dutka, B.J., and S.E. Tobin. 1976. Study on the efficiency of four procedures of enumerating coliforms in water. Can. J. Microbiol. 22:630-635.

17. Fiksdal, L., E.A. Vik, A. Mills, and J.T. Staley. 1982. Nonstandard methods for enumerating bacteria in drinking water. J. Am. Water Works Assoc. 74:313-318.

18. Gainey, P.L., and T.H. Lord. 1952. Microbiology of water and sewage. Prentice-Hall, Inc. New York, New York.

19. Garvie, E. I. 1955. The growth of Escherichia coli in buffer substrate and distilled water. J. Bacteriol. 69:393-398.

20. Geldreich, E.E., H.D. Nash, D.J. Reasoner, and R.H. Taylor. 1972. The necessity of controlling bacterial populations in potable waters; community water supply. J. Am. Water Works Assoc. 64:596-602.

21. Geldreich, E.E., H.D. Nash, D.J. Reasoner, and R.H. Taylor. 1975. The necessity of controlling bacterial populations in potable waters bottled water and emergency water supplies. J. Am. Water Works Assoc. 67:117-124.

22. Green, B.L., E. Clausen, and W. Litsky. 1975. Comparison of the new Millipore HC with conventional membrane filters for the enumeration of fecal coliform bacteria. Appl.Microbiol. 30:697-699.

23. Green, D.E., and P.K. Stumpf. 1946. The mode of action of chlorine. J. Am, Water Works Assoc. 38:1301-1305.

24. Haley, C.E. 1980. Outbreaks of waterborne disease in the United States, 1978. J. Infect. Disease 141:794-797.

25. Herman, L.G. 1976. Sources of the slow-growing pigmented water bacteria. Health Lab. Sci. 13:5-10.

26. Hoadley, A.W., and E. McCoy. 1966. Studies of certain gram-negative bacteria from surface waters. Health Lab. Sci. 3:20-32.

27. Hoadley, A.W., and C.M. Cheng. 1974. The recovery of indicator bacteria on selective media. J. Appl. Bacteriol. 37:45-57.

28. Hurwitz, C., C.L. Rosano, and B. Blattberg. 1957. A test of the validity of reactivation of bacteria. J. Bacteriol. 73:743-746.

29. Jones, J.G. 1970. Studies on fresh water bacteria: effect of medium composition and method of estimation of bacterial population. J. Appl. Bacteriol. 33:679-686.

30. Kabler, P.W. 1954. Water examination by membrane filter and most probable number procedures. Am. J. Public Health. 44: 379-386.

31. Klein, D.A., and S. Wu. 1974. Stress: a factor to be considered in heterotrophic microorganism enumeration from aquatic environments. Appl.Microbiol. 27:429-431.

32. Knox, W.E., P.K. Stumpf, D.E. Green, and Y.H. Auerbach. 1948. The inhibition of sulfhydryl enzymes as the basis of the bacterial action of chlorine. J. Bacteriol. 55:451-458.

33. Lapin, L. 1980. Statistics meaning and method, 2nd ed. Harcourt Brace Jovanovich, Inc. New York, New York.

34. Lamka, K.G., M.W. LeChevallier, and R.J. Seidler. 1980. Bacterial contamination of drinking water supplied in a modern rural neighborhood. Appl. Environ. Microbiol. 39:734-738.

35. LeChevallier, M.W., T.S. Hassenauer, A.K. Camper, and G.A. McFeters. 1984. Disinfection of bacteria attached to granular activated carbon. Appl. Environ. Microbiol. 48:918-923.

36. LeChevallier, M.W., R.J. Seidler, and T.M. Evans. 1980. Enumeration and characterization of standard plate count bacteria in chlorinated and raw water supplies. Appl. Environ. Microbiol. 40:922-930.

37. Lee, S.H., J.T. O'Connor, and S.K. Banerji. 1980. Biologically mediated corrosion and its effects on water quality in distribution systems. J. Am. Water Works Assoc. 72:636-645.

38. Lennette, E.H., A. Balows, W.J. Hausler, Jr., and J.P. Truant, (ed.). 1980. Manual of clinical microbiology, 3rd ed. American Society for Microbiology, Washington, D.C.

