A QUESTIONNAIRE AND A CONCENTRATOR SAMPLING TECHNIQUE USED TO EVALUATE WATER QUALITY DEGRADATION IN WATER DISTRIBUTION SYSTEMS

by

Deborah Kathleen Manning

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APPROVED:

W. R. Knocke, Co-Chairman	G. D. Boardman, Co-Chairman
J. H. Sherrard	R. E. Benoit

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I. INTRODUCTION

Approximately sixty-five percent of the population of Virginia obtains its potable water from a public water supply (1). In all, there are 5,596 public water suppliers serving approximately 3.5 million people (1). Citizens expect the water supply to be of high quality.

The definition of what, precisely, constitutes a high quality water is not easily agreed upon. The basic characteristics have been delineated by organizations concerned with the supply of potable water. The United States Public Health Service (USPHS) Drinking Water Standards of 1962 stated, "Domestic water supplies should protect the health and promote the well-being of individuals and the community (2)." Part of fulfilling this goal is to insure the water supply is not objectionable in terms of sight, taste, or smell. In other words, the water supply must not only be safe from a health standpoint, it must also be aesthetically pleasing. If it is not aesthetically pleasing, consumers may turn to a different, perhaps less safe, water source (3).

The American Water Works Association (AWWA) defined a quality water in the following manner (4):

> Ideally, water delivered to the consumer should be clear, colorless, tasteless, and odorless. It should contain no pathogenic organisms and be free from biological forms which may be harmful to human health or aesthetically objectionable. It should not contain concentrations of chemicals which may be physiologically harmful, aesthetically objectionable, or economically damaging.

> The water should not be corrosive or encrusting to, or leave deposits on, water-conveying structures through which it may be retained, including pipes, tanks, water heaters, and plumbing fixtures. The water should be adequately protected by natural processes, or by treatment processes which ensure consistency in quality.

Key words to note in this definition are "water delivered to the consumer." In cases reported in the literature (5-9), water leaving a treatment plant was a "quality" water in terms of being aesthetically pleasing. However, during the water's passage through the distribution system, the water quality deteriorated. The water may have become turbid, black or red or it may have developed an objectionable taste or odor. In recognition of such deterioration problems, the USPHS has emphasized the need for monitoring within the distribution system (10).

There are many causes of water quality degradation in distribution systems. This paper emphasizes degradation caused by biological agents. Chief among these agents are bacteria of the "nuisance organism" group, including organisms such as the actinomycetes, methane oxidizers, nitrifiers, iron bacteria and sulfur bacteria. These microorganisms are called nuisance organisms because they are not pathogenic by nature; rather they tend to be implicated as potential causative agents of aesthetic problems.

Despite technological advances in other areas of water treatment, aesthetic problems continue to be of concern to both plant operators and researchers as they seek the sources of and solutions to aesthetic problems (11-14). One of the problems encountered when studying

microorganisms in distribution systems is that of detection. Most microorganisms which inhabit water distribution systems attach themselves to pipe walls. Therefore, samples of water from distribution systems often yield low total microbial counts even though the walls of the system's pipes are infested with microorganisms (15). Development of a technique for concentrating large volumes of water in order to more easily detect the bacteria present would be useful.

In order to address some of the problems associated with nuisance bacteria, the following objectives were formulated:

1. To assess the performance of a concentrator to sample large quantities of water for microbiological analysis;
2. To analyze the responses to a questionnaire completed by water treatment plant operators throughout Virginia. The data from the questionnaire responses were used to determine whether water quality parameters currently being monitored were predictive of water quality degradation problems and to determine the effects of treatment methods on such quality problems.

II. LITERATURE REVIEW

A. INTRODUCTION

This chapter contains a review of the literature concerned with water quality degradation in water distribution systems. Biologically-caused degradation and the nuisance bacteria held responsible for this problem are described. In addition, sampling techniques and literature accounts of the use of the Pellicon concentrator in research work are reviewed.

B. NUISANCE BACTERIA

1. General Characteristics of the Water Distribution System and of Nuisance Bacteria

Although many water consumers believe potable water is "sterile," the water in a distribution system and the water itself support a diverse group of microorganisms. The water in the distribution system can supply a microorganism with all its nutrient requirements and the physical structure of the system supplies an attachment site for microbial growth. Wilson (15) stated, "It is safe to say there is no water supply in the world which does not contain sufficient food to support active bacterial growth." Thus, a water distribution system and the life it supports can be thought of as an ecosystem. Russell (5) described the development of a bacterial ecosystem within a water

distribution system and its effect on the water quality in the following manner:

> It would appear that cells and nutrients accumulate on surfaces within the distribution system. Continuous supply of nutrients from the water being transported through the system allows certain cells to grow and multiply. Ultimately, complex biological societies can be formed, each helping to supply the needs of another. Development of slime layers, tubercules, and sludges can tend to help protect new cells thus allowing further growth.
>
> Eventually, certain essential nutrients such as oxygen become depleted. Putrefaction can begin to occur resulting in dead organic matter which can act as a nutrient for additional forms of life. The net effect of this process is a complete change in the inorganic and organic content of the water observed as a rapid deterioration in water quality.

Other researchers (6,8,16) have not only confirmed the existence of bacteria in water distribution systems but have found a diversity of bacteria. O'Connor et al. (6) noted that this range and diversity "...would appear to complicate, if not preclude, the complete eradication of all bacterial species from the system."

Other characteristics of a water distribution system, besides a flow of water and potential attachment sites, encourage microbial growth. The manner in which the water treatment plant is operated is an important factor (5). The amount of calcium entering the system relative to the pH of the water should be considered. Too little calcium may cause rapid corrosion of the pipe, thereby encouraging growth of iron bacteria, when the pipe material is iron-based. The pipe material and presence or absence of pipe liners also affect microbial

growth (5). In addition, the flow rate for which the distribution system is designed encourages microbial growth (5). Distribution systems are by necessity designed for fire flows and for future flow requirements. This means that for a significant portion of its life, low flow velocities are the norm in a distribution system. By not inducing the sloughing of microbial growths, low flow velocities enhance the development of bacterial ecosystems.

As mentioned in Russells' description above, bacterial cells "accumulate on surfaces within the distribution system (5)." Nuisance organisms "accumulate" by means of attachment mechanisms (17). Attachment is enhanced by the formation of a slime (18). A microorganism's ability to attach to a surface and produce a slime affords several advantages. Attached to a surface, bacteria can grow at substrate concentrations which are too dilute to support suspended growth (18). In a water distribution system, the amount of nutrients bacteria are exposed to when attached to the pipe surface is different from the amount of nutrients they would be exposed to if suspended in the water. The difference can be an advantage for an attached microorganism. The formation of a slime also serves to attract other microorganisms and nutrients (18). Slimes are primarily polysaccharide, a substance which is "tacky" and makes other substances which contact it bond to the slime (18).

Other than possessing attachment capabilities and often producing a slime, nuisance bacteria have few common characteristics. Among the

nuisance bacteria one will find aerobes and anaerobes, phototrophs and chemoorganotrophs, heterotrophs and autotrophs. Such diversity makes the nuisance bacteria an interesting group within the microbial kingdom.

Nuisance bacteria cause a variety of problems, including the formation of turbidity, color, taste and odor and the depletion of dissolved oxygen (5,7-9). The following paragraphs contain descriptions of these problems.

Turbidity is both a result of bacterial growth and a factor contributing to the growth. Bacteria can cause turbidity when they are sloughed off pipe walls (5,7). Turbidity caused by suspended matter such as clay and silt supplies both nutrients for bacteria to grow on and protected areas for them to grow in (16). Turbidity caused by either bacteria or non-living matter protects bacteria from chlorine (16,19). Because turbidity caused by bacteria may shield pathogens from the disinfectant power of chlorine, nuisance bacteria are indirectly related to disease (16).

Color in water can be caused by numerous factors. Nuisance bacteria contribute to color problems by causing the precipitation of iron and manganese (5). Red water is often caused by the precipitation of ferric compounds, whereas black water may be caused by the precipitation of manganic compounds (5). These precipitates, in turn, can afford bacteria protection from disinfectants.

Perhaps one of the leading problems caused by nuisance bacteria is the formation of taste- and odor-causing compounds. Geosmin and

mucidone, metabolic products of the actinomycetes, have been isolated under laboratory conditions and identified as taste- and odor-causing substances (20-23). The growth of sulfate reducers and sulfur oxidizers can result in the evolution of hydrogen sulfide, H_2S (24). Hydrogen sulfide is responsible for a "rotten egg" odor.

Dissolved oxygen depletion can occur as a result of microbial activities. Oxygen is utilized in oxidation reactions performed by iron bacteria, nitrifiers, the thiobacilli and methane oxidizers (17). Dissolved oxygen depletion causes anaerobic conditions and spurs the growth of anaerobic bacteria within the water distribution system (16).

Nuisance bacteria can be found throughout water distribution systems (25). Consequently, the problems nuisance microorganisms cause and complaints regarding these problems can occur at any point in the distribution system. Dead-end areas are often mentioned as being particularly susceptible to water quality degradation problems; distribution system designers are exhorted to produce designs which minimize dead ends (5,26).

O'Connor et al. (6) have provided a model for the interior of a pipe. The model, shown in Figure 1, shows the bacterial ecosystem which develops in distribution systems and some of the interactions of these bacteria with their environment.

2. Bacteria that Transform Iron and Manganese

Bacteria that transform iron and manganese cause the precipitation of ferric and manganic oxides. The precipitation of these compounds can

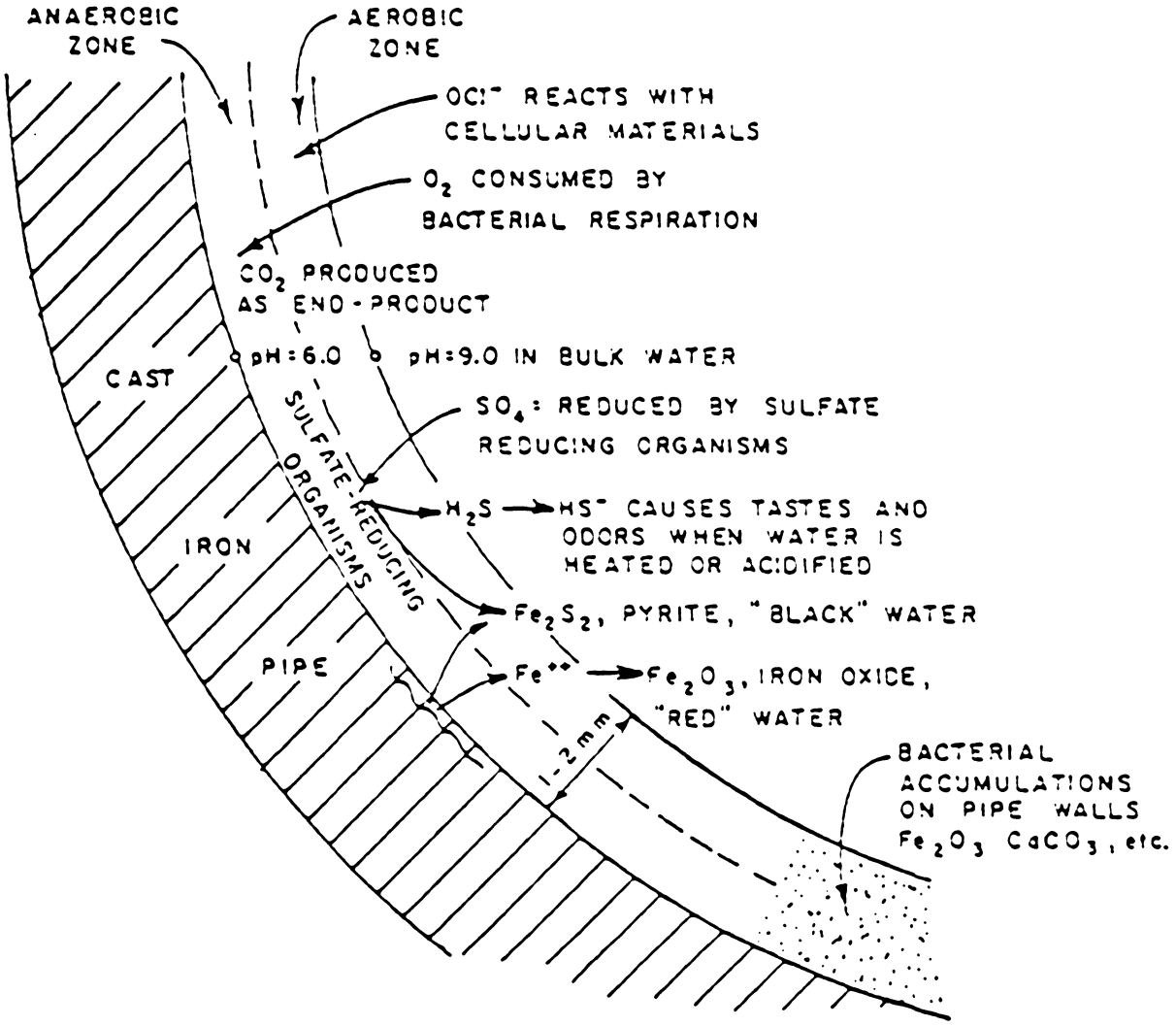


Figure 1. Model of Pipe Interior (After O'Connor, et al. (6)).

result in the encrustation of pipes or wells, decreasing water-carrying capacity (8,27,28). The bacteria which transform iron and manganese may incorporate the precipitate into their cell structure and form slimes or filaments (29). Both slimes and filaments can cause a decrease in a pipe's water-carrying capacity (15,25). Sloughing of slimes or filaments causes turbidity and "red" or "black" water (16). Another effect of iron oxidation is the staining of plumbing fixtures (5). In order to avoid these problems, many water treatment plants incorporate units for the oxidation of iron and manganese (17).

