DEVELOPMENT OF A WATER SAMPLE CONCENTRATOR FOR THE CONCENTRATION OF BACTERIA IN DRINKING WATER

by

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DEDICATION

To my Mother and Father, with much love.

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I. INTRODUCTION

Concentration is a technique used in the analysis of natural and treated water to improve the sensitivity of an analytical method or to isolate a species which interferes with the analysis. Several concentration techniques are cited in the literature including methods such as membrane adsorption, foam separation, freeze separation, carbon adsorption, ultrafiltration, osmotic filtration, liquid-liquid extraction, distillation, evaporation and sublimation (1). The purpose of this study was to develop a water sample concentrator for the collection of a representative bacterial sample from large quantities of water in a water distribution system. These samples would be necessary for assessing microbiological growths within the distribution system. A reliable concentration method could be used to test for coliform or nuisance bacteria for determining potential health hazards, sources for taste and odor problems, the need for maintaining a chlorine residual throughout the distribution system and the need for water main flushing. Following concentration, the bacteria must be recovered and enumerated. The method of concentration, therefore, must avoid bacterial contamination from outside sources and operate under conditions which allow the bacteria to survive.

The concentration technique used in this study was a membrane filtration adsorption/elutriation procedure. Membrane filters have been widely used for the concentration of bacteria

and viruses from water. Standard Methods (42) describes microporous filter methods for the detection of viruses and bacteria. The concentrator fabricated for this study was based on information given in Standard Methods (42) for concentrating virus samples but was adapted for bacterial concentration. Concentration of the organisms on membrane filters is based on physical filtration due to membrane porosity and electrostatic attraction.

Recently, authorities have questioned the reliability of the Most Probable Number test (MPN) and the Membrane Filtration test (MF). It has been stated that MPN estimates are not precise and some of them are inherently improbable due both to the wide range the 95 percent confidence limits cover and to the frequent occurrence of improbable positive tube combinations (47). Studies have shown that with the MF test the brand of membrane filter utilized has a significant effect on the ability to recovery bacteria (12). Must debate has resulted over comparison of MPN and MF results and several studies demonstrate that these two procedures can yield significantly different coliform numbers (35). Also, the issue of stressed organisms and its impact on the enumeration of bacterial populations raises doubts about the reliability of MPN and MF results. Then, even after enumeration there is the question as to whether the indicator organism-pathogen relationship reliably indicates the presence of a health hazard (35). Increasingly, these issues are casting serious doubt on the dependability of the standard bacterial testing

procedures and people involved with water analysis realize the need for a reliable quantitative technique for bacterial enumeration.

Present methods of bacterial analysis are limited in the volume of water examined. A technique is needed which permits high volume sampling for quantitation of microorganisms present at low densities. Under the procedures for sample collection in Standard Methods (42), an investigator is directed to obtain a volume of sample sufficient to carry out all tests required and preferably not less than 100 milliliters of water for samples intended for bacteriological examination. In the specific tests, pour plates for the standard plate count require only one to two milliliters, test tube analysis such as the MPN technique only require 10 to 100 milliliters, and the membrane filtration technique requires only 20 to 500 milliliters. A technique such as the membrane adsorption/elutriation technique may yield a more representative bacterial assay because large volumes of water can be processed quickly and easily. The bacteria are trapped on the upper surface of the membrane which contains a uniform, fine pore structure. The lower portion of the membrane has a looser pore structure which facilitates the high flow rate.

The objectives of this study were to develop and field test a water sample concentrator. An important goal in the development of the concentrator was to utilize a concentration method which would yield reliable and efficient recoveries of bacteria

with the ability to process from 20 L to 100 L of water. Also, the water sample concentrator should be economical and practical for field use. Various commercially available filter materials, elutriation techniques, additive solutions, and operating conditions were tested and analyzed using the bacteria, Escherichia coli and Streptococcus faecalis. Due to the controversy over the MPN and MF techniques, tests were conducted to evaluate concentration efficiency based on both procedures.

II. LITERATURE REVIEW

Microbiological analysis of drinking water to insure the safety of the public health requires reliable and precise enumeration procedures. Potable water of good bacteriological quality is generally associated with attainment of less than one total coliform per 100 mL of water sample (7). The use of the coliform test has been a major force in controlling epidemic disease in the United States by indicating the possibility of contamination from many types of pathogens. Several of the Enterobacteriaceae are pathogenic bacteria. For example, members of the genus Salmonella produce typhoid or salmonellosis, and Shigella and Proteus species produce dysentery or severe enteritis. Several pathogenic viruses are also transmitted via the intestinal route (9).

Research has shown that potable water transmitted through the distribution system may undergo changes in its microbiological quality (17). The deterioration of the water quality may be attributed to the dissipation of the chlorine residual and regrowth of the microorganisms, corrosion, dead ends, and creation and sloughing of a pipe slime layer which can result in health hazards, taste and odor problems and increased turbidity. The Safe Drinking Water Act requires that the water quality at the user's tap must be insured (33).

Investigations into the deterioration of water quality are producing evidence to indicate that a more rigorous control

should be exercised over the general microbiological quality of the water (21). Therefore, reliable microbiological monitoring procedures are necessary for the detection of nuisance organisms within the distribution system as well as for the production of water free of pathogens.

Current Methods

The most popular methods today for the bacterial analysis of drinking water are the most probable number (MPN) method, the membrane filtration (MF) method and the standard plate count. The most probable number (MPN) method for estimating bacterial densities was introduced by McCrady in 1915 (34) and since that time has gained wide acceptance and has long been an approved procedure for the microbiological examination of water. However, the MPN method is an indirect technique for estimating the concentrations of bacteria in water based on statistical probabilities of the number of positive tubes in each dilution and the precision of this method has been questioned (47). The membrane filtration technique (18) has been included in Standard Methods since the tenth edition. The coliform recoveries by membrane filtration methods are influenced by the brand of membrane used, the sterilization procedure for the membrane, the growth medium, the temperature of incubation, the sources of the coliform bacteria and the surface pore morphology of the membrane filter (29). The standard plate count is a pour plate technique

for estimating the general bacterial population. Geldreich et al. (17) have recommended that although the detection of coliform bacteria is a major concern in potable water quality measurements, the standard plate count should also be evaluated for control of the general bacterial populations.

MPN Versus MF

The most probable number and membrane filtration methods actually isolate different types of coliforms and each method defines coliform bacteria in their own way.

The multiple tube dilution method using the most probable number depends on the ability of an organism to ferment lactose producing lactase and permease with gas formation. Organisms within the coliform group which do not ferment lactose have lost their ability to produce permease. With the MPN method, the definition of the total coliform organism is "all of the aerobic and facultative anaerobic gram-negative, non-spore forming, rod shaped bacteria that ferment lactose with gas formation within 48 hours at 35°C" (32).

The membrane filtration method depends upon the breakdown of lactose, producing acetaldehyde which oxidizes sodium sulfite-acetaldehyde which in turn oxidizes the sodium sulfite-based fuchsin complex in the medium and results in the green metallic sheen of the colonies (32). A definition of the total coliform group using the MF technique is that it "comprises all the

aerobic and facultative anaerobic, gram-negative, non-spore forming, rod shaped bacteria that produce a dark colony with a metallic sheen within 24 hours on an Endo-type medium containing lactose" (42).

Researchers disagree on whether the analytical results obtained from the MPN or MF method are more reliable. Several comparisons of the results obtained with these methods have been made which show considerable variation, especially with chlorinated samples (4,26,35,36). Hoffman et al. (23) conducted experiments to compare the MF and MPN techniques and determined that the MF method gives an evaluation of coliforms that is equal to or higher than the MPN results except for heavily chlorinated sewage samples. Bissonette et al. (4) reported that in general MPN techniques gave better recovery than plating or membrane filtration procedures. In work by Lin (30), the membrane filtration method commonly yielded results 10 to 1000 times less than the bacterial counts obtained by the MPN method while the MF results were very similar to the MPN results when using a two step MF method. Lin's two step MF method provides an enrichment step which allows a recovery period for cell repair of stressed organisms (43). Stressed organisms will be discussed further in a later section of this chapter.

Mowat (36) claims the MF procedure to have advantages over the MPN procedure which include simplicity, speed, accuracy, reproducibility, and reduction of labor, space and material.

Also due to the simplicity, the MF method can be conducted by lesser trained personnel than is required by the MPN method.

Need for Improved Microbiological Examinations

The need for quantitative techniques for the enumeration of small numbers of enteric pathogens and indicator organisms in large volumes of water and wastewater has been pointed out by Dutka (10,11). Both the MPN and MF procedures are limited in the volumes of samples which are examined and improved sensitivity would be obtained if larger volumes of water were examined (1).

In recently reported epidemics, pathogens have been isolated from waters which, based on coliform standards, should have been safe (11). Mack (32) reported two outbreaks of gastroenteritis where standard test procedures did not indicate that the drinking water was contaminated. In both of these outbreaks, the indicator organism was present, but not found because of the small number of organisms in a large volume of water. Subsequent investigations revealed no coliform organism detection by the standard MPN test; however, after concentration, the MPN test indicated that coliform bacteria were present. Standard Methods (42) recommends sampling 400 liters of water to recover virus, but only 50 to 100 mL are needed to perform the MPN and MF tests. Geldreich (17) has shown that there will be interference in detecting low densities of total coliform organisms if the water sample contains 500 or more noncoliform organisms per mL.

There is a general dissatisfaction with the total coliform bacteria as an index organism as well as the various procedures to estimate its population in various water samples. However, no one has provided a better indicator organism than the coliform for drinking water. Among the major criticisms against the coliforms are that they are not detected when they are in limited numbers in large volumes of water or in the presence of interfering noncoliform bacteria. Mack (32) recommends that these shortcomings be corrected by concentrating the few coliforms present in large volumes of water before testing for their presence and by determining the total bacterial count of the water sample to detect interference.

Pathogens and indicator organisms are not the only microorganisms which need improved quantitative techniques. For nuisance organisms, Lueschow and Mackenthum (31) were unable to quantitatively determine low concentrations of iron bacteria using standard procedures when attempting to control taste, odor and discoloration problems in municipal water systems. Therefore, they developed a membrane filter technique which could sample several hundred milliliters of water and accurately enumerate the iron bacteria with a calibrated microscope.

Microbiological Concentration Methods

Much research has been conducted recently on the concentration of microbiological samples to improve the sensitivity of

the analysis. Levin (28) has developed a filtration technique for high volume sampling of water that, when combined with the MPN procedure, permits the quantification of microorganisms present at very low densities (less than one coliform per 100 ml). Much work has been done with the concentration of virus from large volumes of tap water (14). In many cases, virus levels in potable water may be extremely low, even under 10 plaque forming units in 100 liters (40). This necessitates concentration in order to detect and enumerate viruses because they would not be reliably and accurately detected by conventional methods.

Two virus adsorbents have been suggested for field use because they can bind viruses from water even at high flow rates; these are cellulose membranes and insoluble polyelectrolyte layers (45). Hill et al. (22) have developed an apparatus for enteric virus detection utilizing the adsorption of the virus to a membrane filter and the subsequent elutriation of the virus from the filter for enumeration. A similar procedure is listed as a tentative technique in the 15th edition of Standard Methods (42). The efficiency of virus adsorption has been shown to be enhanced by controlling pH in the range of 3.5 to 4.5 and adding cations at predetermined concentrations (46). Experiments have been conducted which utilized $MgCl_2$, $CaCl_2$ and $AlCl_3$ salts in concentrations from 0.00005M to 0.05M to enhance virus adsorption (46).