39. Levin, A. 1978. The rural water survey. J. Am. Water Works Assoc. 70:446-452.

40. Lewis, R.F. 1965. Control of sulfate-reducing bacteria. J. Am. Water Works Assoc. 57:1011-1015.

41. Lin, S.D. 1976. Evaluation of Millipore HA and HC membrane filters for the enumeration of indicator bacteria. Appl. Environ. Microbiol. 32:300-302.

42. Lin, S.D. 1977. Tastes and odors in water supplies: a review. Water Sewage Works. 124:R141-R163.

43. Mara, D.D., and D.J.A. Williams. 1970. The evaluation of media used to enumerate sulphate reducing bacteria. J. Appl. Bacteriol. 33:543-552.

44. Matsen, 3.M. 1975. Hospital infections with pigmented water bacteria. Health Lab. Sci. 12:305-310.

45. Mattson, J.S., and H.B. Mark, Jr. 1971. Activated carbon. Marcel Dekker, Inc. New York, New York.

46. Maxcy, R.B. 1973. Condition of coliform organisms influencing recovery of subcultures on selective media. J. Milk Food Technol. 36:414-416.

47. McCabe, L.J., J.M. Symons, R.D. Lee, and G.G. Robeck. 1970. Survey of community water supply systems. J. Am. Water Works Assoc. 62:670-687.

48. McFeters, G.A., S.C. Cameron, and M.W. LeChevallier. 1982. Influence of diluents, media and membrane filters on detection of injured waterborne coliform bacteria. Appl. Environ. Microbiol. 43:97-103.

49. Means, E.G., L. Hanami, H.F. Ridgeway, and B.H. Olson. 1981. Evaluating mediums and plating techniques for enumerating bacteria in water distribution systems. J. Am. Water Works Assoc. 73:585-590.

50. Milbauer, R., and D. Grossowicz. 1959. Reactivation of chlorine-inactivated Escherichia coli. Appl. Microbiol. 7:67-70.

51. Morris, R.L., J.D. Dougherty, and G.W. Roland. 1963. Chemical aspects of actinomycete metabolites as contributors to taste and odor. J. Am. Water Works Assoc. 55:1380-1390.

52. Mowat, A. 1976. Most probable number versus membrane filter on chlorinated effluents. J. Water Pollut. Control Fed. 48:724-728.

53. O'Connor, J.T., L. Hash, and A.B. Edwards. 1975. Deterioration of water quality in distribution systems. J. Am. Water Works Assoc. 67:113-116.

54. Oxoid Ltd. 1977. The Oxoid manual of culture media, ingredients, and other laboratory services, 4th ed. Oxoid Ltd. London, England.

55. Ray, B., and M.L. Speck. 1973. Discrepancies in the enumeration of Escherichia coli. Appl. Microbiol. 25:494-498.

56. Reasoner, D.J., and E.E. Geldreich. 1985. A new medium for the enumeration and subculture of bacteria from potable water. Appl. Environ. Microbiol. 49: 1-7.

57. Robick, G.G., K.A. Dostal, and R.L. Woodward. 1964. Studies of modifications in water filtration. J. Am. Water Works Assoc. 56:198-213.

58. Romano, A.H. and R.S. Safferman. 1963. Studies on actinomycetes and their odors. J. Am. Water Works Assoc. 55:169-176.

59. Schalekamp, M. 1979. The use of GAC filtration to ensure quality in drinking water from surface sources. J . Am. Water Works Assoc. 71:638-647.

60. Scheusner, D.L., F.F. Busta, and M.L. Speck. 1971. Injury of bacteria by sanitizers. Appl. Microbiol. 21:41-45.

61. Shayegani, M., A.M. Lee, and L.M. Parsons. 1977. A scheme for identification of non-fermentative bacteria. Health Lab. Sci. 14:83-94.

62. Shipe, E.L., Jr., and G.M. Cameron. 1954. A comparison of the membrane filter with the most probable number method for coliform determinations from several waters. Appl. Microbiol. 2:85-88.

63. Silvey, J.K.G., J.C. Russell, D.R. Redden, and W.C. McCormick. 1950. Actinomycetes and common tastes and odors. J. Am. Water Works Assoc. 42:1018-1026.