Bacteria that transform manganese also transform iron. They are referred to, however, as "iron" bacteria, not "manganese" bacteria. Most emphasis in the literature is placed on the iron-related nature of these microorganisms. Because of this and because the iron-precipitating capability of a particular nuisance bacteria played an important role in this thesis, bacteria associated with iron and manganese will be discussed here mainly in terms of their relationship to iron.

Researchers have tried to determine the limiting iron concentration below which bacteria associated with iron will not grow. Harder (30) reported growth at 1.3 mg/L, in contrast, Starkey and Halvoroson (31) found 1.0 mg/L Fe to be the lower limit. Schorler (32), as reported by Mackenthum and Keup (8), indicated an iron concentration of 0.2 mg/L was limiting to the growth of iron bacteria. Wolfe (33) reported growth of iron bacteria at iron concentrations less than 0.02 mg/L and added that

iron-depositing bacteria may be found in waters where the iron concentration is too low to be detected because "the organisms are sessile and may concentrate iron and manganese from a large volume of water that flows over them." Determining a lower limit iron concentration for growth has proved difficult.

It has also been difficult for researchers to classify bacteria associated with iron and manganese. This has been the case because some of these bacteria are particularly susceptible to modifications in their appearances, or morphological changes, due to different environmental conditions (33). Wolfe (33) explained, "Thus, the same organism has been given different names by different workers and the same organism has been regarded as several different species by a single worker all as a result of a lack of knowledge of the role played by the environment in modifying the appearance of the organism."

Classification has also been difficult because some iron bacteria are notoriously difficult to grow in the laboratory (34). Without knowledge of a microorganism's characteristics under known conditions, there is no reference point from which morphological changes can be ascertained.

Starkey (29) divided bacteria that transform iron or manganese into the following four groups: iron bacteria, non-specific bacteria, sulfur bacteria and sulfate-reducing bacteria.

Iron bacteria directly oxidize ferrous iron to ferric oxides or manganous compounds to manganic oxides. They are aerobic, often

filamentous and widely distributed in nature. Iron bacteria are capable of accumulating large amounts of ferric or manganic precipitate around their cells (29). On a weight basis, as much as 500 times as much precipitate may be present in a colony compared to cells of iron bacteria (29).

Iron bacteria deposit iron or manganese in a morphologically defined way (35). This is a key concept in understanding these microorganisms. The different ways in which species of iron bacteria deposit iron also serve as a basis on which to categorize bacteria within the iron bacteria group and also on which to distinguish between iron bacteria and other iron- and manganese-transforming bacteria. Iron bacteria incorporate the ferric or manganic precipitate into the cell structure in the form of filaments or stalks or in sheaths surrounding their cells. Other bacteria involved with transformations of iron or manganese do not incorporate the precipitate into their cell structure. As stated above, iron bacteria are categorized according to their morphological characteristics. Siderocapsa accumulate the ferric or manganic precipitate outside a mucoid capsule which surrounds coccoid or rod-shaped cells (29). Sphaerotilus, Clonothrix, Leptothrix and Crenothrix are members of the Chlamydobacteriaceaeles order, commonly known as the filamentous bacteria (29,36,37). These microorganisms deposit precipitates in the form of filaments. Gallionella is a member of the Caulobacteriales, or stalked bacteria (29). It consists of

kidney-shaped cells at the end of a twisted stalk of ferric or manganic precipitate (29).

Although iron bacteria precipitate iron and manganese in a way which is morphologically related to their cells, the physiological connection between iron or manganese oxidation and the metabolism of the bacteria is still debated (36,38,29). More specifically, the debate centers on whether iron bacteria use the oxidation of iron or manganese as a source of energy. Starkey (29) explained that in 1888, Winogradsky (40) published a paper proposing that iron bacteria are autotrophic, using iron and manganese oxidation as their source of energy. Simply put, such oxidation involves the following reactions:

$$2\ Fe^{++} + 1/2\ O_2 + 5\ H_2O \rightarrow 2\ Fe(OH)_{3(S)} + 4\ H^+ + \text{Energy} \quad [1]$$

and

$$Mn^{++} + 1/2\ O_2 + H_2O \rightarrow MnO_{2(S)} + 2H^+ + \text{energy} \quad [2]$$

However, the hypothesis that all iron bacteria are autotrophic has never been indisputably proven (36,38,39).

As mentioned previously, iron bacteria are widespread in nature. That so little can be said regarding the metabolic relationship between these microorganisms and iron and manganese indicates more research is needed.

The second category of bacteria that transform iron and manganese are the non-specific bacteria. These bacteria do not fall into a major type, either on a morphological or physiological basis. However,

through their normal activities, they alter their environment in such a way that iron or manganese is precipitated (29,34). Normally, non-specific bacteria oxidize organic matter which was previously bound in a stable complex with iron or manganese. The oxidation of the organic matter causes the destabilization and subsequent precipitation of the iron or manganese (34,41).

Of particular interest to this thesis is the non-specific bacteria Pseudomonas cepacia. P. cepacia, an iron precipitator, was given the name "cepacia" because this word means "of or like onion" and many P. cepacia strains were isolated from rotten onions (42). In addition to being found in water distribution systems, P. cepacia is widely distributed in soils (42).

P. cepacia is a gram-negative, obligate aerobe with rod-shaped cells measuring 0.8 to 1.0m by 1.6 to 3.2m. It is motile by means of multitrichous polar flagella (42). The optimal temperature for growth is 30°C to 35°C. It can grow on a wide range of organic compounds and it produces a variety of pigments dependent on the media used. Some strains of P. cepacia are reported to produce slime on a 2 to 4 percent sucrose media (42).

Sulfur bacteria and sulfate-reducing bacteria can directly or indirectly cause deposition of iron and manganese. The sulfur bacteria Thiobacillus ferrooxidans is capable of autotrophically using iron oxidation as its source of energy. The reaction proceeds as follows (24):

$$12\ FeSO_4 + 3\ O_2 + 6_2O \rightarrow 4\ Fe_2(SO_4)_3 + FeOH)_{3(S)} \qquad [3]$$

Sulfate-reducing bacteria can produce hydrogen sulfide, a reducing agent which, in turn, can cause precipitation of ferrous and manganous sulfides and disulfides (41).

3. Bacteria that Transform Sulfur

Sulfur is a physiological requirement of all living organisms (37). Consequently, it is transformed in some way by all of them. However, in utilizing sulfur, some bacteria produce hydrogen sulfide, H_2S (24). The presence of hydrogen sulfide in drinking water can cause taste and odor problems, staining of silverware, clothes and plumbing fixtures, reduction of chlorine residuals and an accelerated rate of pipeline corrosion (5,6,9,24,43).

Sulfur-oxidizing bacteria, including the photosynthetic sulfur bacteria, the colorless sulfur bacteria, and the Thiobacilli, oxidize organic and inorganic sulfur compounds and can produce numerous other sulfur compounds, (24). Such bacteria are autotrophic and may be aerobic or anaerobic (37).

The reduction of sulfate is performed by a more diverse and less specific group of microorganisms than the sulfur oxidizers (24). Again, hydrogen sulfide may be an end product of the reduction. For example, the Desulfovibrio perform the following typical general reaction (24):

$$\text{Alcohols or fatty acids} + SO_4^{=} \rightarrow CO_2 + H_2S + H_2O \quad [4]$$

4. Bacteria that Oxidize Methane

Water that contains methane can support methane-oxidizine bacteria (5), which reportedly cause problems in ice machines, flush tanks and within the water distribution system (17).

Methane oxidation is performed by the microorganisms Methylomonas and Methylococcus (5). Both are strict aerobes and methane and methanol are their only known sources of carbon and energy (42). A typical oxidation/reduction reaction for methane oxidizers is as follows (17):

$$CH_4 + 2\ O_2 \rightarrow CO_2 + 2\ H_2O \quad [5]$$

5. Bacteria that Transform Nitrogen

Nitrifying bacteria are not often-reported nuisance bacteria. They can, however, cause depletion of dissolved oxygen (6) and have been linked with methemoglobinemia, or "blue baby," in infants (44).

Nitrification, as performed by Nitrosomonas and Nitrobacter, is a two-step process in which ammonia is converted first to nitrite and then to nitrate (37). The reactions are as follow (37):

$$2\ NH_3 + 3\ O_2 \rightarrow 2\ HNO_2 + 2\ H_2O \quad [6]$$

$$HNO_2 + 1/2\ O_2 \rightarrow HNO_3 \quad [7]$$

All species of Nitrosomonas and Nitrobacter are strict autotrophs except Nitrobacter winogradskyi which can utilize organic substrates as well (37).

6. Actinomycetes

Actinomycetes are technically classified as bacteria but they bear a resemblence to fungi in that they are filamentous and branched microorganisms that produce moldlike spores called conidia (37,45). They have long been held responsible for taste and odor problems (46). In 1950, Silvey et al. (45) described the role of actinomycetes in the production of tastes and odors which have been termed in various places fishy, grassy, marshy, musty, potato bin and woody (45,77). Actinomycetes produce two substances, geosmin and mucidone, which have been isolated from cultures grown in the laboratory. It is these substances which have been identified as causing certain taste and odor problems. Although geosmin and mucidone have been isolated from actinomycetes cultures grown in the laboratory, neither has been found in natural waters (46).

7. Summary Table for Nuisance Bacteria

Table I summarizes basic information regarding the metabolism of nuisance bacteria.

Table I. Summary Table for Nuisance Bacteria

Bacteria	Energy Source Oxidation/ Reduction Reactions	Carbon Relationship	O_2 Relationship
1. Iron oxidizers, e.g., <u>Thiobacillus ferrooxidans</u>	$Fe(II) \rightarrow Fe(III)$ or $Mn(II) \rightarrow Mn(III)$ (OXID.); $O_2 \rightarrow H_2O$ RED.	Autotroph	Aerobe
2. Non-specific iron transformers e.g., <u>Pseudomonas cepacia</u>	Carbohydrates, Alcohols, Fatty acids $\rightarrow CO_2$ (OXID.); $O_2 \rightarrow H_2O$ (RED.)	Heterotroph	Obligate Aerobe
3. Sulfur-oxidizing bacteria e.g., purple sulfur bacteria	$H_2S \rightarrow S$ (OXID.); $O_2 \rightarrow H_2O$ (RED.)	Autotroph	Anaerobe or Aerobe
4. Sulfate-reducing bacteria e.g., <u>Desulfovibrio</u>	Alcohols & fatty acids $\rightarrow CO_2$ (OXID.); $SO_4^{2} \rightarrow H_2S$ (RED.)	Heterotroph	Obligate Anaerobe
5. Nitrifiers e.g., <u>Nitrosomonas</u>	$NH_3 \rightarrow NO_2$ (OXID.); $O_2 \rightarrow H_2O$ (RED.)	Autotroph	Aerobe
6. Methane oxidizers e.g., <u>Methylomonas</u>	$CH_4 \rightarrow CO_2$ (OXID.); $O_2 \rightarrow H_2O$ (RED.)	Heterotroph	Aerobe
7. Actinomycetes	Complex organics $\rightarrow CO_2 + H_2O$ + energy	Heterotroph	Aerobe

C. SOURCES OF MICROBIAL CONTAMINATION

Microbial contamination of water distribution systems occurs in several ways, some of which can be avoided while others cannot. The finished water entering the distribution system most likely contains microorganisms that have survived disinfection, if indeed disinfection is used (16). Improper disinfection of new mains (48) may mean that, from the beginning of operation, bacteria were present in the distribution system. Connections to supplies which are not controlled are undesirable from all points of view but may not be detectable (48). Cross connections can allow back syphonage of contaminated water into the distribution system (48). Finally, special attention should be paid during and after line breaks and repairs to assure that contamination does not occur (16).