Electrophoretic studies of viruses indicate that most viruses are negatively charged near pH 7.0. At pH 3.5, where adsorption to the filter surface is optimum, most viruses have a net positively charged (25). The membrane filters are negatively charged and the acidification may cause an electrostatic attraction between the virus and filters at the lower pH. In the neutral pH range the multivalent cations may enhance adsorption between the negatively charged filter and the negatively charged viruses (41). In recovery studies using poliovirus type I and the above concentration technique, virus recoveries ranged from 25 to 50 percent with virus inputs of 16 to 50 plaque forming units per 100 gallons when 100 gallons were sampled (22). A similar apparatus developed by Farrah et al. (14) utilized a 10-inch pleated epoxy-fiberglass filter at flow rates up to 37.8 liters/minute. In this apparatus, viruses in 500 gallons of tap water were concentrated 100,000-fold in 3 hours with an average recovery of 40 to 50 percent. The three main problems with the use of the adsorption-elution concentration method are: 1) the pH must be lowered to 3.5 to adsorb the viruses, 2) the adsorbent filters have a tendency to become clogged, and 3) the presence of organics in the water interferes considerably with the ability of the filters to adsorb viruses (15,40).

Sobsey (41) was able to eliminate the need for acid or salt addition for virus adsorption by effectively concentrating

viruses with zeta-plus filters. Zeta-plus filters are microporous filters which are electropositively charged. Zeta-plus filters have also been used for the concentration of bacteriophages from large volumes of water (19).

Another successful microbiological concentration method recently researched is ultrafiltration. Belfort et al. (40) accomplished virus concentration by using a capillary ultrafiltration unit which has the advantage over the adsorption-elutriation method of not requiring any preliminary steps such as pH adjustment. A mean virus recovery of 80 percent was obtained with 50 liter samples containing 39 to 200 plaque forming units per 100 liters by using the ultrafiltration technique.

Zierdt (48) reported that filtration with membrane filters is not strictly a mechanical process, but is greatly influenced by the electrochemical forces at work between the particles and the filters. He noted a strong adherence of bacteria, yeast, erythrocytes, leukocytes, platelets, spores and polystyrene spheres to membrane filter materials during filtration through membranes with pore size diameters much larger than the particles themselves.

Several studies have been conducted to compare various commercially available cellulose acetate 0.45μm porosity membrane filters for the enumeration of indicator bacteria (20,30, 38,44). A study by Green et al. (20) ranked six types of membrane filters in order of decreasing effectiveness as follows: Millipore HC >

Gelman ≃ Johns-Manville > Sartorius > Millipore HA > Schleicher and Schuell. Tobin and Dutka (44) confirmed Green's results with a study that indicated that the five best membranes for coliform recovery could be placed in two groups: Millipore HC and Gelman, followed by Johns-Manville SG and AG and Sartorius. Tobin and Dutka (44) also concluded that membrane filters have different structural conformations which seem to affect flow rates and bacterial recovery. Also, in general, the faster the flow rate, the greater the bacterial recovery because less bactericidal material has an opportunity to adsorb to the filter. Presswood and Brown (38) reported that the Gelman GN-6 filters recovered significantly more coliform bacteria than did Millipore HA filters. They concluded that one obvious difference in the Millipore and Gelman filters is the method of sterilization. The Millipore HA filters are sterilized with ethylene oxide while the Gelman filters are sterilized in an autoclave. Although all cellulose brands of membrane filters are basically similar, additives such as wetting agents, inks used for grid markings, or ethylene oxide residues may be toxic to the bacteria and account for the differences in recoveries.

Green et al. (20) citing Sladek et al. stated that the recovery of coliforms could be increased by modifying the surface pore morphology of the filter. The Millipore Corporation (Bedford, Mass.) developed the type HC filter based on this theory. The Millipore HC filter has an absolute retention pore

size of 0.7μm and a surface opening diameter size of 2.4μm. Lin (30) evaluated Millipore type HC and conventional 0.45μm type HA filters and found the Millipore HC to be superior to the type HA filter for fecal coliform recovery. The type HC membrane can also be used for total coliform and fecal streptococcus determinations.

Following concentration on the membrane filter, the microorganisms must be eluted and enumerated. Solutions containing either a mixture of amino acids or individual amino acids such as fetal calf serum, beef extract, tryptose-phosphate broth or glycine have been used as eluents (16). Common eluents include 0.5 M glycine buffer at pH 11.5 and 3 percent bovine serum at pH 9.0. Beef extract concentrations of less than 3 percent were found to be efficient for recovery of absorbed viruses from membrane filters (27). Recoveries with beef extract could not be significantly improved by varying the type of beef extract or by extending the elution time to 30 minutes (27). Although elutriation at a pH as high as 11.5 had no detrimental effect on virus concentration, a pH that high could be lethal to bacteria thereby greatly decreasing the efficiency of recovery.

Indicator Organisms

Pathogenic bacteria excreted in human feces are found in low concentrations in wastewater and many are known to be more sensitive to environmental conditions than Escherichia coli.

Escherichia coli is excreted by both humans and warm blooded animals and is used as an indicator of fecal wastes which may contain pathogens. However, many researchers feel that no single indicator is adequate to access the bacteriological quality of water (11,12,17).

The minimum requirement for an indicator is that it must be a biotype that is prevalent in sewage and excreted by humans or warm-blooded animals. In addition, the indicator should be present in greater numbers than the pathogenic bacteria, not be able to proliferate to any greater extent than the pathogens in the aqueous environment, be more resistant to various disinfectants than the pathogenic bacteria, and quantifiable by simple and rapid laboratory procedures (11,26). Only a few of the numerous fecal microorganisms found in wastewater and capable of contaminating drinking water could be considered as indicators. The less abundant pathogenic bacteria are ruled out because of difficulties in isolation. Indicators most commonly used today are Clostridium perfringens, Streptococcus faecalis, the coliform bacteria, and Escherichia coli.

The significance of the presence of E. coli in surface waters is that fecal contamination due to humans or other warm-blooded animals has occurred and therefore a potential health hazard risk from microbial pathogens exists (8). Total coliform bacteria are often used as indicators of drinking water quality and their presence in any drinking water should initiate an

immediate search for a contaminating source. Their presence in wastewater or surface waters is to be expected and does not have the significance of the presence of fecal coliforms (32).

There is no one universal bacterial indicator system (12). A variety of indicators or techniques such as adenosine triphosphate (ATP), epi-fluorescence, bacterial counts, heterotrophic bacterial counts at 20°C and 35 to 37°C, and Limulus LPS assays can be used to determine the bacterial quality of water and to indicate the presence of toxicants (12).

Indicator bacteria need not be restricted to those which indicate fecal or sewage pollution. The presence of Thiobacillus ferrooxidants in water indicates pollution by sulfuric acid which might be due to acid mine drainage (9). The concept of using microbiological indicators can also be expanded to other areas where indicators are needed.

Streptococcus faecalis as an Indicator

Fecal streptococci are present in the feces of humans and warm blooded animals. They appear able to survive longer than bacterial pathogens and are not considered to be of pathogenic significance themselves. Also, they do not appear to multiply in natural or polluted waters. For these reasons, Streptococcus faecalis has been considered as an indicator organism.

Clausen et al. (6) suggested that fecal streptococci may not be an ideal indicator in all circumstances, but their use may be

beneficial in certain situations. When assessing waters for viral contamination, Streptococcus faecalis may be a good indicator because its survival more closely parallels viruses than does that of coliforms. Researchers disagree on whether coliforms or Streptococcus faecalis are more resistant to chlorination; therefore, Streptococcus faecalis may be a more desirable indicator. When attempting to identify the source of fecal pollution, a determination of the number of fecal streptococci bacteria is helpful because the ratio of fecal coliforms to fecal streptococci levels is different for human and animal feces.

Stressed Organisms

Stressed organisms are microorganisms which have been sublethally injured. The injured cells lose their normal resistance and become sensitive to many chemicals. In addition these cells may lose many smaller molecular cellular components to the environment. Both of these changes have been attributed to impaired permeability. The cells might lose their ability to multiply. In most cases, the damage is to surface structures such as the lipopolysaccharide layer or to the cytoplasmic membrane (39).

Bacteria are capable of repairing damaged cell features if given the proper nutrients and conditions of incubation (43). After repair in a suitable environment, the stressed organisms regain the ability to multiply (3,13,39). The problem is that

the stressed organisms may not be detected during routine analysis and the test will not indicate the actual microbiological quality of the sample. Several researchers are recommending an enrichment period on a rich, non-selective medium before exposure to selective media to improve recovery of indicator organisms with membrane filtration techniques (3,4,43). Again, the literature indicates that the present standard techniques for recovery and enumeration of bacteria need to be improved.

Adenosine Triphosphate Photometric Determinations

A relatively new technique for the enumeration of microorganisms is the chemical analysis of ATP content rather than a bioassay. Adenosine triphosphate (ATP) is a phosphate containing compound found in all living plant and animal cells. The ATP molecule contains pyrophosphate bonds which release large amounts of energy when hydrolyzed as shown in the following equation (2):

$$ATP \xrightarrow{H_2O} ADP + P_i + 7300 \text{ cal mole}^{-1}$$

The ATP molecule stores and supplies energy for cell metabolism, transmission of nerve impulses and muscle contraction.

Researchers have been able to estimate microbial biomass through ATP determinations based on a fairly constant ATP-to-cell carbon ratio among a certain type of organism and the fact that

ATP is found only in living cells (5). The bioluminescence reaction of the firefly luciferin luciferase enzyme system can provide a rapid and sensitive estimate of ATP content, which can be converted to an organic carbon biomass estimate (37). The ATP of cells can be extracted by a tris buffer, acetone, or sulfuric acid solution. A known volume of the extract is injected into an ATP photometer along with the luciferase enzyme. The amount of ATP in the sample is determined by comparing the amount of light emitted per unit time with values obtained when ATP standards were injected into the photometer (24). Immediate information regarding the presence of microorganisms in water is provided while avoiding the time consuming handling and processing of water samples for microbiological analysis. This technique can detect 10^{-12} g ATP per mL and correlates well with direct microscopic counting for deep ocean samples (24). It is uncertain as to the reliability of this method with the low numbers of microorganisms present in drinking water.

Summary

From this review of the literature, it is evident that there is a need for improved bacterial detection and enumeration methods. A common criticism of the present methods is that low numbers of bacteria are not detected in large volumes of water and several researchers have recommended that concentration methods currently used for the isolation of viruses be adapted

for the enumeration of bacteria. Such a method would be beneficial both as a technique for the enumeration of indicator organisms to insure that potable water is free of pathogens and as a technique for the enumeration of nuisance organisms to monitor the deterioration of water quality within distribution systems.

III. MATERIALS AND METHODS

In this chapter, details concerning the methods of sampling and analysis during the development and field testing of a bacterial concentrator are presented. The concentrator used in this study closely resembles the apparatus recommended in Standard Methods (42) for concentrating viruses on a microporous filter. However, modifications to the unit were necessary for work with bacteria. Several laboratory experiments were conducted to determine the efficiency of the concentrator for the recovery of different types of bacteria from drinking water quality samples. Escherichia coli and Streptococcus faecalis were the types of bacteria concentrated but unless otherwise noted, the reader should assume E. coli was used in a given experiment.