64. Silvey, J.K.G. 1966. Taste and odor-effect of organisms. J. Am. Water Works Assoc. 58:706-715.

65. Skinner, Q.D., J.C. Adams, P.A. Rechard, and A.A. Beetle. 1974. Enumeration of selected bacterial populations in a high mountain watershed. Can. J. Microbiol. 20:1487-1492.

66. Stark, W.H., and E. McCoy. 1938. Distribution of bacteria in certain lakes of northern Wisconsin. Zentralol. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. II. 98:201-209.

67. Stuart, D.G., G.A. McFeters, and J.E. Schillinger. 1977. Membrane filter technique for the quantification of stressed fecal coliforms in the aquatic environment. Appl. Environ. Microbiol. 34:42-46.

68. Taylor, R.H., and E..E Geldreich. 1979. A new membrane filter procedure for bacterial counts in potable water and swimming pool samples. J. Am. Water Works Assoc. 71:402-405.

69. Trulear, M.G., and W.G. Characklis. 1982. Dynamics of biofilm processes. J. Water Pollut. Control Fed. 54:1288-1301.

70. Tuovinen, O.H., K.S. Button, A. Vuorinen, L. Carlson, D.M. Mair, and L.A. Yut. 1980. Bacterial, chemical, and mineralogical characteristics of tubercles in distribution pipelines. J. Am. Water Works Assoc. 72:626-635.

71. Van Soestberger, A.A., and C.H. Lee. 1969. Pour plates or streak plates?. Appl.Microbiol. 18:1092-1093.

72. Venkobachar, C., L. Iyengar, and A.V.S.P. Rao. 1975. Mechanism of disinfection. Water Res. 9:119-124.

73. Weber, W.J., Jr., M. Pirbazari, and G.L. Melson. 1978. Biological growth on activated carbon: an investigation by scanning electron microscopy. Environ. Sci. Technol. 12:817-819,

APPENDIX I

Media Formulations

Modified Iron Sulfite Agar (Oxoid)

Iron Sulfite Agar (Oxoid)	23.0 g
$FeSO \cdot 7H_2O$	0.5 g
Sodium Lactate 60%	5.8 ml
$MgSO \cdot 7H_2O$	2.0 g
Distilled water	1.0 l

Adjust pH to 7.5 with 4 N NaOH. Dispense 50 ml amounts into prescription bottles. Autoclave.

Supplement

Ascorbic Acid	7.5 g
Sodium Thioglycollate	7.5 g
Distilled water	100.0 ml

Adjust pH to 7.5. Dispense 5 ml amounts into screw cap tubes. Autoclave.

To use:

Add 0.5 ml of supplement to each 50 ml of melted medium. Dispense 10 ml of medium into inoculated culture tubes. Mix well. Allow to solidify. Add 3-4 ml of agar water for overlay.

Modified Henrici's Medium

Peptone	0.5 g
Glycerine	0.5 ml
Soluble Starch	0.5 g
Sodium Caseinate	0.5 g
K_2HPO_4	0.3 g
$MgSO_4 \cdot 7H_2O$	0.2 g
$FeSO_4 \cdot 7H_2O$	trace
Agar	15.0 g
Distilled water	1.0 1

Adjust pH to 7.4 with 1 N NaOH. Autoclave. Dispense into sterile petri plates.

R2A Medium

Yeast Extract	0.5 g
Proteose Peptone no. 3 (difco)	0.5 g
Casamino Acids	0.5 g
Glucose	0.5 g
Soluble Starch	0.5 g
Sodium Pyruvate	0.3 g
K_2HP0_4	0.3 g
$MgS0_4 . 7H_20$	0.05 g
Agar	15.0 g
Distilled	1.0 1

Adjust pH to 7.2 with crystalline K_2HP0_4 or KH_2PO_4 prior to adding agar. Add agar. Heat to dissolve agar. Autoclave. Dispense into sterile petri plates.

Starch-Casein Agar

Soluble Starch	10.0 g
Casein	0.3 g
KNO_3	2.0 g
NaCl	2.0 g
K_2HP0_4	2.0 g
$MgS0_4 . 7H_20$	0.05 g
$CaC0_3$	0.02 g
$FeS0_4 . 7H_20$	0.01 g
Agar	15.0 g
Distilled water	1.0 1

Dispense 50 ml amounts into prescription bottles and 17.0 amounts into screw cap tubes. Autoclave.