D. MICROBIAL SAMPLING PROCEDURES IN THE DISTRIBUTION SYSTEM

The Commonwealth of Virginia's Waterworks Regulations (49) call for bacteriological sampling at a frequency dependent on the population served by the water distribution system. The regulations state, "The frequency of sampling and the location of sampling points shall be established or approved by the Bureau [of Water Supply] after investigation of the source, method of treatment and storage, and protection of the water concerned... The samples shall be taken at reasonably evenly spaced time intervals throughout the month."

In Virginia, bacteriological testing is required for coliforms only (49). The standard sample required for such bacteriological testing is defined as follows (49):

"The standard sample for the coliform test shall consist of:

a. Not less than 100 milliliters for the membrane filter technique, and

b. Five (5) standard portions for the fermentation tube method of either:

   1. Ten milliliters, or

   2. One hundred milliliters,"

Standard Methods (50) describes techniques for sampling in the distribution systems under Part 900 Microbiological Examination of Water, Section 906 Samples.

Selection of sampling areas is made keeping in mind the need for gathering samples which truly represent the water consumers see. Samples should be collected in areas of low as well as high flows, in areas served by tanks or reservoirs, and in unique areas within the distribution system--such as isolated areas or deadends (26). The actual sampling taps selected should be ones at which water is used all day long (26). Taps in schools and other public buildings are considered best for this reason.

The number of microorganisms detected in a small sample of distribution system water is often very low. Viruses may be present, but in concentrations too dilute to make them detectable (51,52).

Bacteria present in the distribution system are most often attached to the pipe walls. Only small numbers are washed off and carried by the distribution system water (15). Because the concentration of microorganisms is low, it would be desirable to concentrate water samples to more easily and reliably detect the microbes.

A review of research dealing with concentration techniques revealed that most work has been concerned with the recovery of viruses. Although the concentrator experiments performed for this thesis dealt with the recovery of bacteria, some mechanisms of the filtration and concentration processes are similar for both viral and bacterial applications. Also, more research utilizing the Pellicon cassette system concentrator has been performed in the viral than the bacterial area. Results of this research would be valuable for the study at hand and are presented in the subsequent paragraphs.

Millipore's Pellicon concentrator utilizes a type of filtration called molecular filtration by some authors (51,53) and pressure ultrafiltration by others (5,54). In this process, a solution is forced to flow along the surface of a filter or membrane. The membrane is of a certain molecular weight rating and allows molecules of a lower molecular weight to pass through it while the higher molecular weight particles are washed along the membrane, eventually to be collected as a concentrate (54). The Pellicon concentrator, then, divides an influent solution into two separate volumes, the retentate and the filtrate. Theoretically, only the retentate contains the higher-weight molecules.

Gangemi et al. (55) utilized the Pellicon concentrator with certain arenaviruses, specifically Machupo and Tacaribe. Three to six liters containing a total of 10^6 to 10^7 virus particles were pumped through the concentrator. No membrane preparation was performed. The solutions in which the viruses were suspended consisted of maintenance medium E-199 and 5 percent fetal calf solution. The solution was clarified prior to being pumped through the concentrator. Using this procedure, Gangemi et al. found that their retentate solution contained 50 to 100 percent of the influent number of viruses. Higher percent recoveries corresponded to lower concentration factors. For instance, a 36-fold concentration had a corresponding recovery of 100 percent while a 100-fold concentration resulted in a percent recovery of only 50. The concentration of viruses particles in the influent did not affect the percent recovery. Of the Pellicon concentrator, Gangemi et al. concluded, "With its high flow rate and gentle action, this system is well suited for concentration of fragile viruses...".

In studies performed to evaluate the Pellicon concentrator, Berman et al. (51) reported a variety of percent recovery values. These researchers used poliovirus 1 suspended in distilled water during their experiments. The concentration of viruses in the influent ranged from 1 to 10 plaque-forming units per milliliter. A number of concentration techniques were developed. Initially, a sample was passed through the concentrator with no special pretreatment of the filter membrane and with no subsequent washing of the membrane. After 23- to 46-fold

concentrations, virus recoveries in the retentate averaged only 10.5 percent. However, less than 1 percent of the unaccounted-for virus was found in the filtrate. After examining the concentrator for the presence of toxic materials and finding none, it became obvious to Berman et al. that a modification of the concentration technique would be necessary to obtain higher recoveries. One modification consisted of "forward washing" the membrane after the initial sample was pumped through. The forward wash was recovered and assayed. After summing the percent recovery in the retentate and the percent recovery in the forward wash, Berman et al. still found less than 10 percent of the initial number of virus particles. In an attempt to ascertain whether the virus particles were nonreversibly adsorbed to the membrane, experiments were performed in which one percent glycine was used either to pretreat the membrane or as a supplement in the influent solution. Virus recovery in the retentate increased to 39 percent, indicating that the glycine was effective in blocking virus adsorption sites on the membrane. Pretreatment of the membrane with flocculated beef extract resulted in virus recoveries in the retentate of 33 to 75 percent. However, virus recovery in the filtrate also increased, ranging from 10 to 36 percent.

In their final procedural modification, Berman et al. used a membrane with a higher nominal molecular weight limit. They found a lower efficiency of recovery in the retentate (17 percent) but the number of viruses present in the filtrate was negligible. The

researchers concluded that "the described molecular filtration system can effectively concentrate viruses from water, provided membranes are pretreated with flocculated beef extract."

Shibley et al. (53) made use of the Pellicon concentrator during experiments to concentrate solutions containing Epstein-Barr viruses. An important consideration in this work was the development of a concentration method which did not impair the biological activity of the viruses, specifically their infectivity. Shibley et al. avoided the problem of viral adsorption to the filter by instituting a membrane washing technique. Ten liters of virus-containing fluid (the fluid consisted of the viruses and the growth medium, RPMI 1640, to which was added 20 percent heat-inactivated fetal bovine serum and bacterial and fungal growth inhibitors) were passed through the Pellicon concentrator, followed by a series of 200-ml washes. Recirculated filtrate was used for the wash solution. This procedure resulted in over 90 percent recovery in the retentate after the first pass through the concentrator and 99 percent of the remaining trapped viruses were recovered by the third pass. In addition, the biological activity, i.e., the infectivity, of the viruses was unimpaired. Shibley et al. found the molecular filtration process, and, in particular, the Pellicon concentrator, to be "practical and efficient means" of concentrating large volumes of Epstein-Barr viruses.

From the literature reports on the use of the Pellicon concentrator, the following advantages of the system can be inferred:

1. System has good virus recovery
2. Rapid flow rates are possible, allowing for the sampling of large quantities of water
3. High concentration factors are possible and simple to obtain in comparison to other concentration procedures.
4. The concentration process is gentle and viruses are recovered unimpaired.

Disadvantages of the Pellicon concentrator include the following:

1. Viruses can become adsorbed to the filter membrane unless suitable precautions are taken.
2. Recovery efficiency decreases at higher concentration factors.
3. Membranes can become clogged or compacted.
4. The concentrator apparatus is expensive.

III. MATERIALS AND METHODS

This chapter covers two topics. First, the materials and methods used in evaluating the Millipore Pellicon cassette system concentrator will be described. Second, the questionnaire sent to Virginia's water treatment plant operators will be presented and discussed.

A. EVALUATION OF THE MILLIPORE PELLICON CASSETTE SYSTEM CONCENTRATOR

1. The Concentrator and Related Apparatus

The Pellicon concentrator, produced by the Millipore Corporation (Bedford, MA), is simply a filter held securely in place between two pieces of plexiglass. The concentrator separates an influent stream into two product streams, the retentate and the filtrate. Each of the the three streams, the influent, retentate and filtrate, has its own port on the face of the concentrator, as shown in Figure 2. Theoretically, the retentate contains the larger molecules, including the bacteria, that are held back by the filter membrane. The filtrate contains the smaller molecules that pass through the filter membrane. Ideally, particles do not become attached to the filter but are washed along it and brought to the retentate port.

The concentrator used in these experiments was 23 centimeters (cm) wide by 25 cm long by 30 cm high (9x10x12 inches), weighing approximately 9.0 kilograms (20 pounds). The components of the Pellicon concentrator are shown in Figure 3. As mentioned above, the components serve to hold the filter membrane in place or to cause the influent

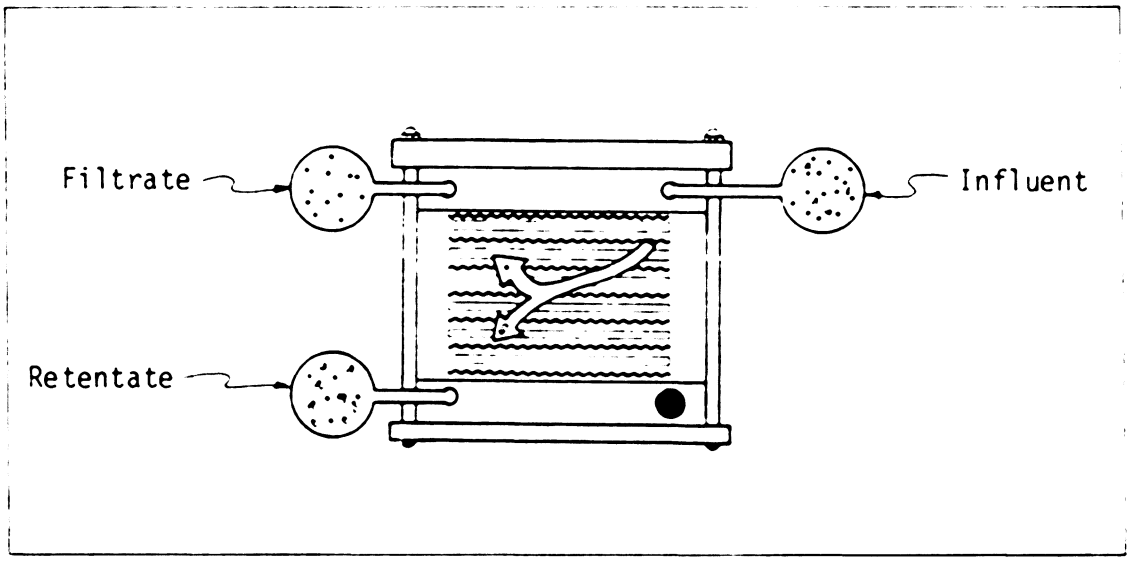


Figure 2. Schematic of Concentrator Showing Port Locations (After Millipore (56)).