Bacterial Types, Maintenance, and Enumeration

Cultures of Escherichia coli and Streptococcus faecalis were obtained from the Virginia Polytechnic Institute and State University Microbiology Department. These cultures were streaked on nutrient-agar slants and maintained in a ten degree centigrade (10°C) refrigerator during the entire study period. Approximately once every two weeks, the bacteria were streaked onto a new slant to maintain a viable supply of bacteria. One day prior to an experiment, a test tube of nutrient broth specific for the

selected bacteria was inoculated from the slant and incubated at 35 ± 0.5°C overnight. Volumes ranging from one loopful to ten mL of the culture, depending on the desired bacterial concentration, were then added to dechlorinated tap water to make up the influent water samples. The influent bacterial concentrations ranged from 93 per 100 mL to 93 x 10^7 per 100 mL. Prior to the experiment, the concentration of bacteria in the influent sample was determined by the most probable number (MPN), membrane filtration (MF) or standard plate count techniques, as described in Standard Methods (42). Escherichia coli was enumerated by the three tube MPN method with lactose broth, the total coliform membrane filter procedure using a sample volume of 100 mL, and the standard plate count method. Streptococcus faecalis was enumerated by the multiple tube technique with three tubes for both the presumptive test using azide dextrose broth and the confirmed test with ethyl violet azide broth.

Sterilization and Disinfection

Aseptic techniques were used in all phases of media preparation and bacterial enumeration. Prior to an experiment, pipets, 100 mL graduated cylinders, a membrane filter, 150 mL beakers with stir bars, and milk dilution bottles containing 99 mL of dilution water were sterilized in a Barnstead autoclave (Barnstead, Division of Synbron Corporation, Boston, Massachusetts) and allowed to cool to room temperature.

To disinfect the apparatus between trials, the concentrator was rinsed with nineteen liters of water containing 50 mL of chlorine bleach. Thirty-eight liters of tap water were then pumped through the system followed by a rinse of nineteen liters of water containing 300 mg/L sodium thiosulfate. In this way bacterial contamination from a previous experiment was eliminated, and any chlorine residual was subsequently neutralized.

Description of the Concentrator

A schematic diagram of the water sample concentrator is shown in Figure 1. The influent and effluent samples were contained in five gallon Nalgene carboys. The sample was forced through the system by a Manostat Varistaltic pump (Manostat, New York, New York). Additions of either alum or calcium carbonate and sodium thiosulfate ($Na_2S_2O_3$) were regulated by a Johnson Model M14 fluid proportioner (Johnson & Son Machine Corporation, Clifton, New Jersey). Various membrane filters were tested in the system and were supported by a Gelman filter holder (Gelman Instrument Company, Ann Arbor, Michigan). Tygon tubing was used for all flow lines.

The fluid proportioner introduced the additive solutions into the sample stream prior to the membrane filter to dechlorinate the influent sample and to enhance the adsorption of the bacteria on the filter. A 10 mg/mL solution of aluminum sulfate was prepared by adding 1.948 grams of alum

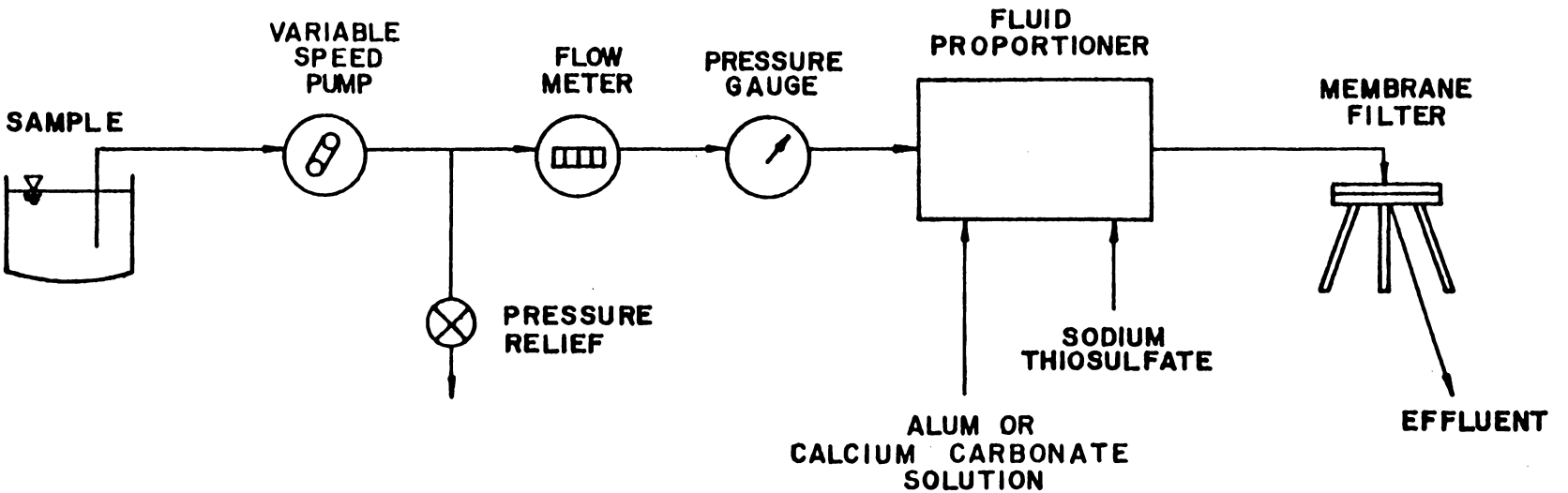

FIGURE I. SCHEMATIC DIAGRAM OF THE BACTERIAL CONCENTRATOR

($Al_2(SO_4)_3 \cdot 18\ H_2O$) per 100 mL of solution and was added to the sample stream at a 1:106 dilution. This resulted in a 0.0016 N solution of aluminum sulfate once the concentrated alum solution mixed with the sample stream. Solutions of calcium carbonate ($CaCO_3$) were prepared in concentrations ranging between 0.005 N and 0.20 N and were added to the sample stream at dilutions of either 1:128 or 1:64. In each case, the $CaCO_3$ solution was adjusted to pH 6.0 with sulfuric acid to convert the $CaCO_3$ to soluble calcium bicarbonate ($Ca(HCO_3)_2$). For dechlorination, a 0.10 N solution of sodium thiosulfate (15 grams of $Na_2S_2O_3$ per liter) was added at a 1:106 dilution to provide a 9×10^{-4} N concentration in the sample stream.

Four commercially available membrane filters were tested in the water sample concentrator. All of the filters were 142 mm in diameter. The filters used in this study are described below:

a. Millipore filter, 0.45μm porosity, Millipore Corporation, Bedford, Massachusetts.

b. Cox filters, M-780 media, 0.45μm and 5.0μm porosities, Cox Instrument, Division of Lynch Corporation, Detroit, Michigan.

c. AMF filters, zeta-plus, 0.4-0.6μm and 0.7-3.0μm porosities, Cuno Division, Meriden, Connecticut.

Typical Concentration Experiment

In a typical experiment, sample volumes of 19 liters were concentrated. As mentioned earlier, the influent sample was tap water which had been dechlorinated with 100 mg/L sodium thiosulfate and seeded with between one loopful and ten mL of bacterial cultures. A MPN and/or MF test was conducted on the influent to determine the initial bacterial concentration and the pH of the sample was determined. The influent sample was pumped through the water sample concentrator at a flowrate of approximately two liters per minute. Additive solutions of alum or calcium carbonate and sodium thiosulfate were introduced to the sample stream by the fluid proportioner just prior to the membrane filter.

As the water sample passed through the filter, the bacteria were strained out due to the small pore size of the filter and/or adsorption to the filter. The alum and calcium carbonate additive solutions were introduced to enhance this adsorption of the bacteria to the filter. Total filtration times were generally about ten minutes. After concentration, the effluent pH was recorded and bacteria in the effluent were assayed. The membrane filter was removed from the filter holder with sterile forceps so the filtered and adsorbed bacteria could be eluted and enumerated.

An experiment, including preparation, autoclave sterilization, concentration, MPN dilution work, disinfection of the

concentration apparatus, and clean up, generally required one day. All materials were autoclave sterilized the night before an experiment to allow ample time for the equipment to cool.

From the information obtained, a mass balance was conducted on the bacteria in the system. The percent bacterial recovery on the filter was calculated from the known fluid volumes and bacterial levels. The percent recovery was the basis for evaluating the various independent variables associated with the development of the concentrator.

Recovery of Adsorbed Bacteria

After bacteria were adsorbed or filtered onto a filter, they were eluted and enumerated. Elutriation was accomplished with a bovine serum solution (Flow Laboratories, Rockville, Maryland) adjusted to pH 9.0. The high pH gave the bacteria more of a negative charge so their affinity for the filter was reduced which allowed the proteins in the serum to compete with bacteria for filter adsorptive sites. The filter was mechanically disrupted by blending or slicing to enhance the release of the bacteria. The bacteria, released into the solution, were enumerated by the MPN, MF, or standard plate count techniques.

During the development of the water sample concentrator, several elutriation techniques were tested and are described below. In all cases, aseptic techniques were used. The filter

was handled with sterile forceps and all glassware and Petri dishes were sterile.

Elutriation in a Large Petri Dish

After concentration, the filter was placed in a sterile 150 by 15 mm Petri dish and cut into approximately 10 mm wide strips. Then 50 mL of a three percent bovine serum solution was added at pH 9.0 and allowed to contact the strips for about fifteen minutes. The bacteria were released into the solution and an MPN, MF, or standard plate count (SPC) test was performed on the solution.

Elutriation in a Graduated Cylinder

After concentration, the filter was cut into approximately 10 mm wide strips which were placed in a sterile, 100 mL graduated cylinder. Fifty mL of a three percent bovine serum solution was added at pH 9.0 to the graduated cylinder and a fifteen minute contact period was allowed. The filter strips and solution were agitated occasionally with a sterile glass rod. An MPN, MF, or SPC test was performed on the solution.

Elutriation in a Blender

After concentration, the filter was placed in a blender with 100 mL of a three percent bovine serum solution at pH 9.0. The solution was then blended for thirty seconds. The mixture of bovine serum and filter pieces was poured into a sterile 150 mL beaker containing a stir bar. The mixture was stirred on a

magnetic stirring plate for a fifteen minute contact period. A sample from the beaker was used for enumeration of the bacteria by the MPN, MF, or standard plate count technique.

After several experiments with this procedure, it was noticed that several fine pieces of the blended filter would adhere to the walls of the blender and would not be poured into the sterile, 150 mL beaker. The procedure was then changed and an additional 25 mL aliquot of the three percent bovine serum solution was used to rinse the beaker to obtain the filter pieces and any bacteria which may have adhered to the blender walls. The total volume of three percent Bovine Serum utilized for elutriation was then 125 mL. Unless otherwise noted, this elutriation technique with the blender was the method used in the experiments.

Special Studies

Among the special studies performed were field trials, vacuum filtration tests and evaluations of the system efficiency by measurement of adenosine triphosphate (ATP) levels. In the field work, grab sample results were compared to the results obtained from a concentrated sample. Vacuum filtration tests were conducted to determine if concentration efficiency was related to the means of inducing sample flow through the concentrator. In the final special study, system efficiency was

evaluated through a comparison of influent and effluent cellular adenosine triphosphate (ATP) levels.