To use:

Melt 50 ml amounts and dispense about 15.0 ml to sterile petri plates. Allow to solidify. Melt 17.0 ml amounts and add 1 ml of sterile Cycloheximide* (1 mg/ml) at the time of inoculation. Add 2 ml of the appropriate dilution. Mix. Layer the previously poured plates with 5 ml of this mixture. Incubate at 20°C for 7 days.

*Actidione, Upjohn Co. or equivalent

Legend for Appendix II

+ = positive reaction or utilization

− = negative reaction or no utilization

N = not done

Morphology	1 rod 2 cocci or coccobacillus 3 branching filamentous hyphae
Pigment	1 colorless, white to cream 2 yellow 3 red, orange
Glucose fermentation	1 no change
Flagella	1 peritrichous
Cell Morphology	1 rod to coccus transformation 2 snapping division

APPENDIX II

	Gram Reaction	Morphology	OF Glucose	Pigment	Oxidase	Indole	Flagella
Actinomycete	+	3	M	N	N	N	N
Arthrobacter spp.	+	1	M	N	N	N	N
Bacillus spp.	+	1	M	N	N	N	N
Corynebacterium spp.	+	1	M	N	N	N	N
Micrococcus luteus	+	2	1	2	N	N	N
Microccus roseus/varians	+	2	1	3	N	N	N
Acinetobacter spp.	–	2	1	N	[illegible]	N	N
Alcaligenes spp.	–	1	1	N	N	N	1
Group M (nonpigmented)	–	1	1	1	N		N
Group M (pigmented)	–	1	1	3	N		N
Moraxella sp. (nonpigmented)	–	1	1	1	+	N	N
Moraxella sp. (pigmented	–	1	1	2	+	N	N

	Cell Morphology	Motility	Endospores
Actinomycete			
Arthrobacter spp.	N	N	N
Bacillus Spp.	1	N	
Corynebacterium spp.	N	N	+
Micrococcus luteus	2	N	
Micrococcus roseus/varians	N	N	N
Acinetobacter spp.	N	N	N
Alcaligenes spp.	N	+	N
Group M (nonpigmented)	N	–	N
Group M (pigmented)	N	–	N
Moraxella sp. (nonpigmented)	N	N	N
Moraxella sp. (pigmented)	N	N	N
	N		N

APPENDIX III

Average Weekly Plate Counts of Selected Bacterial Populations at Various Sites in the Surface Water Treatment Plant

Site	Date	Heterotrophic Plate Counts per 100 ml		Actinomycetes per 100 ml	Sulfate # Reducing Bacteria per 100 ml
		Modified Henrici Agar	R2A agar		
Raw Water	14 May	7.8×10^{5}##@			1.4×10^{3}
	22 May	1.7×10^{6}			2.1×10^{3}
	31 May	1.8×10^{6}			2.2×10^{3}
	6 June	1.0×10^{6}		2.0×10^{2}	3.3×10^{3}
	13 June	3.8×10^{5}		1.8×10^{3}	1.5×10^{3}
	20 June	8.2×10^{4}		8.0×10^{2}	2.0×10^{3}
	26 June	1.2×10^{6}		1.4×10^{3}	2.4×10^{3}
	5 July	2.0×10^{6}		2.2×10^{3}	1.4×10^{3}
	12 July	5.9×10^{5}		8.0×10^{2}	1.1×10^{3}
	19 July	1.7×10^{6}		8.0×10^{2}	3.3×10^{3}
	25 July	1.6×10^{6}	1.1×10^{6}	2.6×10^{3}	3.6×10^{3}
	6 August	7.6×10^{5}	6.8×10^{5}	1.0×10^{3}	7.6×10^{3}
	9 August	4.0×10^{5}	1.3×10^{6}	8.0×10^{2}	1.0×10^{3}
	14 August	3.9×10^{5}	3.4×10^{5}	8.0×10^{2}	1.2×10^{3}
	21 August	6.0×10^{5}	9.8×10^{5}	1.2×10^{3}	1.0×10^{3}
	30 August	9.8×10^{4}	4.7×10^{5}	8.0×10^{2}	6.6×10^{2}
	5 September	2.3×10^{6}	4.3×10^{5}	1.0×10^{3}	1.2×10^{3}
	11 September	9.3×10^{5}	7.1×10^{5}	7.0×10^{2}	3.6×10^{2}