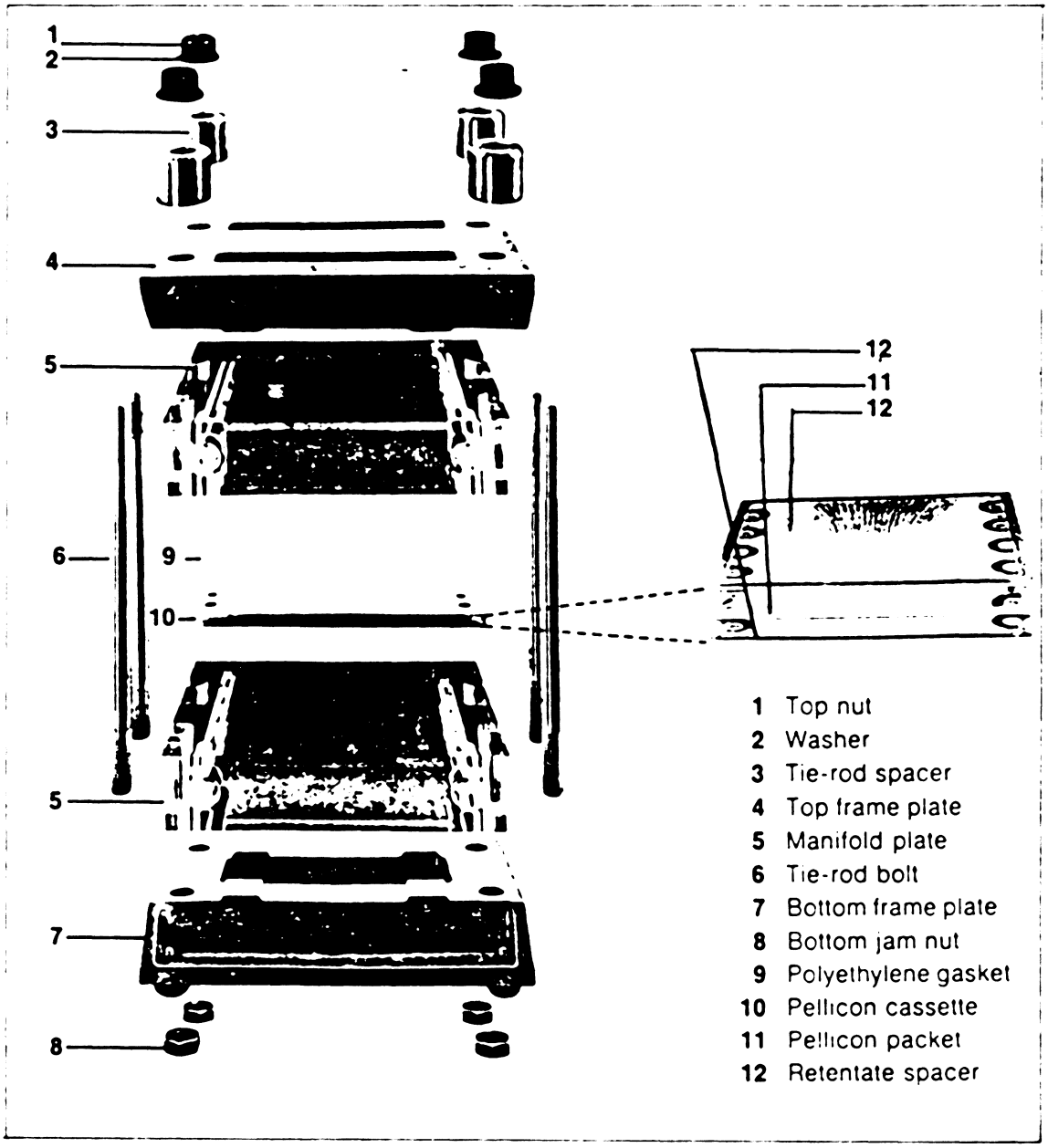


Figure 3. Components of Pellicon Cassette System Concentrator (After Millipore (56)).

stream to separate into the retentate and filtrate flows. The top nut, washer, tie-rod spacer and bottom jam nut were made of stainless steel while the top and bottom frame plates were made of nylon-coated aluminum. The filter membrane, sandwiched between two acrylic manifold plates, was made of polysulfone with a 100,000 molecular weight pore size. The total filter area was 0.5 square meters (5 square feet). Connections to the concentrator were made using polypropylene hose connectors. The hoses themselves were Tygon tubing. All fittings and connectors were wrapped with Teflon tape.

Other components of the concentrator apparatus included the following:

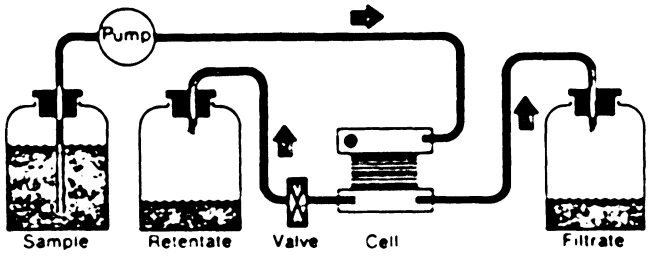
a. a valve on the retentate hose used to create back pressure to control the retentate volume;

b. a pressure gauge (U.S. gauge) on the influent line;

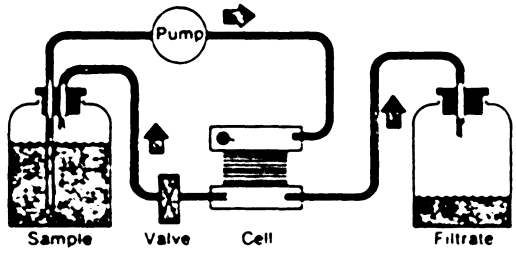
c. a variable speed, peristaltic pump by Manostat with pump speed normally set so that the pressure gauge read 1.05 kilograms per square centimeter (15 pounds per square inch); and

d. Nalgene or glass carboys to hold the influent, retentate and filtrate.

The Pellicon concentrator can operate under various modes, including single-pass, recirculating and constant-volume molecular wash modes. The single-pass mode was used during the laboratory studies. The recirculating mode was used to concentrate 10 liter and 15 liter sample volumes during the field studies. Schematics of these modes are shown in Figure 4. Basically, the recirculating mode is the same as the



Single-Pass Mode



Recirculating-Flow Mode

Figure 4. Schematic Showing Modes of Operation of Pellicon Concentrator (After Millipore (56)).

single-pass mode. However, during the recirculating mode, when the first pass is complete, the influent hose is placed in the retentate container and the retentate fluid is recirculated until the desired retentate volume is reached.

2. Microbial Cultures

Two types of microorganisms were used in the laboratory studies to evaluate the performance of the Pellicon concentrator: Pseudomonas cepacia and Escherichia coli-B.

Pseudomonas cepacia, an iron precipitator, was isolated from a water distribution system by James Rickloff, a graduate student at Virginia Tech. Since the concentrator was envisioned for use ultimately in the isolation of microorganisms from water distribution systems, it was considered desirable to test the concentrator in the laboratory using a microorganism actually isolated from a distribution system.

The P. cepacia culture was maintained on standard plate count agar slants (see Appendix A, Culture Media Specifications). When iron precipitators were needed for an experiment, tryptone glucose yeast broth (see Appendix A) was inoculated with P. cepacia from an agar slant and incubated for approximately 48 hours at 20°C (Precision Scientific Incubator).

Agar utilized for the pour plates from which the P. cepacia colonies were counted was a ferric ammonium citrate (FAC) agar (see Appendix A). Pour plates were inverted and incubated five to seven days at 20°C (Precision Scientific Incubator). The colonies were circular

with smooth edges and a yellowish-brown color. Plate counts were performed using a Spencer Darkfield Quebec Colony Counter.

Escherichia coli-B was used in evaluating the concentrator because it offered several advantages which P. cepacia did not. First, E. coli does not form viscous colonies, as P. cepacia does, that are difficult to disperse in large volumes of water. E. coli is simple to culture and quantitative results can be obtained within 24 hours. Finally, E. coli is a well-known indicator organism.

The E. coli-B culture was obtained from the Virginia Tech Microbiology Department. Bacteria from this culture were transferred to nutrient agar slants (see Appendix A). When E. coli-B were needed for an experiment, a lactose broth tube (see Appendix A) was inoculated with bacteria from a nutrient agar slant. The broth tube was then incubated for 24 to 48 hours at 35°C (Nalco incubator).

Quantitative testing for E. coli was performed using the presumptive portion of the multiple-tube fermentation technique for members of the coliform group, as specified in Section 908 of Standard Methods (50).

3. Experiments Performed

Experiments were performed to determine the Pellicon concentrator's ability to produce a concentrated solution of bacteria from a more dilute one. Among the parameters noted for the influent, retentate and filtrate in each experiment were the concentration of bacteria and the

volume. A typical sequence for a single-pass, laboratory experiment was as follows.

a. The influent, retentate and filtrate carboys were disinfected by rinsing each with a dilute bleach solution. The concentrator was disinfected by pumping a dilute bleach solution through it.

b. Each carboy was rinsed with a dilute sodium thiosulfate solution to neutralize any residual chlorine. The same was done for the concentrator by pumping a dilute sodium thiosulfate solution through it.

c. The influent carboy was filled with the desired volume of tap water (usually 15 L). Enough sodium thiosulfate was added to neutralize the chlorine residual in the tap water. (See Section 906 of Standard Methods (50). For a 15 L sample, 35 ml of 0.025 N sodium thiosulfate were used.)

d. The contents of a broth tube in which the desired microbial culture were growing was transferred first to a dilution blank bottle filled with 90 ml of autoclaved, distilled water. The bottle was then shaken vigorously. This was done to help break apart the P. cepacia cells which grew in a viscous colony and to make easier and more accurate the transferring of bacteria by pipet.

e. The desired amount of the bacteria/distilled water solution was then transferred by pipet to the influent carboy. The influent carboy was shaken to disperse the bacteria. Pour

plates (for P. cepacia) were made or broth tubes (for E. coli) were inoculated to determine the concentration of bacteria in the influent.

f. Pumping proceeded until all the influent had passed through the concentrator.

g. Pour plates were made or broth tubes were inoculated to determine the concentration of bacteria in the retentate and filtrate. The retentate and filtrate volumes were measured.

When the results from early experiments indicated that not all of the bacteria were being recovered in the retentate and filtrate solutions, a second procedure was initiated to determine whether bacteria were being retained in the concentrator itself. This procedure, called the multiple-pass experiment, was performed as follows.

a. Steps a through g for the single-pass experiment were performed.

b. The influent, retentate and filtrate carboys were chlorinated and dechlorinated using a dilute bleach solution and a sodium thiosulfate solution, respectively.

c. The influent carboy was filled with the desired amount of tap water to which enough sodium thiosulfate was added to neutralize the chlorine residual.

d. Pumping proceeded until all the influent had passed through the concentrator.

e. Pour plates were made or broth tubes were inoculated to determine the concentration of bacteria in the retentate and filtrate. The retentate and filtrate volumes were measured.

f. Steps b through e were performed the desired number of times. Each time a volume of tap water was pumped through the concentrator, a "pass" was said to have been completed. The author generally pumped two volumes through the concentrator after the initial influent volume inoculated with bacteria had been pumped through the concentrator. Thus, a multiple-pass experiment meant that three separate influent volumes were pumped through the concentrator, or three passes were made.

An important consideration in the evaluation of the concentrator was its performance in the field. Samples of water from a city in Virginia were taken at two locations: a city-owned maintenance garage approximately three miles from the water treatment plant discharge, and a privately-owned building approximately two miles from the plant discharge. In these field experiments, influent volumes of 10 and 15 liters of tap water were reduced to less than one liter of retentate volume using the recirculating mode. A one liter sample was taken at each site for direct testing.

The field experiment procedure was the same as for the single-pass, laboratory experiments. The concentrator was chlorinated and dechlorinated before each field experiment. Also, because of the problem, encountered in the laboratory, of bacteria becoming caught in the concentrator, samples of the sodium thiosulfate rinse solution were

assayed for bacteria of the iron-precipitating (e.g., P. cepacia) or lauryl tryptose broth fermenting (e.g., E. coli) type. This was done in order to see whether bacteria from previous experiments were caught inside the concentrator and were still being washed off the filter.

The 1 liter sample volumes and the retentate volumes were transferred, on ice, to Virginia Tech in plastic containers to which sodium thiosulfate had been added and which had been autoclaved. These containers were prepared as specified in Section 906 of Standard Methods (50). At Virginia Tech, tests were performed to detect the presence of iron precipitators (e.g., P. cepacia) or lauryl tryptose broth fermenters (e.g., E. coli).

B. ANALYSIS OF THE QUESTIONNAIRE

A copy of the questionnaire sent to Virginia's water treatment plant operators is contained in Appendix B. This questionnaire was composed and sent to the operators by Doctors Knocke and Boardman of the Virginia Tech Civil Engineering Department. Much of the data collection and assembly was performed by James Rickloff and Michael Daugherty, graduate students at Virginia Tech.