The field concentration trials were performed at a municipal water treatment plant and an elementary school located in a small Virginia community. The concentration experiments were conducted in basically the same way as in the laboratory. After concentration, the filter was placed in a sterile 150 by 15 mm Petri dish and stored on ice. Influent and effluent grab samples were taken and stored on ice in Whirl pack sample bags. Bacterial enumeration tests were conducted within 12 hours of the time the samples were collected.

The vacuum pump experiments were conducted with no additive solutions and at flowrates of 0.17 to 0.25 L/min. E. coli was the bacterium used and the blender/bovine serum method was used for elutriation. A schematic diagram of the apparatus is shown in Figure 2. The effluent carboy was capped with a two hole stopper. Tubing from one of the holes was attached to the vacuum pump and tubing from the other was connected to the effluent side of the filter holder. Tubing on the influent side of the filter holder was submerged in the influent carboy. This created a closed system so that when the vacuum pump operated, a suction was created which drew the influent sample through the membrane filter.

In those experiments in which ATP was measured to serve as an index of concentrator efficiency, the bacterial cells were

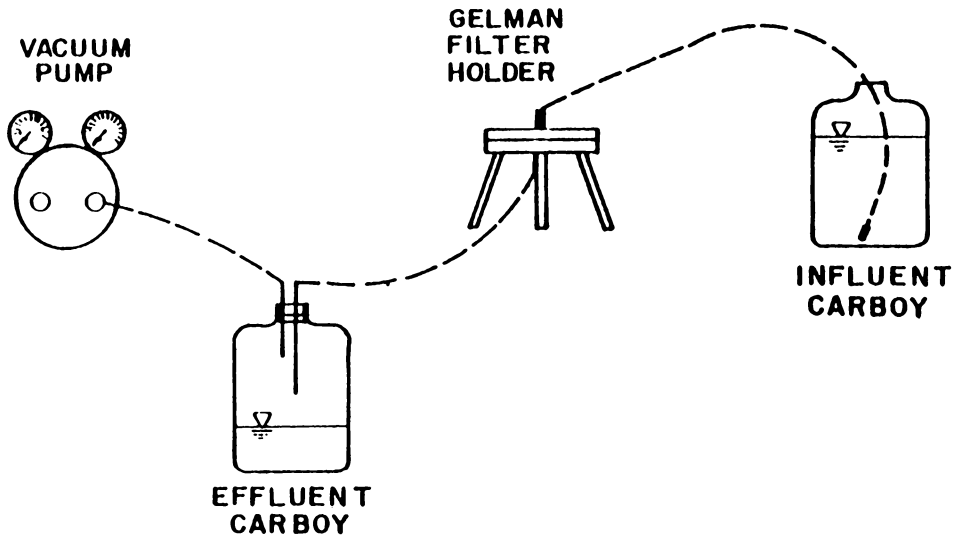

FIGURE 2. APPARATUS FOR CONCENTRATION WITH VACUUM FILTRATION

lysed to release the ATP content of the cells into solution. This was accomplished by mixing 0.1 mL samples of the influent and effluent carboys with 0.1 mL of ATP-releasing agent. A two minute contact period was allowed for the reaction to go to completion. The mixture was then injected into the photometer and the number of counts was recorded. Five tests were conducted on influent and effluent samples to obtain a reliable average value. After concentration, the filter was sliced into small pieces and submerged in 7.0 mL of distilled water and 7.0 mL of releasing agent. The solution was mixed for two minutes with sterile forceps before injection of a sample into the photometer. Twenty samples were tested and the ATP counts recorded for each sample.

In other concentration trials utilizing the ATP photometer, one cm^2 squares were cut from the filter and mixed with 0.1 mL of releasing agent. The sample was injected into the photometer and the ATP counts recorded. The relative number of bacteria on the filter was then calculated by extrapolating the number of ATP counts for the 1.0 cm^2 square over the entire effective surface area of the filter. The bacterium used in all the ATP tests was E. coli.

Mass Balance on Bacteria in the Concentration System

A mass balance around the concentrator was conducted on the bacteria after each experiment. Unfortunately, the bacterial

levels of the influent and effluent streams never balanced and in many experiments the number of bacteria in the effluent and recovered from the filter was insufficient to account for a large percentage of the influent bacteria. The equation used for the mass balance was:

$$\text{Influent} = \text{effluent} + \text{accumulation}$$

or,

$$\text{no. influent bacteria} = \text{no. effluent bacteria} + \text{no. bacteria recovered from filter}$$

An example calculation for determining the percent bacterial recovery for a typical experiment is outlined below.

Example Calculation for a Typical Experiment

Sample	MPN per 100 ml
Influent	1.5×10^7
Effluent	4.0×10^4
Filter	7.5×10^8

Total Bacteria In

Influent: $1.5 \times 10^7/100\ \text{mL} \times 19.0\ \text{L} \times 1000\ \text{mL/L} = 2.8 \times 10^9$

Total Bacteria In

Effluent: $4.0 \times 10^4/100\ \text{mL} \times 19.0\ \text{L} \times 1000\ \text{mL/L} = 7.6 \times 10^6$

Total Bacteria On

Filter: $7.5 \times 10^8/100\ \text{mL} \times 100\ \text{mL} = 7.5 \times 10^8$

Mass Balance

Influent = Effluent + Accumulation

$2.85 \times 10^9 = 7.6 \times 10^6 + 7.5 \times 10^8$

$2.85 \times 10^9 \neq 7.57 \times 10^8$

Therefore,

% Bacteria Not Accounted in Mass Balance = 73.0%

% Bacteria Passing Filter = $(7.6 \times 10^6 / 2.85 \times 10^9) \times 100$ = 0.27%

% Bacteria Recovered on Filter = $(7.5 \times 10^8 / 2.85 \times 10^9) \times 100$ = 26.0%

IV. RESULTS AND DISCUSSION

In this chapter, results obtained through experiments conducted during the development and field testing of the bacterial concentrator are presented and discussed. During the development of the bacterial concentrator, experiments were conducted to determine the effects of various additive solutions on bacterial recovery, to compare different elutriation methods and to compare various types of commercially available filter medias. Also, the reliability of the bacterial enumeration techniques used in this work, the potential use of ATP as an indicator of concentrator efficiency, the possibility of a vacuum system for bacterial concentration and the use of the concentrator in the field were investigated.

Effect of Bovine Serum on *E. coli*

An experiment was conducted to determine the optimum pH of the bovine serum solution used during elutriation. In virus concentration work, a three percent solution of bovine serum at pH 9.0 is a commonly used eluent, but it was necessary to determine if this solution was appropriate for bacterial recovery. In order to study the viability of *E. coli* in the eluent, 90 mL of three percent bovine serum solutions at pH 7.0, 8.0, and 9.0 were mixed with ten ml of an *E. coli* culture grown overnight in lactose broth at 35°C. A control consisting of distilled water

and ten mL of the E. coli culture was also prepared. After a fifteen minute contact period, the numbers of E. coli in each mixture were determined by standard plate count procedures. The results are shown in Table 1. The standard plate counts were reasonably close for all the solutions tested. It was decided to continue with the three percent bovine serum solution at pH 9.0 because it had no adverse effect on E. coli and the eluent should be more effective at higher pH levels.

Effect of the Alum Additions on E. coli

Alum is known to be a very effective additive solution for enhancing the concentration of viruses on membranes. However, when alum was used as an additive solution in this work, very low bacterial recoveries were achieved. An experiment was then conducted to determine the effect of alum on E. coli. One mL volumes of an overnight E. coli culture were placed in 100 mL of distilled water, and 0.02 N, 0.004 N, and 0.20 N solutions of alum. After a contact time of 20 minutes, the E. coli concentration was determined by the MPN technique. The results are given in Table 2. These data indicate that the bacteria were not stable in the alum solutions; e.g., the numbers of bacteria in the 0.2 N alum solution were two logs less than the bacterial numbers in the control. This is not too surprising because, as noted in Table 2, the pH levels of the alum solutions were rather low (3.4-4.2). Based on these data, it appears alum was added by

TABLE 1

EFFECT OF BOVINE SERUM ON *E. COLI*

Solution	Standard Plate Count (colonies/mL)
Distilled Water	1.14×10^7
pH 9.0, 3% Bovine Serum	9.20×10^6
pH 8.0, 3% Bovine Serum	7.25×10^6
pH 7.0, 3% Bovine Serum	9.10×10^6

TABLE 2

EFFECT OF ALUM ADDITIONS ON *E. COLI*

Solution	pH	MPN Per 100 mL
Distilled Water	5.5	4.3×10^8
0.20 N Alum Solution	3.4	4.3×10^6
0.004 N Alum Solution	4.0	9.3×10^7
0.002 N Alum Solution	4.2	2.3×10^8

the concentrator at a level that would probably reduce the influent E. coli population by about 50 percent. Because of its adverse effect on E. coli, alum was no longer used as an additive solution. This is not to say, however, that alum should never be added, because with proper pH adjustment, alum might enhance bacterial recoveries.

Comparison of Results Obtained with Different Additive Solutions

Experiments were conducted to determine if the sample stream should be chemically conditioned to enhance the adsorption of the bacteria to the membrane fibers. The efficiency of the concentrator when no additions were made was compared to the performance of the concentrator when alum or calcium carbonate was added. In each of these experiments E. coli was adsorbed to 0.45μm Millipore filters and eluted by the blender technique. The results are given in Table 3. When no additions were made, only 10.0 percent of the bacteria entering the system were recovered. When alum was added, the percent recoveries were 5, 6, and 139 percent. Bacterial recoveries achieved when calcium carbonate was added ranged from 4 to 294 percent with an average of 59 percent. Therefore, calcium carbonate was selected as the best additive solution for use in the concentrator. The calcium carbonate solutions were always adjusted to pH 6.0 with 0.2 N sulfuric acid to convert the calcium carbonate to calcium bicarbonate before use.