APPENDIX III - continued

Site	Date	Heterotrophic Plate Counts per 100 ml		Actinomycetes per 100 ml	Sulfate # Reducing Bacteria per 100 ml
		Modified Henrici Agar	R2A agar		
Effluent From Sedimentation Basin	14 May	2.8×10^5			1.0×10^2
	22 May	2.6×10^5			5.7×10^2
	31 May	2.6×10^5			5.3×10^2
	6 June	1.6×10^4		*	1.0×10^2
	13 June	4.5×10^3		*	3.0×10^2
	20 June	2.7×10^4		*	2.3×10^2
	26 June	3.8×10^4		1.0×10^2	2.0×10^2
	5 July	1.4×10^5		1.0×10^2	1.7×10^2
	12 July	6.4×10^4		*	6.7×10^2
	19 July	3.4×10^5		1.0×10^2	1.3×10^2
	25 July	6.2×10^5	3.1×10^5	4.0×10^2	4.3×10^2
	6 August	1.4×10^5	1.5×10^5	*	1.3×10^2
	9 August	1.1×10^5	3.0×10^5	1.0×10^2	1.0×10^2
	14 August	2.2×10^5	1.0×10^5	1.0×10^2	*
	21 August	1.2×10^5	2.1×10^5	1.0×10^2	3.3×10^1
	30 August	4.8×10^4	1.5×10^5	*	1.0×10^2
	5 September	7.1×10^5	5.7×10^5	*	2.0×10^2
	11 September	5.2×10^5	1.8×10^5	*	1.0×10^2

APPENDIX III - continued

Site	Date	Heterotrophic Plate Counts per 100 ml		Actinomycetes per 100 ml	Sulfate # Reducing Bacteria per 100 ml
		Modified Henrici Agar	R2A agar		
Effluent From Filters	14 May	7.3×10^4			*
	22 May	4.7×10^4			3.3×10^1
	31 May	2.3×10^4			*
	6 June	8.1×10^4		*	3.7×10^2
	13 June	4.5×10^3		*	1.3×10^2
	20 June	2.0×10^4		*	*
	26 June	1.8×10^4		*	1.7×10^2
	5 July	2.8×10^4		*	1.0×10^2
	12 July	2.0×10^0		*	1.0×10^2
	19 July	5.0×10^3		*	*
	25 July	5.0×10^3	1.5×10^5	*	6.7×10^1
	6 August	3.4×10^5	1.9×10^5	*	*
	9 August	7.3×10^5	1.9×10^5	1.0×10^2	*
	14 August	2.0×10^5	4.8×10^4	*	*
	21 August	2.0×10^5	6.8×10^4	*	*
	30 August	2.1×10^4	5.1×10^4	*	3.3×10^1
	5 September	2.6×10^4	1.3×10^5	*	*
	11 September	4.5×10^5	9.4×10^4	*	*

APPENDIX III - continued

Site	Date	Heterotrophic Plate Counts per 100 ml		Actinomycetes per 100 ml	Sulfate # Reducing Bacteria per 100 ml
		Modified Henrici Agar	R2A agar		
Finished Water	14 May	*			*
	22 May	1.0×10^{3}			*
	31 May	*			*
	6 June	5.0×10^{2}		*	*
	13 June	1.0×10^{3}		1.0×10^{2}	*
	20 June	5.0×10^{2}		*	*
	26 June	5.0×10^{-1}		3.2×10^{1}	*
	5 July	5.0×10		*	*
	12 July	2.0×10^{0}		*	*
	19 July	1.1×10^{2}	7.8×10^{1}	*	*
	25 July	4.8×10^{1}	7.1×10^{1}	*	*
	6 August	2.0×10^{0}	4.5×10^{0}	*	*
	9 August	1.2×10^{1}	1.6×10^{1}	*	*
	14 August	1.0×10^{1}	9.0×10^{0}	*	*
	21 August	3.0×10^{0}	2.0×10^{0}	*	*
	30 August	4.0×10^{0}	2.0×10^{0}	*	*
	5 September	2.2×10^{1}	3.0×10^{0}	*	*
	11 September	1.1×10^{1}	3.5×10^{0}	*	*

Multiple tube determination
@ Values are the average of duplicate plate
* None detected