The questionnaire was designed so that most questions required a simple "yes" or "no" answer. Although a list of water parameters was presented in the questionnaire and the operators were asked to indicate the average raw and finished water levels of these parameters, not all operators filled in this information because they did not measure them. When this occurred, supplemental information was obtained from the

Commonwealth of Virginia's Department of Health. The Department of Health is the regulatory agency responsible for supervising water treatment plants and for testing for and maintaining records of water quality parameters which some plants do not have the resources to do.

The operators were also asked to indicate which unit processes were employed at their water treatment plant. A list of possible processes was supplied and the operators were asked to check the applicable ones. For processes which might be instituted as a remedial measure for a water quality problem, such as powdered or granular activated carbon, no provision was made to allow the operators to comment on whether the processes were effective.

A great deal of information was available from the completed questionnaires. Correlation of the data and discussion regarding it are contained in the following chapters.

IV. RESULTS

This chapter presents results from the experiments performed to evaluate the Pellicon concentrator and results from the questionnaires completed by the water treatment plant operators. The chapter is divided into two parts: Section A deals with the concentrator experiments while Section B covers the questionnaire results. Each section explains what parameters were examined and why they were considered important. Discussion of the results is contained in Chapter V - Discussion.

A. RESULTS FROM THE PELLICON CONCENTRATOR EXPERIMENTS

A plate count technique was used in experiments in which enumeration of Pseudomonas cepacia was necessary. The results of these experiments depended on the variability inherent in the plate count procedure, which is a function of many factors including the bacterium being used and the person performing the procedure. An experiment was set up to determine this variability. In the experiment, five replicate samples were drawn from a bulk solution of tap water to which P. cepacia had been added. From these samples, dilutions were made and plated. The experiment was performed three times with different bulk solutions. The results from these experiments are shown in Table II. For each experiment, the dilutions plated and the plate counts at each dilution are presented. The symbols "S1", "S2", etc. represent Sample number 1, Sample number 2, etc.

Table II. Experiments to Determine Variability of Plating Technique for *P. cepacia*

Dilution	S1	S2	S3	S4	S5
Experiment No. 1					
10^{-1}	390	399	409	394	380
10^{-2}	39	34	33	37	48
10^{-3}	3	3	6	3	4
Experiment No. 2					
10^{-1}	396	393	405	409	404
10^{-2}	40	36	36	50	35
10^{-3}	5	2	7	1	4
Experiment No. 3					
10^{-2}	26	31	36	46	29
10^{-3}	6	3	4	4	3

EXPERIMENT	COEFFICIENT OF VARIATION, percent
#1, 10^{-1} Dilution	2.7
#1, 10^{-2} Dilution	15.7
#2, 10^{-1} Dilution	1.7
#2, 10^{-2} Dilution	15.8
#3, 10^{-2} Dilution	22.9
#3, 10^{-3} Dilution	30.5

Also shown in Table II are the coefficients of variation for these experiments. These calculations were performed to indicate whether the author's plating technique using P. cepacia was acceptable. For experiments in the biological field, coefficients of variation typically range from 10 to 30 percent (57). From this standpoint, the author's technique was adequate. It should be noted, however, that at the higher dilutions, when the number of colonies on a plate fell below 30, the coefficient of variation was high. Standard Methods (50) recommends that plates with less than 30 colonies not be counted. The calculations shown in Table II confirm this recommendation on the basis that the scatter of the data acquired from such plates was high.

Since, in the multiple-pass experiments, tap water was used to rinse the concentrator, it was necessary to determine whether any of the microorganisms inherently present in tap water would either form colonies on ferric ammonium citrate agar or would ferment lactose broth. On two occasions, dechlorinated tap water was plated on FAC agar. On both occasions, no growth appeared. On three occasions, lactose broth tubes were inoculated with dechlorinated tap water. On all three occasions, no growth or gas production occurred. Based on these results, it was assumed that any bacterial growth on the FAC agar and any fermentation of the lactose broth that occurred during laboratory experiments was due to the P. cepacia or E. coli purposefully introduced into the experiments.

The main objective of the concentrator experiments was to ascertain what percent of influent bacteria could be recovered in the retentate or

the filtrate. Each time an experiment was performed, the number of P. cepacia or E. coli in the retentate as a percentage of the original number in the influent was determined. This value, called "percent recovery in the retentate" was determined as follows:

$$\text{Percent recovery in the retentate} = \frac{\text{total number of bacteria in retentate}}{\text{total number of bacteria in influent}} \times 100 \quad [8]$$

In the series of nineteen single-pass P. cepacia experiments, the percent recovery in the retentate ranged from a low of 7 to a high of 85. More detailed information from these experiments is included in Appendix C.

In the series of eleven single-pass E. coli experiments, the percent recovery in the retentate ranged from a low of 14 to a high of 126. The experimental conditions and results of these experiments are tabulated in Appendix D.

The percent recovery in the filtrate was determined in a similar manner for early experiments. Because this number was either zero or a small percentage of the influent number, it was not considered significant and will not be reported in the following tables. For the reader's information, Appendices C and D list the concentration of P. cepacia or E. coli in the filtrate.

Since percent recovery in the retentate was considered the most important parameter with which to evaluate the concentrator's performance, it would be of value to know what factors influence the percent recovery. It was thought that two system parameters, the concentration of P. cepacia or E. coli in the influent and the percent

reduction in volume, should be examined for their effect. The percent reduction in volume is a term describing the difference between the filtrate volume and the retentate volume. It was calculated using the following equation:

$$\text{Percent reduction in volume} = \left[1 - \frac{\text{retentate volume}}{\text{influent volume}} \times 100\right] \qquad [9]$$

In order to examine the relationship between percent recovery in the retentate and the influent concentration for the P. cepacia experiments, Figure 5 was developed based on raw data in Appendix C. For the points shown on Figure 5, the percent reduction in volume was held between 82 and 88 percent. A linear regression curve was fit to these points and is shown on the figure.

Figure 6 was developed to examine the relationship between percent recovery in the retentate and the influent concentration for the E. coli experiments. The percent volume reduction for these experiments was held between 54 and 64 percent. A linear regression model was fit to these points; this line is shown on the graph. Raw data for Figure 6 are found in Appendix D.

With the Pellicon concentrator, it was not possible to exactly control the percent reduction in volume. This parameter could vary from experiment to experiment even though no process modifications, such as valve adjustments, were made. Consequently, in Figures 5 and 6, an unintentional variation in the precent reduction in volume is a factor.

As mentioned above, the percent reduction in volume was the second system parameter thought to have an effect on the percent recovery in

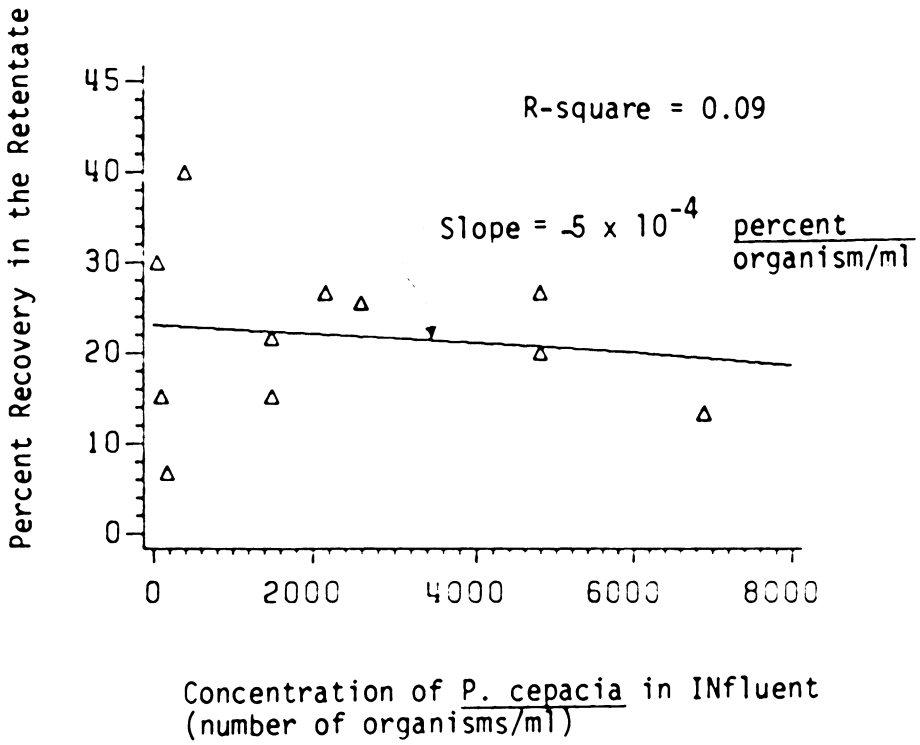


Figure 5. Percent Recovery versus Influent Concentration--
Single-Pass, P. cepacia Experiments.

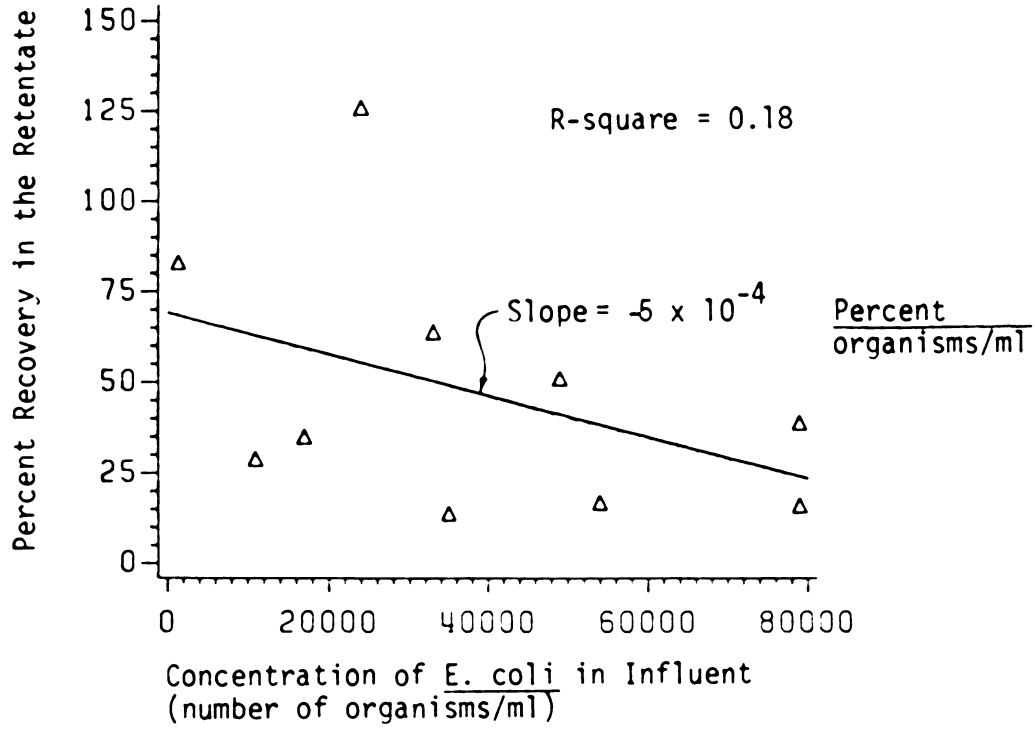


Figure 6. Percent Recovery versus Influent Concentration--
Single-Pass, E. coli Experiments.

the retentate. Figure 7 is a plot of the percent recovery in the retentate versus percent reduction in volume for the single-pass, P. cepacia experiments. A cubic regression equation was used to fit a curve through the points since, after examining linear, quadratic and cubic regression curves, the cubic curve was found to best describe the relationship. Because the influent concentration of P. cepacia varied from experiment to experiment, the concentration corresponding to each point plotted is shown next to the point. These concentration values are in terms of number of P. cepacia per milliliter of influent. Data for Figure 7 are contained in Appendix C.

Figure 8 is a plot similar to Figure 7. It presents data from the single-pass, E. coli experiments. Raw data for this figure are shown in Appendix D. As in the P. cepacia experiments, the concentration of E. coli in the influent varied from experiment to experiment. For this reason, the number of E. coli per milliliter present in the influent corresponding to each point plotted appears next to the point. A linear regression equation was developed for the data in the figure and is shown on the graph. Quadratic and cubic regression equations were also examined but discarded since the linear equation appeared to best fit the relationship. However, due to the large amount of scatter in the data points, the line plotted should be looked at in terms of indicating the trend of the relationship. The line does not, by any means, represent an exact mathematical expression of the results.