TABLE 3

RECOVERY OF *E. COLI* WHEN A 0.45μm MILLIPORE FILTER WAS USED[1]

Additive Solutions	*E. coli* Concentration (MPN/100 mL)		pH		Recovery (%)
	Influent	Effluent	Influent	Effluent	
none	2.3×10^7	3.9×10^3	--	--	10.0
0.0016 N Alum 5.0	9.3×10^7	$>2.4 \times 10^4$	--	--	
0.0016 N Alum	1.5×10^6	90	--	--	139.0
0.0016 N Alum + 0.0016 N Phosphate buffer	9.3×10^6	$>2.4 \times 10^5$	7.3	6.0	6.0
0.0016 N $CaCO_3$	4.3×10^6	9.3×10^3	7.6	7.8	184.0
0.0016 N $CaCO_3$ + 0.0009 N $Na_2S_2O_3$	4.3×10^7	1.5×10^4	7.5	7.6	11.0
0.0016 N $CaCO_3$ + 0.0009 N $Na_2S_2O_3$	2.3×10^7	4.3×10^4	7.2	7.5	110.0
0.0016 N $CaCO_3$ + 0.0009 N $Na_2S_2O_3$	2.3×10^7	4.0×10^3	7.6	7.8	50.0
0.0016 N $CaCO_3$ + 0.0009 N $Na_2S_2O_3$	2.3×10^7	1.5×10^4	7.6	7.7	20.0
0.0016 N $CaCO_3$ + 0.0009 N $Na_2S_2O_3$	9.3×10^6	<3.0	7.9	8.0	17.0
0.0016 N $CaCO_3$ + 0.0009 N $Na_2S_2O_3$	7.5×10^6	9.3×10^2	7.8	7.9	61.0
0.0016 N $CaCO_3$ + 0.0009 N $Na_2S_2O_3$	9.3×10^6	1.5×10^4	7.8	7.9	42.0
0.0016 N $CaCO_3$ + 0.0009 N $Na_2S_2O_3$	4.3×10^6	<3.0	7.6	7.6	294.0

TABLE 3 (Con't)

Additive Solutions	*E. coli* Concentration (MPN/100 ml)		pH		Recovery (%)
	Influent	Effluent	Influent	Effluent	
0.0016 N $CaCO_3$ + 0.0009 N $Na_2S_2O_3$	$1.5x10^5$	23	7.8	7.8	20.0
0.0016 N $CaCO_3$ + 0.0009 N $Na_2S_2O_3$	$1.5x10^5$	15	7.6	7.7	17.0
0.0016 N $CaCO_3$ + 0.0009 N $Na_2S_2O_3$	$1.5x10^5$	4	7.6	7.7	41.0
0.0016 N $CaCO_3$ + 0.0009 N $Na_2S_2O_3$	$9.3x10^3$	<3	7.8	7.8	16.0
0.0016 N $CaCO_3$ + 0.0009 N $Na_2S_2O_3$	$4.3x10^3$	<3	7.9	--	66.0
0.0016 N $CaCO_3$ + 0.0009 N $Na_2S_2O_3$	$4.3x10^2$	<3	7.6	7.8	4.0
0.0016 N $CaCO_3$ + 0.0009 N $Na_2S_2O_3$	$4.3x10^2$	<3	--	7.2	41.0
0.0016 N $CaCO_3$ + 0.0009 N $Na_2S_2O_3$	$4.3x10^2$	<3	8.1	7.9	14.0

[1]Experimental Conditions: flowrate - 2 L/min; elutriation conditions - 3% bovine serum, pH 9.0, 15 min. contact, blender technique.

Comparison of Elutriation Techniques

Experiments were conducted to determine the most efficient of three elutriation techniques. The results of these experiments are shown in Tables 4 and 5 and are discussed below. In Table 4, the results obtained with 0.45μm Cox filters show a 1.0 percent bacterial recovery when elutriation was accomplished by slicing the filter into strips and agitating the filter strips with 50 mL of 3 percent bovine serum solution in a graduated cylinder. Recoveries of 40 percent and 23 percent were obtained when the filter was blended in the bovine serum solution and then mixed gently in the solution for 15 minutes. Although very few experiments were conducted with Cox filters, the results indicated that the blender technique was much more efficient than using the Petri dish elutriation technique.

As is illustrated by the data in Table 5, the blender technique appeared to also be superior to the Petri dish technique when Millipore filters were used. In a concentration experiment with no additive solutions, a 0.1 percent bacterial recovery was obtained when elutriation was accomplished by slicing the filter in a Petri dish and adding 50 mL of bovine serum solution for a 15 minute contact time. The bacterial recovery improved to 10.0 percent when a blender was used in the elutriation step as described above.

Also in Table 5, it is shown that the blender technique provided better bacterial recoveries than the Petri dish

TABLE 4

RECOVERY OF *E. COLI* WHEN COX FILTERS WERE USED[1]

Filter Porosity (u)	Additive Solutions	*E. coli* Concentration (MPN/100 mL)		Elutriation Method		Filter Recovery (%)
		Influent	Effluent	Contact Time (min)	Technique	
5.0 3.0	None	9.3×10^6	9.3×10^6	15	Petri dish	
5.0	0.0016 N Alum	2.3×10^7	9.3×10^5	15	blender	1.0
0.45	0.0016 N Alum	9.3×10^6	$<3 \times 10^2$	15	grad. cyl.	1.0
0.45	0.0006 N $CaCO_3$ + 0.0009 N $Na_2S_2O_3$	1.5×10^7	4×10^2	15	blender	40.0
				40	blender	23.0

[1]Experimental Conditions: flow rate - 2 l/min; elutriation conditions - 3% bovine serum, pH 9.0.

TABLE 5

RECOVERY OF *E. COLI* WHEN 0.45μm MILLIPORE FILTERS AND DIFFERENT ELUTRIATION METHODS WERE USED[1]

Additive Solutions	*E. coli* Concentration (MPN/100 mL) Influent	Effluent	Flowrate (L/min)	Elutriation Method Contact Time (min)	Technique	Filter Recovery (%)
none	9.3×10^6	$<3.0 \times 10^6$	1.5	15	petri dish	0.1
none	2.3×10^7	3.9×10^3	2.0	15	blender	10.0
				30	blender	10.0
0.0016 N Alum	1.5×10^6	$<3.0 \times 10^2$	0.3	15	petri dish	2.0
0.0006 N Alum	9.3×10^7	$>2.4 \times 10^4$	1.7	15	blender	5.0

[1]Elutriation Conditions: 3% bovine serum, pH 9.0.

technique when a 10 mg/mL solution of alum sulfate was added during concentration. However, in this experiment, the recovery was only marginally better which was probably due to the effect of the alum additive solution.

An experiment was conducted to determine if bacteria were inactivated during the blending of the filters. A sample was prepared by inoculating 19.0 liters of dechlorinated tap water with 10 mL of E. coli culture grown overnight in lactose broth at 35°C. Ten ml volumes of the sample were then diluted in 90 mL of distilled water and 90 mL of three percent bovine serum solution. Bacterial levels in the two mixtures were then determined by SPC methods before and after the dilutions were mixed in an electric blender for 20 seconds. To detect any effect a membrane filter might have on bacterial recoveries during the blending operation, a sterile 0.45μm Millipore filter was placed in 10 mL of the sample and 90 mL of three percent bovine serum and blended. It appeared from the results of this experiment (see Table 6) that the bacteria were not inactivated by blending.

After these few initial comparison tests were completed, the blender technique was used in nearly all experiments for bacterial elutriation. Unless otherwise noted in the following sections, the reader should assume that the blender technique was used in the elutriation step.

TABLE 6

EFFECTS OF USING A BLENDER TO ELUTE E. COLI

Test Conditions	Standard Plate Count (colonies per ml)
Place 10 ml of sample in 90 ml of distilled water. Allow a contact time of 15 minutes.	1.6×10^4
Place 10 ml of sample in 90 ml of 3% bovine serum at pH 9.0 Allow a contact time of 15 minutes.	2.0×10^4
Place 10 ml of sample and 90 ml of distilled water in blender. Mix 30 seconds. Allow a contact time of 15 minutes.	1.6×10^4
Place 10 ml of sample and 90 ml of 3% bovine serum at pH 9.0 in blender. Mix 30 seconds. Allow a contact time of 15 minutes.	2.0×10^4
Place 10 ml of sample, 90 ml of 3% bovine serum at pH 9.0, and a sterile 0.45μm, 142 mm Millipore filter in blender. Mix 30 seconds. Allow a contact time of 15 minutes.	2.5×10^4

Variations in the Blender Technique for Elutriation

After the blender technique was selected as the best method of elutriation, slight variations of the technique were tested in an attempt to improve bacterial recoveries. Changes in the contact time, the temperature during the period of contact, and the percent of bovine serum in solution were evaluated. The results of these experiments are discussed in the following paragraphs.

Effects of Temperature on Elutriation Efficiency

After being mixed in a blender, the bovine serum and filter pieces were poured into a sterile 150 mL beaker with a stir bar and mixed on a magnetic stirrer for various contact periods. Experiments were conducted to determine if the release of the bacteria into the solution would be better at room temperature or at 4°C during the contact period. The results in Table 7 show that the room temperature tests always provided equal or better recoveries of the bacteria. In these experiments, the assumption was made that the bacteria would be uniformly distributed across the surface area of the filter. The filter was sliced in half and one half was placed in a 4°C refrigerator during the contact period, while the other half was kept at room temperature. The room temperature varied from 27 to 30°C during these experiments.

TABLE 7

EFFECT OF TEMPERATURE ON THE RECOVERY OF *E. COLI* ADSORBED TO 0.45μm MILLIPORE FILTERS[1]

Additive Solutions	*E. coli* Concentration (MPN/100 mL)		pH		Elutriation Method Contact Time (min)	Temperature (°C)	Filter Recovery (%)
	Influent	Effluent	Influent	Effluent			
0.0016 N $CaCO_3$ + 0.0009 N $Na_2S_2O_3$	4.3×10^7	1.5×10^4	7.5	7.6	15	4	11.0
					15	room	53.0
0.0016 N $CaCO_3$ + 0.0009 N $Na_2S_2O_3$	2.3×10^7	1.5×10^4	7.6	7.7	15	4	20.0
					35	4	4.0
					15	room	20.0
0.0016 N $CaCO_3$ + 0.0009 N $Na_2S_2O_3$	7.5×10^6	9.3×10^3	7.8	7.9	15	4	61.0
					35	4	132.0

[1]Experimental Conditions: flowrate - 2 L/min; elutriation conditions - 3% bovine serum, pH 9.0, blender technique

Because bacterial recoveries were usually better at room temperature, the elutriation step in studies conducted hereafter was generally performed at room temperature.

Effect of Bovine Serum Strength on Elutriation

The strength of the bovine serum solution used to elute the bacteria from the filter was varied to determine if serum level could be correlated to bacterial recovery. It was thought that a more concentrated solution of bovine serum might compete more effectively for membrane adsorption sites than weaker solutions. The data presented in Table 8 indicate that the 3 percent bovine serum solution was more effective than the 6 percent solution. It is interesting to note that the 6 percent solution appeared to yield better recoveries at 4°C than at room temperature. However, this matter was not pursued since the 3 percent bovine serum appeared to be more effective.

Effect of Contact Time During Elutriation

Studies were conducted to determine whether contact time between filters and the eluent could be correlated to bacterial recovery. However, no obvious correlation between contact time and percent recoveries was noted (Table 9). Although rather arbitrarily selected, a fifteen-minute contact period was generally used in the concentration trials.

TABLE 8

EFFECT OF BOVINE SERUM CONCENTRATION ON THE RECOVERY OF *E. COLI* ADSORBED TO 0.45μm MILLIPORE FILTERS[1]

Additive Solutions	*E. coli* Concentration (MPN/100 ml) Influent	Effluent	pH Influent	Effluent	Bovine Serum (%)	Elutriation Contact Time (min)	Temperature (°C)	Filter Recovery (%)
0.0016 N $CaCO_3$ + 0.0009 N $Na_2S_2O_3$	2.3×10^7	4.3×10^4	--	8.1	6	15	room	21.0
					6	25	4	55.0
0.0016 N $CaCO_3$ + 0.0009 N $Na_2S_2O_3$	2.3×10^7	4.3×10^4	7.2	7.5	3	15	room	110.0
					3	35	room	20.0
					6	15	room	10.0

[1]Experimental Conditions: flowrate - 2 L/min; elutriation conditions - pH 9.0, blender technique

TABLE 9

EFFECT OF ELUTRIATION CONTACT TIME ON THE RECOVERY OF *E. COLI* ABSORBED TO 0.45μm MILLIPORE AND COX FILTERS[1]

Filter Type	Additive Solutions	E. coli Concentration (MPN/100 ml) Influent	E. coli Concentration (MPN/100 ml) Effluent	Elutriation Contact Time (min)	Filter Recovery (%)
Millipore	0.0016 N $CaCO_3$ 0.0009 N $Na_2S_2O_3$	2.3×10^7	4.3×10^4	15 35	110.0 20.0
Millipore	0.0016 N $CaCO_3$ 0.0009 N $Na_2S_2O_3$	2.3×10^7	1.5×10^4	15 35	20.0 4.0
Millipore	0.0016 N $CaCO_3$ 0.0009 N $Na_2S_2O_3$	9.3×10^6	<3.0	25 45	17.0 5.0
Millipore	0.0016 N $CaCO_3$ 0.0009 N $Na_2S_2O_3$	7.5×10^6	9.3×10^3	15 35	61.0 132.0
Cox	0.0016 N $CaCO_3$ 0.0009 N $Na_2S_2O_3$	1.5×10^7	4.0×10^2	15 40	40.0 23.0
Millipore	0.0016 N $CaCO_3$ 0.0009 N $Na_2S_2O_3$	9.3×10^6	1.5×10^4	15 35	42.0 109.0
Millipore	0.0016 N $CaCO_3$ 0.0009 N $Na_2S_2O_3$	4.3×10^6	<3.0	15 30	294.0 593.0
Millipore	0.0020 N $CaCO_3$ 0.0009 N $Na_2S_2O_3$	$<3.0 \times 10^4$	<300	15 30 45	163.0 163.0 75.0
Millipore	0.0020 N $CaCO_3$ 0.0009 N $Na_2S_2O_3$	4.6×10^7 9.3×10^5	--	15 30 45	21.0 45.0 45.0

[1]Experimental Conditions: flow rate - 2 L/min; influent and effluent pH - 7.5; elutriation conditions - 3% bovine serum, pH 9.0, blender techniques.