A final point to be noted regarding Figure 8 is the relatively small range of percent reduction in volume values. This small range is

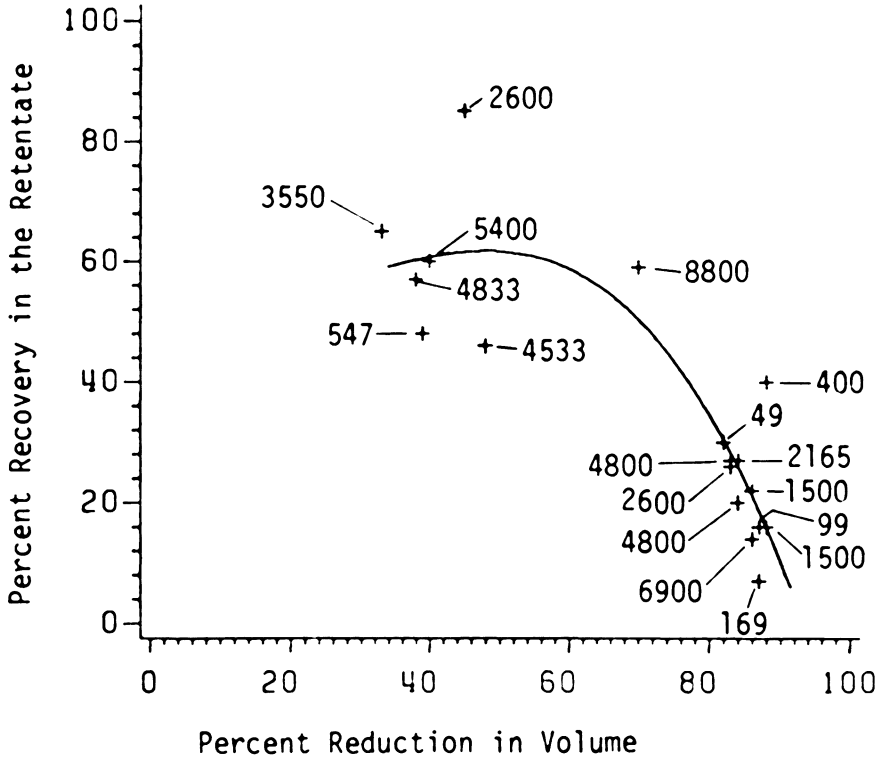


Figure 7. Percent Recovery versus Percent Reduction in Volume--Single-Pass, P. cepacia Experiments. (Numbers shown next to points represent the number of bacteria/ml.)

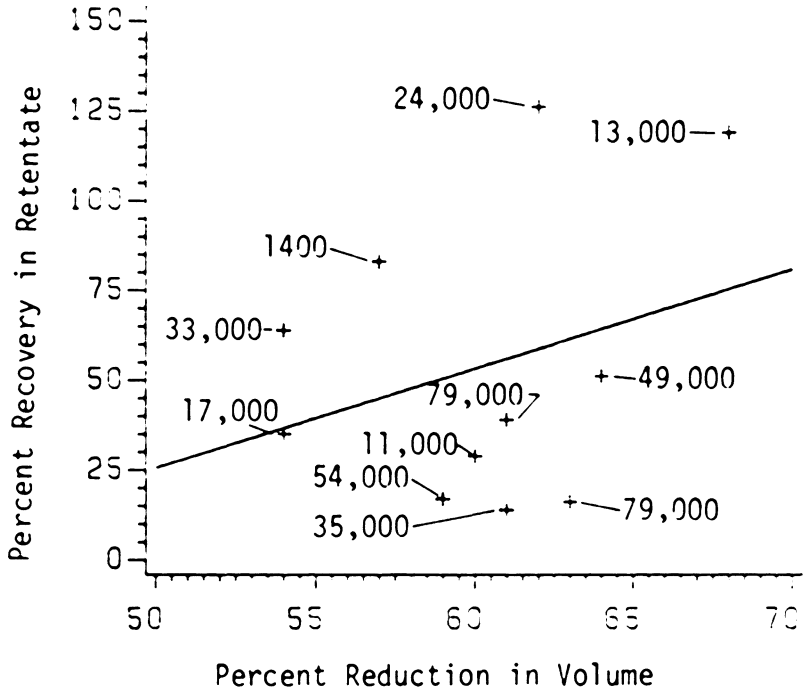


Figure 8. Percent Recovery versus Percent Reduction in Volume--Single-Pass, E. coli Experiments. (Numbers shown next to points represent the number of bacteria/ml.)

a result of the fact that the single-pass, E. coli experiments were performed to gather information about the percent recovery alone. Emphasis was placed on getting this information without varying experimental conditions to a great extent. Therefore, both the range of the percent reduction in volume values and the range of the influent concentration values are small.

As mentioned in Chapter III - Materials and Methods, when early experimental results indicated the number of P. cepacia or E. coli in the retentate and filtrate were less than the original number in the influent, a multiple-pass type of experiment was devised. Three multiple-pass experiments were performed using P. cepacia. The results of these experiments are presented in Table III. This table shows the effect of passing successive volumes of water through the concentrator. When a pass through the concentrator was made, a certain amount of water, inoculated with bacteria or not, was completely pumped through the concentrator. Each pass involved pumping a new quantity of water (generally 5 to 10 L) through the concentrator. In effect, after the initial volume of water, which contained P. cepacia and E. coli, was passed through the concentrator, the subsequent passes served to rinse the concentrator. Data for Table III are contained in Appendix E.

Two multiple-pass experiments were performed using E. coli. The results of these experiments are shown in Table IV. Again, the effect of passing successive volumes of water through the concentrator is shown. Appendix F contains the data for Table IV.

Table III. Results from Multiple-Pass, *P. cepacia* Experiments

Pass	Results
Experiment No. 1	
1	59% of influent bacteria recovered in retentate
2	43% increase in bacteria recovered
3	73% increase in bacteria recovered
Experiment No. 2	
1	57% of influent bacteria recovered in retentate
2	78% increase in bacteria recovered
3	51% increase in bacteria recovered
Experiment No. 3	
1	60% of influent bacteria recovered in retentate
2	94% increase in bacteria recovered
3	88% increase in bacteria recovered

Table IV. Results from Multiple-Pass, *E. coli* Experiments

Experiment No. 1	
Pass	Results
1	119% of influent bacteria recovered in retentate
2	14% increase in bacteria recovered
3	4% increase in bacteria recovered
Experiment No. 2	
Pass	Results
1	14% of influent bacteria recovered in retentate
2	35% increase in bacteria recovered
3	2% increase in bacteria recovered

After laboratory testing of the concentrator was complete, field experiments were performed. Results from these experiments are shown in Tables V and VI. Table V presents the results of tests for the presence of iron precipitators (for example, P. cepacia). Table VI presents the results of tests for the presence of lauryl tryptose broth fermenters (for example, E. coli). Starting with a sample volume of either 10 or 15 L, the sample was recirculated through the concentrator until approximately 1 L of retentate was obtained. This represents a substantial reduction in volume ranging from 91 to 94 percent. The time required for such a reduction in volume was approximately 15 minutes.

For Tables V and VI, it is important to note that the retentate values were calculated by comparing the retentate's bacterial counts with the grab sample's bacterial count. Separate samples of the influent for each experiment were not collected. This was done assuming the water was homogeneous and did not vary with time. It was necessary in order to make the field sampling and corresponding laboratory work possible to complete by one person.

As mentioned in Chapter III - Materials and Methods, samples of the sodium thiosulfate rinse solution used in the field were tested for the presence of iron precipitators and lauryl tryptose broth fermenters in order to determine whether bacteria had become caught in the concentrator. The results of these tests showed no growth of iron precipitators or lauryl tryptose broth fermenters. Based on this information, it was assumed that no bacteria were caught in the concentrator before each experiment was begun. Also, based on this

Table V. Results from Field Experiment Tests for Presence of Iron Precipitators

Sample	Average Plate Count	% Reduction In Volume	% Recovery In Retentate
Site 1, Grab Sample (influent)	6	-	-
Site 1, Retentate from 10 L Experiment	91	91	138
Site 1, Retentate from 15 L Experiment	34.5	94	33
Site 2, Grab Sample (influent)	0	-	-
Site 2, Retentate from 10 L Experiment	0	93	0
Site 2, Retentate from 15 L Experiment	0	94	0

Table VI. Results from Field Experiment Tests for Lauryl Tryptose Broth Fermenters

Site	Volume of Sample, liters	% Reduction in Volume	% Recovery
1	15	94	No fermenters detected in sample or retentate
1	10	91	No fermenters detected in sample or retentate
2	15	94	No fermenters detected in sample or retentate
2	10	93	No fermenters detected in sample or retentate

information, one can view the rinse water testing as additional distribution system water sampling.

B. RESULTS FROM THE QUESTIONNAIRE

The following tables and figures were derived from responses to the questionnaire sent to water treatment plant operators throughout Virginia. Operators from eighty-nine communities provided information about their water treatment plants and distribution systems. Of these eighty-nine, the responses from operators in sixty-seven communities were used in this analysis. The decision not to include a community was made largely on the basis of incompleteness of the response.

Table VII shows the different raw water sources which were reported as well as the percentage of total plants represented by each source. Ground water was the most common raw water source reported. Rivers and lakes were, respectively, the second and third most frequent raw water sources. Since the last five categories listed in the table made up such small percentages of the total reported sources, they were not considered in any of the following analyses.

The questionnaire listed six classes of water quality complaints: taste, odor, turbidity, red water, black water and other. Table VIII presents a summation of the various types of quality complaints reported. Two numbers are reported for each type of complaint--the number of plants reporting that type of complaint and the percentage of the total plants that number represents. Taste complaints were the most frequently reported. The least frequent complaint was black water.

Table VII. Raw Water Sources

Raw Water Source	Number of Plants Using This Source	Percentage of Total Plants
Groundwater	25	36.76
River	21	30.88
Lake or Impoundment	13	19.12
Groundwater and River	1	1.47
Groundwater and Lake	3	4.41
Groundwater, River and Lake	2	2.94
River and Lake	2	2.94
Other	1	1.47

Table VIII. Numbers and Percentages of Each Type of Quality Complaint

Type of Complaint	Number of Plants Reporting Said Complaint	Percentage of Total Plants
Taste	45	67
Odor	38	57
Turbidity	31	46
Red Water	17	25
Black Water	9	13
Other	15	22

Reports in the literature often stated that complaints about water quality occur in specific areas of the distribution system. To determine if such was the case in this survey, the water treatment plant operators were asked to specify whether complaints received were mainly from one area, were distributed or were from dead-end areas. Table IX summarizes the responses to this question.

The literature also contained reports that certain raw water sources were more susceptible to particular water quality problems than others. For example, utilities using lakes and impoundments as their raw water source would assumedly receive more taste and odor complaints than a utility using a river as its raw water source. To determine whether receipt of quality complaints reported in this study followed a pattern dependent on the raw water source, a tabulation was produced pairing raw water sources with receipt of water quality complaints. The water utilities were divided into three groups by raw water source. The number of plants using each source and receiving a specific water quality complaint was tabulated. Also, the number of plants using the source which did not receive the complaint was determined. These numbers are shown in Table X.

One of the main goals of the analysis of the questionnaire data was to determine if one could predict under what conditions a water utility was likely to receive a complaint regarding the quality of the water. In order to do this, a relationship between the receipt of a quality complaint and some parameter or method of treatment must be established. Several likely finished water parameter/complaint pairs were examined to

Table IX. Location of Complaints

Location	Number of Plants Reporting Complaints Mostly from the Location	Number of Plants Reporting Complaints Not from the Location
One area	12	50
Distributed	51	11
Dead-end areas	24	38

Table X. Yes/No Breakdown for Receipt of Specific Types of Complaints for Each Raw Water Source.