Effectiveness of Filters

Experiments were conducted to determine the most efficient type of filter to use with the water sample concentrator. As indicated earlier, the filters tested were manufactured by AMF, Cox Instrument, and Millipore Corporation. The filters obtained from AMF were referred to as zeta-plus filters. The results of experiments with the zeta-plus filters are provided in Table 10. Zeta-plus filters with a pore size range of 0.4-0.6μm yielded a bacterial recovery of about one to three percent. The zeta-plus filters were approximately 1/4 inch thick and required a special rubber gasket for use in the Gelman filter holder. During elutriation with the bovine serum solution in the blender, the zeta-plus filters came apart in thick fibers which were very hard to mix thoroughly and made the dilution work for MPN testing difficult. The low recoveries are attributed in part to the poor mixing achieved due to the thick fiber structure of the zeta-plus filters. It is likely that many bacteria were simply locked into the filter matrix. It is also likely that many bacteria were able to escape through the filter with a larger porosity. Due to the low levels of recovery and problems experienced during blending, the zeta- plus filters were not selected for further study.

As was shown in Table 4, the 5.0μm Cox filter yielded a bacterial recovery of only 3.0 percent. As indicated by the levels of bacteria in the effluent, most, if not all, of the

TABLE 10

CONCENTRATION OF *E. COLI* USING ZETA-PLUS FILTERS[1]

Filter Porosity	Additive Solutions	*E. coli* Concentration (MPN/100 ml)		pH		Elutriation Method		Filter Recovery (%)
		Influent	Effluent	Influent	Effluent	Contact Time (min)	Technique	
0.4-0.6 μm	0.0009 N $Na_2S_2O_3$	1.5×10^7	9.3×10^3	7.7	7.7	15	blender	13.0
0.7-0.3 μm	0.0009 N $Na_2S_2O_3$	2.3×10^7	1.1×10^5	7.6	7.6	15	grad. cyl.	3.0
						35	grad. cyl.	<1.0
						50	grad. cyl.	3.0

[1]Experimental Conditions: flowrate - 2 L/min; elutriation conditions - 3% bovine serum, pH 9.0.

bacteria were able to pass through the filter. Hence, adsorption of the bacteria on the filter was not as important a mechanism for recovering bacteria as was originally anticipated. The data in Table 4 also indicate that when the 0.45μm Cox filter and blender elutriation technique were used, bacterial recoveries of 23 and 40 percent were accomplished.

Bacterial recoveries with 0.45μm Millipore filters ranged from less than one percent to greater than 100 percent. The Millipore filter was thinner and more brittle than either the Cox or zeta-plus filters. During elutriation in the blender, the Millipore filter was shredded into very fine pieces and this seemed to allow good contact between the filter and bovine serum solution. Hence, although the Millipore filter was very fragile and more susceptible to tearing than the other filters, it was much easier to use in the concentrator and yielded better bacterial recoveries. Most of the subsequent experiments in this study were therefore conducted with the 0.45μm Millipore membrane filter.

Fate of Bacteria in Concentrator without Filter

After the bacterial recoveries with the water sample concentrator were found to be much lower than expected, an experiment was conducted to determine if the bacteria were surviving the concentration apparatus and reaching the filter in a viable state. One concentration trial was conducted with alum and

sodium thiosulfate additive solutions and another was conducted with calcium carbonate and sodium thiosulfate additive solutions. In these experiments, the 19.0 liters of influent sample was pumped through the concentration apparatus as in the usual concentration experiments except a filter was not placed in the filter holder. Samples of the influent were taken for MPN analysis at time zero and after seven minutes of pumping. The effluent MPN sample was taken as soon as the pumping was completed. The results as shown in Table 11 indicate that the bacteria survived the concentration and probably became trapped on the filters during normal concentration experiments. The poor recoveries can probably be attributed to the inability to efficiently remove the adsorbed bacteria from the filter or to inaccuracies in the MPN enumeration. The test with the alum additive solution had a low effluent pH of 4.3. From previous results, one would expect this low pH to have an adverse effect on the bacteria but this was not shown in this experiment. However, this result may not be accurate due to the variations in MPN assays.

Vacuum Filtration Experiments

Vacuum filtration experiments were conducted to determine if pulling the sample through the filter in the Gelman filter holder would allow better recoveries than forcing the sample through the filter with the Manostat pump. The results of these experiments

TABLE 11

FATE OF *E. COLI* IN THE CONCENTRATION APPARATUS WITHOUT A FILTER

Additive Solutions	*E. coli* Concentration (MPN/100 mL)			pH	
	Influent				
	Initial	After 7 min.	Effluent	Influent	Effluent
0.0017 N Alum + 0.0009 N $Na_2S_2O_3$	9.3×10^6	4.3×10^6	9.3×10^6	7.6	4.3
0.0016 N $CaCO_3$ + 0.0009 N $Na_2S_2O_3$	2.3×10^6	9.3×10^6	7.5×10^6	7.3	7.5

are shown in Table 12. For the three experiments, the average recovery was better than 58 percent. Although the recoveries were good with vacuum filtration, the system operated at a flowrate of 0.2 L/min which was much lower than the 2 L/min flowrates that were readily achieved when a pump was used to drive fluid through the system. Because a high flowrate was essential in order for the concentrator to process large volumes of water in a reasonable period of time, the vacuum filtration technique was not pursued further in this study.

Reproducibility of the Most Probable Number Test

Throughout this study, the three tube MPN test was used for enumeration of bacteria. The three tube test was selected over the five tube test for the conveniences of reducing dilution work and the volume of lactose broth required in each experiment. After widely varying bacterial recoveries were obtained in the concentration experiments, three experiments were conducted to determine the reproducibility of the three tube MPN test. In each experiment 19 L of dechlorinated tap water was inoculated with 1.0 mL of an overnight E. coli culture. Several samples of the water were then taken for bacterial enumeration by the MPN method. The results of these three trials are given in Table 13. The three tube MPN test was found to be highly variable. In the first experiment, the average MPN was 1.6×10^6 per 100 mL with a range of 9.3×10^4 to 4.3×10^6 per 100 mL and a standard

TABLE 12

EFFECT OF VACUUM INDUCED FLOW ON THE RECOVERY OF *E. COLI* by 0.45μm MILLIPORE FILTERS[1]

Additive Solutions	*E. coli* Concentration (MPN/100 mL) Influent	Effluent	Flowrate (L/min)	Filter Recovery (%)
none	4.3×10^6	<3.0	0.20	112.0
none	9.3×10^6	40	0.20	34.0
none	1.5×10^7	--	0.25	29.0

[1]Elutriation conditions: 3% bovine serum, pH 9.0, 15 minutes contact time, blender technique.

TABLE 13

REPRODUCIBILITY OF THE MOST PROBABLE NUMBER TEST

FOR THE ENUMERATION OF *E. COLI*

Trial 1		Trial 2		Trial 3	
Sample	MPN/100 ml	Sample	MPN/100 ml	Sample	MPN/100 ml
1	4.3×10^6	1	9.3×10^5	1	2.4×10^7
2	7.5×10^5	2	4.6×10^7	2	4.6×10^7
3	9.3×10^4	3	9.3×10^5	3	2.4×10^7
4	2.4×10^6	4	2.4×10^7	4	9.3×10^6
5	9.3×10^5	Average =	1.8×10^7	5	9.3×10^6
6	2.4×10^6			6	2.4×10^7
7	9.3×10^5			7	4.6×10^7
8	9.3×10^5			8	2.4×10^7
Average =	1.6×10^6			9	2.4×10^7
				10	1.1×10^8
				Average =	3.4×10^7

deviation of 1.3 x 10^6 per 100 mL. In the second experiment an average MPN of 1.8 x 10^7 per 100 mL with a range of 9.3 x 10^5 to 4.6 x 10^7 per 100 mL was obtained and the standard deviation was 2.1 x 10^7 per 100 mL. The third experiment's average MPN was 3.4 x 10^7 per 100 mL with a range of 9.3 x 10^6 to 1.1 x 10^8 per 100 mL and a standard deviation of 2.9 x 10^7 per 100 mL. Of course, the wide variance in these results is due, in part, to dilution errors which cannot be avoided, but these data serve as a good example of the variation associated with three tube MPN testing. Unfortunately, this large variation tends to cast doubt on the reliability of some of the results obtained in the concentration trials.

Bacterial Enumeration by Membrane Filtration and MPN Methods

Experiments were conducted to determine concentrator performance based on enumeration of the bacteria by both the membrane filtration and most probable number techniques. In recent years, there has been a controversy over which of these two techniques provide the more reliable results. One of the main issues in the debate is the question of which technique is better able to detect stressed microorganisms. It is generally recognized that the MPN method is better able to detect stressed organisms.

Table 14 shows the results of trials in which MF and MPN analyses were conducted on the same water samples. In approximately 40 percent of the trials the MF and MPN results were

TABLE 14

TITRATION OF *E. COLI* USING MPN AND MF METHODS

Trial Number	Organisms/100 mL	
	MPN	MF
1	230	200
2	430	153
3	150	142
4	93	166
5	430	113
6	230	108
7	93	95
8	230	82
9	230	95

almost identical. In the other trials there was a large difference between the MF and MPN results. Generally the MF value was considerably lower than the MPN result.

Table 15 shows the results of concentration experiments in which both MF and MPN tests were performed. Generally, the MF and MPN results for the influent and effluent E. coli concentrations agreed fairly well. However, there was a wide variance in the calculated bacterial recoveries based on either MF or MPN data. Note that generally the MPN data appeared to provide more believable recoveries than were determined through use of the MF data.

Determination of Concentrator Performance Through ATP Analyses

Three experiments were conducted to evaluate the efficiency of the concentrator by measuring adenosine triphosphate (ATP) levels. Samples of the influent, filter, and effluent in a typical concentration trial were analyzed for ATP with a photometer. The water samples were digested in a releasing agent (manufactured by SAI) for two minutes to allow the E. coli cells to release their ATP into solution.

The results of these experiments are shown in Table 16. In the first trial, more ATP was detected in the effluent than in the influent and 11.2 percent of the ATP was found on the filter. According to a mass balance, this result is impossible, but the results may have been due to assay problems and/or contamination

TABLE 15

EVALUATION OF CONCENTRATOR PERFORMANCE BY MPN AND MF METHODS[1] WHEN 0.45μm MILLIPORE FILTERS WERE USED

Method	No. E. coli/100 mL		Flowrate (L/min)	pH		Filter Recovery (%)
	Influent	Effluent		Influent	Effluent	
MPN	230	<3	2	8.1	--	26
MF	50	18				22,400
MPN	230	<3	2	--	--	123
MF	200	--				109
MPN	150	4	2	8.0	8.0	1,052
MF	142	20				3,098
MPN	230	<3	2	8.1	8.1	47
MF	113	<3				847
MPN	93	<3	2	8.3	8.2	68
MF	95	<3				--
MPN	230	<3	2	--	--	43
MF	82	-				344
MPN	430	<3	1.2	7.8	7.8	66
MF	153	4				6,800
MPN	93	<3	1.2	8.0	--	658
MF	4,067	--				107
MPN	93	<3	1.2	7.5	8.0	149
MF	166	--				284
MPN	230	<3	1.2	--	8.0	43
MF	95	3				111

[1]Experimental Conditions: additive solutions - 0.0001 N $CaCO_3$, 0.0009 N $Na_2S_2O_3$; elutriation conditions - 3% bovine serum, pH 9.0, 15 minute contact time, blender technique.

TABLE 16

DETERMINATION OF CONCENTRATOR PERFORMANCE THROUGH ATP ANALYSES[1]

Trial	Sample	ATP Level (ng)	ATP Recovery Efficiency (%)	Organism Level (MPN/100 ml)
1	Influent	5452	--	--
	Effluent	7509	>100	--
	Filter	610	11.2	--
2	Influent	ND[2]	--	9.3×10^1
	Effluent	ND	--	3
	Filter	ND	--	--
3	Influent	2528	--	2.3×10^7
	Effluent	18	0.7	9.3×10^3
	Filter	499	19.8	--

[1]Experimental Conditions: organism - E. coli; flowrate - 2 L/min; sample size - 19L; additive solutions - 0.0001 $CaCO_3$, 0.0009 $Na_2S_2O_3$; filter - 0.45μm Millipore; filter digested in SAI releasing agent to liberate ATP.

[2]ND = not detectable.

of the effluent carboy. The influent bacterial concentration was greatly reduced in the second trial to 93 per 100 mL. This low level of bacteria was not detectable on the ATP photometer. Therefore, the ATP photometer generally cannot be used in the bacterial analysis of drinking water. Good results were obtained in the third trial and an ATP recovery of approximately 20 percent was obtained on the filter. Hence, there may be some potential for using ATP to monitor concentrator performance in lab situations where biomass levels are high, but it does not appear that the technique can be directly applied in the testing of potable waters.

Concentration Experiments with Streptococcus Faecalis

In most of the experiments E. Coli was utilized, but three experiments were conducted in which Streptococcus faecalis was the bacterium concentrated. These experiments were conducted using conditions thought to be optimal for the concentration and elutriation of E. Coli. Influent Streptococcus faeacalis concentrations ranged between 4.3×10^5 and 2.1×10^6 organisms per 100 mL. The results of these experiments are shown in Table 17. The concentrator was very effective in concentrating Streptococcus faecalis, yielding an average recovery of greater than 78 percent.

TABLE 17

CONCENTRATION OF *STREPTOCOCCUS FAECALIS* ON 0.45μm MILLIPORE FILTERS[1]

Additive Solutions	*S. faecalis* Concentration (MPN/100 mL) Influent	Effluent	Filter Recovery (%)
0.0006 N $CaCO_3$ + 0.0009 N $Na_2S_2O_3$	2.1×10^6	2.3×10^2	60.0
0.0006 N $CaCO_3$ + 0.0009 N $Na_2S_2O_3$	4.3×10^5	4.0×10^1	44.0
0.0006 N $CaCO_3$ + 0.0009 N $Na_2S_2O_3$	9.3×10^5	3	130.0

[1]Experimental Conditions: flowrate - 2 L/min; elutriation conditions - 3% bovine serum, pH 9.0, 15 minute contact time, blender technique.

Field Concentration Experiments

The water sample concentrator was field tested in a small town in Virginia where there had been recent complaints of bad taste and odor in the water. Two concentration experiments using Millipore filters with 0.45μm porosity were conducted at the water treatment plant on the finished water, and one concentration experiment was conducted in the distribution system at an elementary school. The results of these experiments are shown in Table 18.

No coliforms were detected in grab samples by means of the standard MPN test in the finished water at the water treatment plant. However, after concentration of 19.0 liters of the finished water, coliforms were detected. Two concentration trials were conducted, resulting in the detection of nine coliforms per 100 mL and 3 coliforms per 100 mL, respectively.

Of two grab samples taken for standard MPN analysis at the elementary school, one resulted in no coliform detection and the other detected four coliforms per 100 mL. Following concentration of 19.0 liters of the elementary school tap water, an MPN analysis resulted in the detection of 93 coliforms per 100 mL.

Grab samples and concentrated samples were analyzed for Streptococcus faecalis at both the water treatment plant and the elementary school but no Streptococcus faecalis bacteria was detected in any of the samples.

TABLE 18

RESULTS OF THREE FIELD EXPERIMENTS IN WHICH

0.45μmMILLIPORE FILTERS WERE USED[1]

Sample Collection Site	*E. coli* Concentration (MPN/100 mL)		Filter Recovery (MPN/100 mL)
	Influent	Effluent	
Finished water at treatment plant	<3	--	9
Finished water at treatment plant	<3	--	3
Tap water at elementary school	<3 4	3	93

[1]Experimental Conditions: flowrate - 2 L/min; influent and effluent pH - 8.5; turbidity - 0.29 NTU; additive solutions - 0.0001 N $CaCO_3$, 0.0009 N $Na_2S_2O_3$; elutriation conditions - 3% bovine serum pH 9.0, 15 minute contact time, blender technique.

V. SUMMARY

The purpose of this study was to develop a water sample concentrator for the collection of representative bacterial samples. The concentrator would sample large quantities of water (19 liters) in order to improve the reliability of the present methods of bacterial enumeration which only require samples of 100 mL or less. The concentration method studied was a membrane adsorption/elutriation technique. The water sample was pumped through 142 mm diameter membrane filters of various porosities and then eluted from the filter with a three percent bovine serum solution at pH 9.0. Either a standard MPN or MF test was then performed to determine the level of bacteria in the bovine serum solution.

Of the three methods of elutriation studied, the best results were obtained when the filter and bovine serum solution were mixed in an electric blender. Adequate contact between the filter and the bovine serum could not be obtained when using the technique of slicing the filter into strips and eluating in a large Petri dish or a graduated cylinder. The 0.45μm Millipore filter was best suited for the blender elutriation technique because it easily separated into fine pieces during blending. The Cox filters were thicker and tougher than the Millipore filters and were difficult to shred with the blender. The zeta-plus filters were fluffy and the fibers made the MPN test on the bovine serum solution nearly impossible. The good contact

of the Millipore filter with the serum is postulated to be the reason for the better elutriation of the bacteria.

The additive solutions which were tested during the development of concentrator were alum, calcium carbonate and sodium thiosulfate. Sodium thiosulfate was added to dechlorinate the sample prior to the filter to avoid any disinfection during concentration. Alum and calcium carbonate were added to enhance the adsorption of the bacteria to the filter. Results with alum addition were usually poor and this is attributed to the low pH of the effluent created by the alum addition. Calcium carbonate was selected as the better additive solution, but from the data it would be hard to defend whether or not calcium carbonate additions actually had any effect because such low concentrations were used.

Various elutriation experiments were conducted with different concentrations of bovine serum, varying contact times and different temperatures. It was determined that the best elutriation was obtained when 100 mL of three percent bovine serum solution was mixed with the filter in a blender for 30 seconds and then decanted into a sterile 250 mL beaker. A 25 mL portion of three percent bovine serum solution was then used to rinse the blender to obtain any bacteria which had adhered to the sides. This 25 mL portion was also decanted into the 250 mL beaker and the contents of the beaker were mixed with a stir bar

to allow 15 minutes contact. A MPN test was then conducted on the contents of the beaker.

The best results of this study were obtained when the bacterial concentrator containing a 0.45μm Millipore filter sampled a 19 liter sample at a flowrate of two liters per minute while proportionately adding calcium carbonate and sodium thiosulfate solutions. As mentioned above, the best elutriation and recovery of bacteria was obtained when 125 mL of three percent bovine serum solution at pH 9.0 were mixed with the filter in an electric blender for 30 seconds and then allowed 15 minutes of contact. The water sample concentrator operated in the above manner yielded recoveries of E. coli in the area of 17 percent to 110 percent with an average recovery of 48 percent. In experiments with Streptococcus faecalis bacterial recoveries ranged from 44 percent to 130 percent with an average of 78 percent.

Experiments conducted to determine the reproducibility of the MPN and MF results indicated a wide variance in the results. This wide variance makes much of the concentrator work questionable. Statistically, sufficient concentration experiments were probably not conducted to determine whether certain variations in the concentration technique actually affected the recovery efficiency. Based on the limited number of trials and the results obtained, it is felt that the best experimental

conditions were selected for the successful operation of the bacterial concentrator.

A major weakness of this study was that the efficiency of the concentrator was based on the MPN test. That is, the MPN test was used to determine the number of bacteria in the influent sample. After concentration, the MPN test was used to determine the number of bacteria eluted from the filter and in the effluent from the concentrator. Any error or variance in the MPN test results would affect the calculation of bacterial recovery for a given experiment. Therefore, any weaknesses inherent to the MPN test would be translated into a concentrator weakness.

VI. CONCLUSIONS

An analysis of the information presented in this thesis has led the researcher to draw the following conclusions:

1. The importance of developing a water sample concentrator for the routine analysis of drinking water was shown in the field trials in which coliform bacteria were detected in water distribution system samples upon concentration of 19 liters of water, while no coliforms were detected in a grab sample by the standard MPN method.
2. The bacterial recovery accomplished by the water sample concentrator, when E. coli was the bacterium concentrated, ranged from four to greater than 100 percent with an average of 48 percent. Streptococcus faecalis was recovered in the range from 60 to greater than 100 percent with an average recovery of 78 percent.
3. The mass balance of bacteria in and out of the bacterial concentrator was never achieved due to the variability of the MPN and MF results and errors associated with the elutriation technique. Few to no bacteria were detected in the effluents from the concentrator so bacteria were apparently trapped on the membrane filter.

4. An average E. coli recovery of 58 percent was obtained when 19 liters of water sample were concentrated by vacuum induced flow rather than using a varistaltic pump. Although good recoveries were obtained with this technique, the flow rates were low so sampling large volumes of fluid in the field would not be practical.
5. Monitoring bacterial levels through adenosine triphosphate analysis was possible with extremely high bacterial concentrations, but bioassays by ATP analysis would not be possible at the low level of bacteria normally present in potable water.

REFERENCES

1. Andelman, J. B., and Caruso, S. C., "Concentration and Separation Techniques." In Water and Water Pollution Handbook - Volume 2, L. L. Ciaccio, ed., Marcel Dekker, Inc., New York, NY, pp. 483-582 (1972).