Raw Water Source	Yes/No Breakdown for Receipt of Said Quality Complaints					
	Taste	Odor	Turbidity	Red Water	Black Water	Other
Groundwater	Yes 13 No 10	Yes 9 No 14	Yes 10 No 13	Yes 4 No 19	Yes 0 No 23	Yes 5 No 18
River	Yes 15 No 6	Yes 12 No 9	Yes 7 No 14	Yes 4 No 17	Yes 2 No 19	Yes 4 No 17
Lake or Impoundment	Yes 11 No 2	Yes 11 No 2	Yes 8 No 5	Yes 5 No 8	Yes 5 No 8	Yes 2 No 11

ascertain whether a correlation existed. In addition to finished water parameters, treatment process/complaints pairs were examined to see if their use had an effect on receipt of quality complaints. The following paragraphs contain more detailed information regarding on what basis parameter/complaint or treatment process/complaint pairs were examined to ascertain whether a correlation existed.

Table XI summarizes the relationships between the use of certain unit operations and the receipt of water quality complaints. The questionnaire contained a list of possible treatment operations and the operators were asked to indicate which operations their plants used. This information was paired with the response to the question of whether they received certain water quality complaints. Table XI lists a number of operations and divides the operators' responses into yes/no categories depending on whether the operation is used or not. Each "yes" or "no" category is further broken down into whether the operator also received a given water quality complaint or not. Thus, from the table it can be seen, for instance, how many plants using coagulation received turbidity complaints and how many did not. The table also shows how many of the plants that did not use coagulation received turbidity complaints and how many did not. From these types of pairings, insights into the relationships between unit operations and quality complaints were sought.

The use of aeration was paired with the receipt of taste, odor, red water and black water complaints. One of the reasons for using aeration is to oxidize or strip out organic compounds which can either directly

Table XI. Relationships Between Use of Unit Operations and Receipt of Water Quality Complaints.

Use of Operation		Receipt of Water Quality Complaints: Taste Yes	Taste No	Odor Yes	Odor No	Turbidity Yes	Turbidity No	Red Water Yes	Red Water No	Black Water Yes	Black Water No
Aeration:	Yes	4	0	4	0			2	2	1	3
	No	39	19	32	36			13	45	7	51
Coagulation:	Yes					16	22				
	No					12	12				
Prechlorination:	Yes	26	10	23	13						
	No	17	9	13	13						
Granular Media:	Yes					17	21				
	No					11	13				
PAC/GAC:	Yes	18	3	16	5						
	No	25	15	20	21						
Chlorination:	Yes	42	15					15	42	7	50
	No	1	3					0	5	1	4

cause taste and odor problems or can serve as food for organisms that, in turn, cause taste and odor problems. Aeration is also used to oxidize iron and manganese, forming ferric and manganic precipitates which can be removed from the water before it enters the water distribution system. If iron and manganese are not precipitated prior to entering the distribution system, they can be precipitated in the system, causing red water and black water problems. Because of all of the above, it was thought the relationships between the use of aeration and the receipt of taste, odor, red water and black water complaints would be interesting.

Coagulation is used to facilitate the removal of particulate matter from water. The use of coagulation, therefore, might be expected to affect receipt of turbidity complaints. For this reason, coagulation and turbidity are paired in Table XI.

Prechlorination is another oxidation process which reportedly aids in alleviating taste and odor complaints. These relationships are presented in Table XI.

The use of granular media as a means of removing particulate matter should affect the turbidity of the water and a pairing of the use of granular media and receipt of turbidity complaints is found in the table.

Use of powdered activated carbon (PAC) and granular activated carbon (GAC) is often initiated solely as a remedial measure for taste and odor problems. The relationships between use of PAC or GAC and

receipt of taste and odor problems were considered important and are included in Table XI.

The final operation listed in Table XI is chlorination. Chlorination should not only cause the oxidation of taste- and odor-causing substances and iron and manganese, it should also provide protection in the distribution system against the growth of nuisance bacteria which cause taste, red water and black water problems. For these reasons, chlorination and receipt of taste, red water, and black water complaints were paired.

Figures 9 through 13 present relationships between finished water parameters and quality complaints. These figures have several common components which will be explained here first, followed by a more specific discussion of each graph. The finished water parameter values are average values obtained from one of two places: either from information supplied by the plant operators via the questionnaire or from the Commonwealth of Virginia's Department of Health. The units for the quality complaints shown in Figures 9 through 13 are "number of complaints per person per month." These values were derived from information from the questionnaires. Operators were asked to enumerate how many complaints their utility received per month. They were then asked what percentage of the total complaints were taste complaints, odor complaints, etc. These values, together with the population figures also reported on the questionnaire, were used to calculate the number of a specific type of complaint a utility received per person per

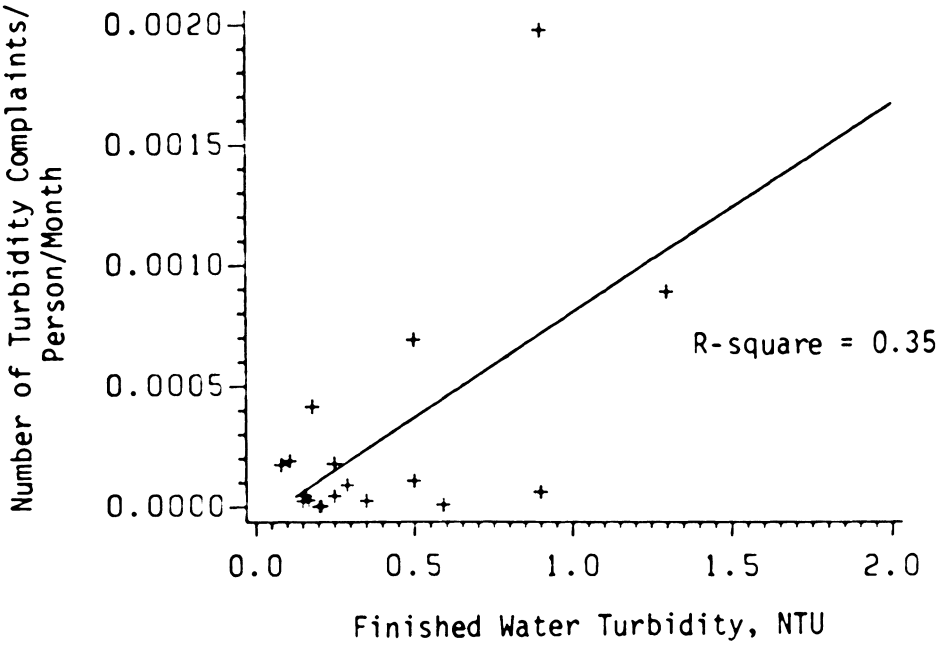


Figure 9. Number of Turbidity Complaints versus Finished Water Turbidity.

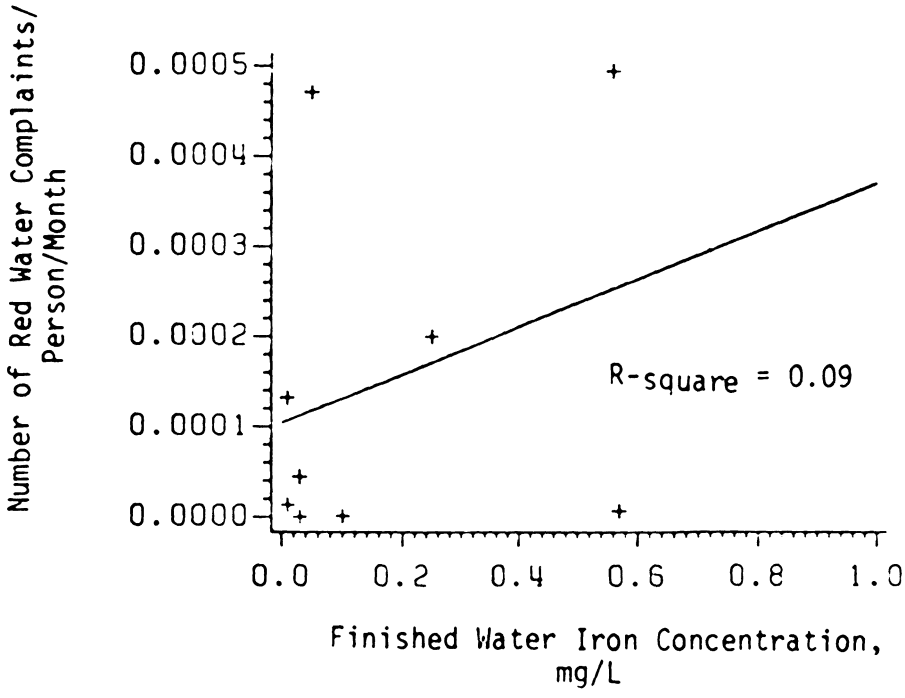


Figure 10. Number of Red Water Complaints versus Finished Water Iron Concentration.

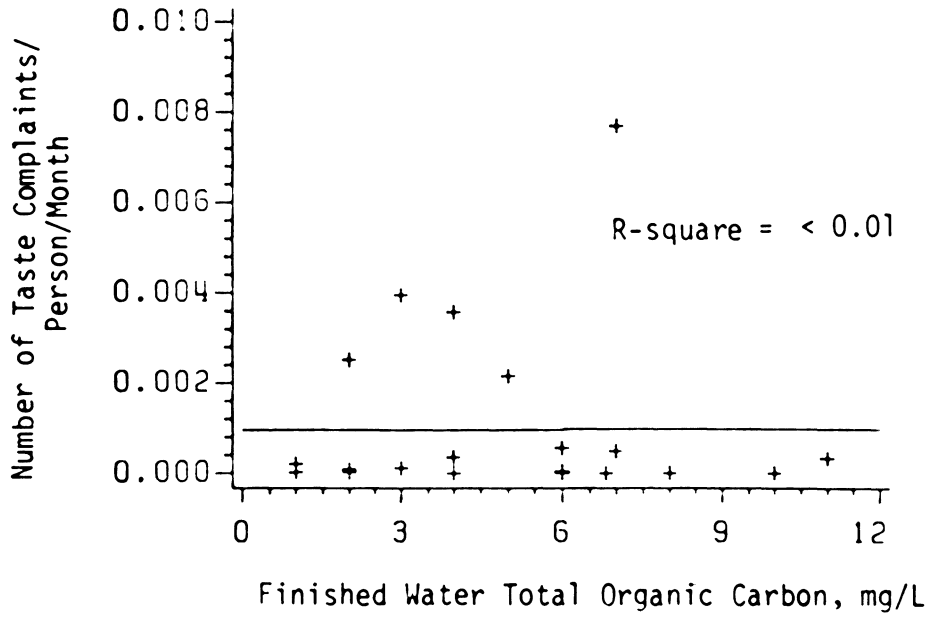


Figure 11. Number of Taste Complaints versus Finished Water Total Organic Carbon.

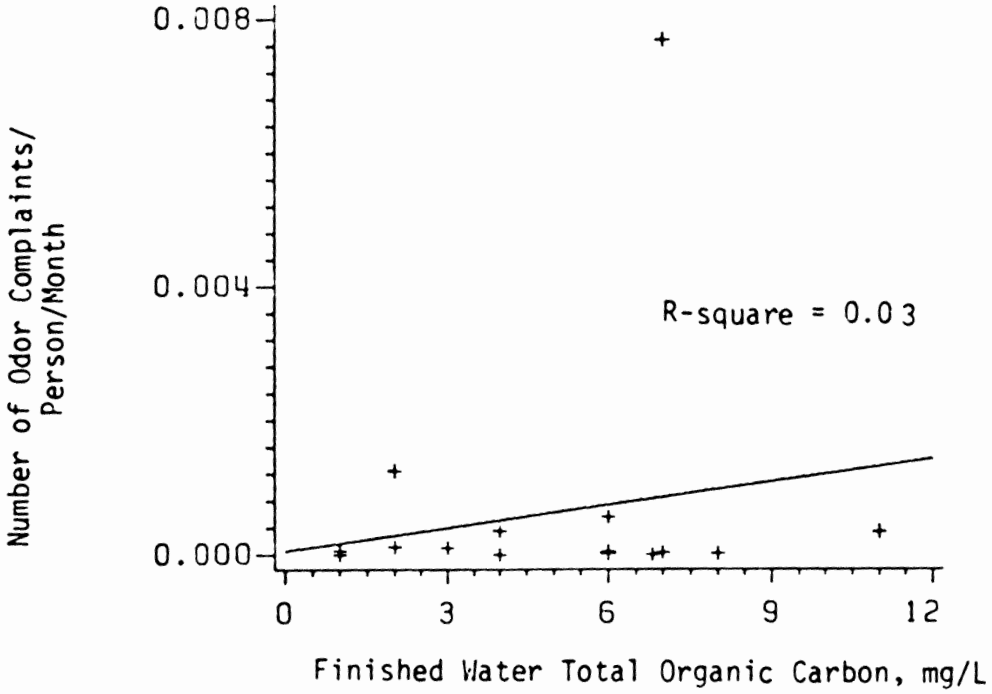


Figure 12. Number of Odor Complaints versus Finished Water Total Organic Carbon.