2. Baum, S. J., Introduction to Organic and Biological Chemistry, Second Edition, MacMillan Publishing Co., Inc., New York, NY, p. 230 (1978).

3. Bissonette, G. K., Jezeski, J. J., McFeters, G. A., and Stuart, D. G., "Influence of Environmental Stress on Enumeration of Indicator Bacteria from Natural Waters." Applied Microbiology, 29, 186-194 (1975).

4. Bissonnette, G. K., Jezeski, J. J., McFeters, G. A., and Stuart, D. G., "Evaluation of Recovery Methods to Detect Coliforms in Water." Applied and Environmental Microbiology, 33, 590-595 (1977).

5. Clark, J. R., "Estimating the Biomass of Aufwuchs using Adenosine Triphosphate Assays." Thesis, Virginia Polytechnic Institute and State University, Blacksburg, Virginia, December (1976).

6. Clausen, E. M., Green, B. L., and Litsky, W., "Fecal Streptococci: Indicators of Pollution." in Bacterial Indicators/Health Hazards Associated with Water, ASTM STP 635, A. W. Hoadley and B. J. Dutka, eds., American Society for Testing and Materials, Philadelphia, PA, pp. 247-264 (1977).

7. Commonwealth of Virginia/State Board of Health, "Waterworks Regulations." Section 4.05 "Bacteriological Quality", Richmond, Virginia (1982).

8. Dufour, A. P., "Escherichia Coli: The Fecal Coliform." in Bacterial Indicators/Health Hazards Associated with Water, ASTM STP 635, A. W. Hoadley and B. J. Dutka, eds., American Society for Testing and Materials, Philadelphia, PA, pp. 48-58 (1977).

9. Dugan, P. R., "Bacterial, Non-Bacterial, and Chemical Indicators of Environmental Quality." Organisms and Biological Communities as Indicators of Environmental Quality - A Symposium, Ohio State University, March 25, 1974, 34-38 (1974).

10. Dutka, B. J., and Bell, J. A., "Isolation of Salmonellae from Moderately Polluted Waters." Journal Water Pollution Control Federation, 45, 316-323 (1973).

11. Dutka, B. J., "Coliforms Are an Inadequate Index of Water Quality." Journal of Environmental Health, 36, 39-46, (1973).

12. Dutka, B. J., "Microbiological Indicators Problems and Potential of New Microbial Indicators of Water Quality." in Biological Indicators of Water Quality. A. James and L. Evison, eds., John Wiley & Sons Publishers, New York, NY, p. 18-1 - 18-21 (1979).

13. Egan, A. F., "Enumeration of Stressed Cells of Escherichia Coli." Canadian Journal of Microbiology, 25, 116-118 (1979).

14. Farrah, S. R., Geeba, C. P., Wallis, C., and Melvick, J. C., "Concentration of Viruses from Large Volumes of Tap Water Using Pleated Membrane Filters." Applied and Environmental Microbiology, 31, 221-226 (1976).

15. Farrah, S. R., Geeba, C. P., Goyal, S. M., Wallis, C., and Melvick, J. L., "Regeneration of Pleated Filters Used to Concentrate Enteroviruses from Large Volumes of Tap Water." Applied and Environmental Microbiology, 33, 308-311 (1977).

16. Farrah, S. R., and Bitton, G., "Elution of Poliovirus Adsorbed to Membrane Filters." Applied and Environmental Microbiology, 36, 982-984 (1978).

17. Geldreich, E. E., Nash, H. D., Reasoner, D. J., and Taylor, R. H., "The Necessity of Controlling Bacterial Populations in Potable Waters: Community Water Supply." Journal of the American Water Works Association, 64, 596-601 (1972).

18. Goetz, A. and Tsuneishi, N., "Application of Molecular Filter Membranes to Bacteriological Analysis of Water." Journal of the American Water Works Association, 43, 943 (1951).

19. Goyal, S. M., Zerda, K. S., and Gerba, C. P., "Concentration of Coliphages from Large Volumes of Water and Wastewater." Applied and Environmental Microbiology, 39, 85-91 (1980).

20. Green, B. L., Glausen, E., and Litsky, W., "Comparison of the New Millipore HC with Conventional Membrane Filters for the Enumeration of Fecal Coliform Bacteria." Applied Microbiology, 30, 697-699 (1975).

21. Haney, P. D., "Evaluation o Microbiological Standards for Drinking Water." Water and Sewage Works, 125, R-126 - R-134

22. Hill, W. F., Akin, E. W., Benton, W. H., Mayhew, C. J., and Jakubowski, W., "Apparatus for Conditioning Unlimited Quantities of Finished Waters for Enteric Virus Detection." Applied Microbiology, 27, 1177-1178 (1974).

23. Hoffman, D. A., Kuhns, J. H., Stewart, R. C., and Crossley, E. I., "A Comparison of Membrane Filter Counts and Most Probable Numbers of Coliform in San Diego's Sewage and Receiving Waters." Journal Water Pollution Control Federation, 36, 109-117 (1964).

24. Holm-Hansen, O., "Determination of Total Microbial Biomass by Measurement of Adenosine Triphosphate." in Estuarine Microbial Ecology, H. L. Stevenson and R. R. Colwell, eds., University of South Carolina Press, Columbia, S.C., pp. 73-88 (1973).

25. Kessick, M. A., and Wagner, R. A., "Electrophoretic Mobilities of Virus Adsorbing Filter Materials." Water Research, 12, 263-268 (1978).

26. Kott, Y., "Current Concepts of Indicator Bacteria." in Bacterial Indicators/Health Hazards Associated with Water, ASTM STP 635, A. W. Hoadley and B. J. Dutka, eds., American Society for Testing and Materials, Philadelphia, PA, pp. 3-14 (1977).

27. Landry, E. F., Vaughn, J. M., McHarrell, Z. T., and Vicale, T. J., "Efficiency of Beef Extract for the Recovery of Poliovirus from Wastewater Effluents." Applied and Environmental Microbiology, 36, 544-548 (1978).

28. Levin, M. A., Fischer, J. R., and Cabelli, V. J., "Quantitative Large-Volume Sampling Technique." Applied Microbiology, 28, 515-517 (1974).

29. Lin, S. D., "Comparison of Membranes for Fecal Coliform Recovery in Chlorinated Effluents." Journal Water Pollution Control Federation, 49, 2255-2264 (1977).

30. Lin, S. D., "Evaluation of Millipore HA and HC Membrane Filters for the Enumeration of Indicator Bacteria." Applied and Environmental Microbiology, 32, 300-302 (1976).

31. Lueschow, L. A., and Mackenthun, K. M., "Detection and Enumeration of Iron Bacteria in Municipal Water Supplies." Journal of the American Water Works Association, 54, 751-756 (1962).

32. Mack, W. N., "Total Coliform Bacteria." in Bacterial Indicators/Health Hazards Associated with Water, ASTM STP 635, A. W. Hoadley and B. J. Dutka, eds., American Society for Testing and Materials, Philadelphia, PA, pp. 59-64 (1977).

33. McClelland, N. I., and Pawlowski, H. M., "Water Quality Changes in Distribution Systems," Water and Sewage Works, 126, 50-53 (1979).

34. McCrady, M. H., "The Numerical Interpretation of Fermentation Tube Results." Journal of Infectious Diseases, 17, 183 (1915).

35. Middlebrooks, E. J., Middlebrooks, C. H., Johnson, B. A., Wright, Jr., and Reynolds, J. H., "MPN and MF Coliform Concentrations in Lagoon Effluents." Journal Water Pollution Control Federation, 50, 2538-2546 (1978).

36. Mowat, A., "Most Probable Number Versus Membrane Filter on Chlorinated Efflueuts." Journal Water Pollution Control Federation, 48, 724-728 (1976).

37. Oleniacz, W. S., Pisano, M. A., Rosenfeld, M. H., and Elgart, R. L., "Chemiluminescent Method for Detecting Microorganisms in Water." Environmental Science and Technology, 2, 1030-1033 (1968).

38. Presswood, W. G. and Brown, L. R., "Comparison of Gelman and Millipore Membrane Filters for Enumerating Fecal Coliform Bacteria." Applied Microbiology, 26, 332-336 (1973).

39. Ray, B., "Methods to Detect Stressed Microorganisms." Journal of Food Protection, 42, 346-355 (1979).

40. Rotem-Borensztajn, Y., Katzenelson, E., and Belfort, G., "Virus Concentration by Capillary Ultrafiltration." Journal of the Environmental Engineering Division, ASCE, 401-407, April (1979).

41. Sobsey, M. D., and Jones, B. L., "Concentration of Poliovirus from Tap Water Using Positively Changed Microporous Filters." Applied and Environmental Microbiology, 37, 588-595 (1979).

42. "Standard Methods for the Examination of Water and Wastewater." 14th Ed., American Public Health Association, Washington, D.C. (1976).

43. Strathman, D. K., "Two-Step Membrane Filtration Versus Most Probable Number of Unchlorinated Sludge, Soil, and Natural Water Samples." Journal Water Pollution Control Federation, 51, 405 (1979).

44. Tobin, R. S., and Dutka, B. J., "Comparison of the Surface Structure, Metal Binding, and Fecal Coliform Recoveries of Nine Membrane Filters." Applied and Environmental Microbiology, 34, 69-79 (1977).

45. Wallis, C., Homma, A., and Melnick, J. C., "Apparatus for Concentrating Viruses from Large Volumes." Journal of the American Water Works Association, 64, 189-196 (1972).

46. Wallis, C., Homma, A., and Melnick, J. L., "A Portable Virus Concentrator for Testing Water in the Field." Water Research, 6, 1249-1256 (1972).

47. Woodward, R. L., "How Probable is the Most Probable Number?" Journal of the American Water Works Association, 49, 1060-1068 (1957).

48. Zierdt, C. H., "Adherence of Bacteria, Yeast, Blood Cells, and Latex Spheres to Large-Porosity Membrane Filters." Applied and Environmental Microbiology, 38, 1166-1172 (1979).

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DEVELOPMENT OF A WATER SAMPLE CONCENTRATOR

FOR THE CONCENTRATION OF BACTERIA IN DRINKING WATER

by

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(ABSTRACT)

A water sample concentrator utilizing a membrane adsorption/elutriation procedure for bacterial recovery was developed and field tested. Influent sample volumes of 19.0 liters were processed by the concentrator within 10 minutes. Standard MPN and membrane filtration tests were conducted to determine the efficiency of the concentrator. In laboratory experiments, an average bacterial recovery of 48 percent was accomplished when E. Coli was the bacterium concentrated. An average recovery of 78 percent was achieved with Streptococcus faecalis. Various commercially available 142 mm diameter membrane filters were tested with the concentrator. Millipore membrane filters having a porosity of 0.45 m were found to be superior to Cox membrane filters with 0.45 and 5.0 m porosities and to zeta plus filters. The most promising technique for eluting bacteria from the membrane filter appeared to consist of mixing the filter and 125 mL of three percent bovine serum solution at pH 9.0 in an electric blender for 30 seconds and then allowing 15 minutes of contact.

Miscellaneous experiments investigated the addition of multivalent cations to enhance adsorption of bacteria to the membrane filters, the use of vacuum induced flow through the concentrator and the possibility of monitoring bacterial levels through adenosine triphosphate analysis. In the field trials, the water sample concentrator detected coliform bacteria in drinking water in which no coliforms were detected in grab samples by the standard MPN method.