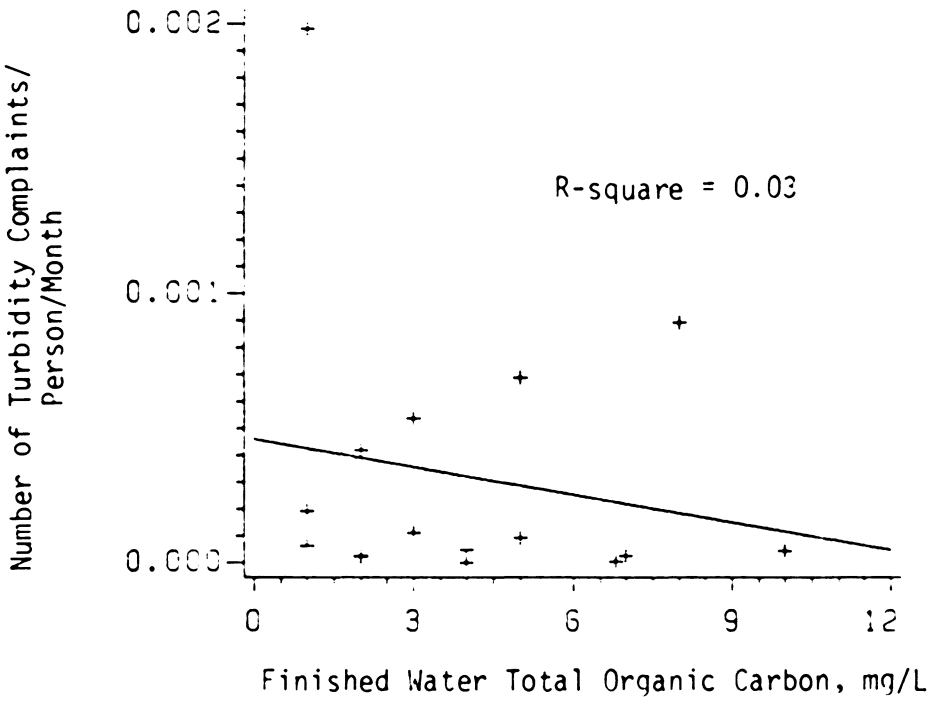


Figure 13. Number of Turbidity Complaints versus Finished Water Total Organic Carbon.

month. For example, the number of turbidity complaints per person per month for a given utility was calculated in the following way:

$$\text{Number of turbidity complaints/person/month} = \frac{\text{Total number of complaints per month} \times \frac{\text{Percent of total complaints that are turbidity complaints}}{100}}{\text{Population served by water utility}} \quad [10]$$

A linear regression equation was fit to each relationship plotted in Figures 9 through 13. At times, the scatter of the data made fitting any curve questionable. It must be kept in mind that these lines are presented only to indicate the trend of the relationships shown. Indications of how well the regression lines fit the existing data are given by the R-square values shown on each graph. The R-square value is a statistical measure which can range from 0 to 1. The closer to 1 the R-square value is, the better the regression line fits the existing data.

Figure 9 shows the relationship between the number of turbidity complaints per person per month and the finished water turbidity. The units of turbidity are nephelometric turbidity units, or NTUs. Finished water turbidity is a well-monitored parameter. Seventy percent of the water treatment plant operators measured this parameter while thirty percent did not. It would seem reasonable to assume that a relationship between finished water turbidity and receipt of turbidity complaints should exist. To test this assumption, Figure 9 was developed.

The number of red water complaints received per person per month as a function of the finished water iron concentration is plotted in Figure

10. Notice the relatively few points available for this graph. As Table VIII indicated, red water is not a common quality complaint. Also, only 51 percent of the water treatment plant operators monitored finished water iron. This combination limited the number of points available for this relationship. However, the relationship itself is one of interest since the level of iron in the effluent would assumedly affect the number of red water complaints.

Figures 11, 12 and 13 illustrate the relationship between finished water total organic carbon concentration and taste, odor and turbidity complaints. Total organic carbon, or TOC, is a measure of the amount of the water's carbon that is tied up in organic molecules and is thus available as a food source for heterotrophic organisms. The relationships shown in Figures 11 through 13 were plotted because taste, odor and turbidity are all problems that can be caused by heterotrophic, nuisance bacteria. It was hoped that the total organic carbon level could be used as an indicator of the potential for the presence of these bacteria in the distribution system based on receipt of these quality complaints. A link could then be made between these bacteria and receipt of quality complaints.

V. DISCUSSION

This chapter discusses the results presented in Chapter IV. Again, because of the nature of this thesis' work, the chapter is divided into two parts: Section A deals with the results of the concentrator studies and Section B discusses the questionnaire results.

A. DISCUSSION OF THE RESULTS FROM THE CONCENTRATOR EXPERIMENTS

The main objectives of the concentrator experiments were to ascertain the percent recovery to be expected from the unit under conditions similar to what would be experienced in the field and to ascertain what factors affected this recovery. It must be stressed that experimental conditions were structured to imitate conditions encountered in water distribution systems. For example, tap water was used and no chemical additions to the sample were made. Accordingly, the results from the concentrator experiments reflect its performance under a specific set of conditions. They are not indicative of the optimal performance level of the Pellicon concentrator.

As mentioned in Chapter IV. Results, the recovery in the retentate for single-pass P. cepacia experiments ranged from 7 to 85 percent. The recovery in the retentate for single-pass E. coli experiments ranged from 14 to 126 percent.

Possible reasons for the broad range of recovery values are discussed in the succeeding paragraphs which deal with factors affecting the percent recovery. Two system parameters, the influent microbial

concentration and the percent reduction in volume, were thought to affect the percent recovery. Figures 5 through 8 were developed to analyze these effects.

Observations regarding percent recovery as a function of influent microbial concentration can be made from Figures 5 and 6. Figure 5, which contains the data from the single-pass, P. cepacia experiments, shows a slight inverse relationship in that when the concentration of bscteria in the influent increased, the percent recovery in the retentate decreased. The slope of the line plotted, which equals -5×10^{-4} percent per organism per ml is slight and the scatter of the points must be considered. If the information in Figure 5 were the only available data illustrating the relationship between influent microbial concentration and percent recovery, one might decide the data are inconclusive. However, Figure 6 presents the results of the E. coli experiments. It, too, indicates that as the influent concentration increased, the percent recovery decreased. The slope of the line shown on Figure 6 is also, quite surprisingly, -5×10^{-4} percent per organism per ml. Taken together, Figures 5 and 6 make a strong case for concluding that increases in influent microbial concentrations had a negative effect on percent recoveries in the retentate.

It might seem likely that as the percent recovery in the retentate decreased, the percent recovery in the filtrate would increase. However, as mentioned in Chapter IV, the number of bacteria that were recovered in the filtrate was consistently zero or negligible. It appears, then, that the decrease in the concentrator's recovery

efficiency with increased bacterial concentration was due to the fact that as more bacteria were introduced to the concentrator, more became impinged on and trapped in the filter membrane. Millipore (56) explained that a key to the concentrator's operation was the "wave-like" motion which washed the particles along the membrane. At higher bacterial concentrations, there was less fluid per bacterial cell to induce this wave-like motion. In slightly different terms, this meant that a given amount of fluid carried a larger number of bacteria along the membrane. This increased load may have led to a bacterial cell's becoming attached to the filter more readily while the chances of its getting washed along the membrane decreased. All of this resulted in a decreased recovery efficiency with increased bacterial concentration.

In water from a distribution system, high concentrations of microorganisms are unlikely. Although very low values of influent bacterial concentration were not utilized in the experiments reported here, it appears that recovery of microorganisms from solutions in which their concentrations are low would be superior to recovery from solutions in which their concentration was high. If this trend holds true even at very low bacterial concentrations, the Pellicon concentrator would be expected to perform well when used for sampling in water distribution systems.

The second system parameter upon which the percent recovery was thought dependent was the percent reduction in volume. Figures 7 and 8 present percent recovery as a function of percent reduction in volume. Figure 7, containing the results of the single-pass P. capecia

experiments, shows a distinct downward trend. As the percent reduction in volume increased, the percent recovery in the retentate decreased. The results of the single-pass E. coli experiments, exhibited in Figure 8, indicate the opposite. As percent reduction increased, percent recovery increased. Once again, it is useful to take into account the scatter of the data in both graphs. The points in Figure 7 conform closely to a curve. The points in Figure 8 do not conform to any distinguishable pattern (a circle is probably the best suited curve). It is possible that the influent E. coli concentrations in Figure 8 were too diverse, making the figure essentially a plot of three variables: percent recovery in the retentate, percent reduction in value and influent bacterial concentration.

As stated above, Figure 7 shows that as the percent reduction in volume increased, the percent recovery in the retentate decreased. The percent reduction in volume was controlled by partially closing a valve in the retentate tubing, causing a back pressure on the concentrator. The higher pressure needed for the sample to pass through the concentrator appears to have caused more bacteria to become impinged on the filter membrane. Filter clogging at higher pressures is not an uncommon occurrence with any type of filter. Indeed, in the operations manual for the Pellicon concentrator (56), the manufacturer warned that poorer recoveries at higher reductions in volume were to be expected. During use of the concentrator for sampling in water distribution systems, either the lower percent reduction in volume must be used or

the trade-off between retentate volume and recovery efficiency must be realized and accounted for.

Tables III and IV contain the results of the multiple-pass experiments. It is evident from Table III, which shows the multiple-pass P. cepacia results, that as more uninoculated tap water was passed through the concentrator, more bacteria were recovered. From this the author concludes that bacteria from the original, inoculated sample became caught in the filter membrane and were washed off by the succeeding quantities of uninoculated tap water that were passed through the concentrator. Table III also shows that, in experiments 2 and 3, the increase in bacteria recovered decreased with each successive pass.

Table IV presents the results from the two multiple-pass experiments that used E. coli. In experiment number 1, over 100 percent recovery was achieved after the first pass. In experiment number 2, however, only 14 percent recovery occurred after the first pass. This variability which was typical of most of the E. coli results, may be partially explained by the fact that the MPN procedure used to assay E. coli is not a direct measure of the number of bacteria present. Instead, MPN values are numbers, derived by statistical methods, which relate how many bacteria would probably be needed to cause the observed result.

The results from the multiple-pass E. coli experiments indicate the same trend as the P. cepacia experiments in that the increase in bacteria recovered decreased with each pass through the concentrator. The values for percent increases in bacteria recovered for the E. coli

experiments were smaller than for the P. cepacia experiments. The percent increase values also decreased more rapidly for the E. coli experiments. This indicates first that the Pellicon concentrator's performance differed depending on the bacterium used. Differences in the outer membranes of the two bacteria may cause the variation in the performance. Table IV also indicates that most of the E. coli recovery can be expected by the second pass. It appears, then, that E. Coli does not become attached to the filter membrane as tenaciously as P. cepacia.

Field experiments were carried out to provide insights into performance of the concentrator under actual in-situ circumstances. Tables V and VI present the results of these field experiments. Table VI shows that no lauryl tryptose broth fermenters were detected in either the sample or the retentate. Although this result speaks well for the distribution system's drinking water quality, it did not aid in evaluating the concentrator performance.

Table V indicates that no iron precipitators were found at site 2. Looking at the site 1 data, one finds a wide range of percent recoveries for a narrow range of percent reduction in volume. Given the very low average plate count for the influent grab sample, this wide range is not surprising. As was seen in Table II, low plate counts caused high coefficients of variation. Unfortunately, (though fortunate from a safety standpoint) it is hard to avoid low plate counts when dealing with drinking water. It was for this very reason that the use of a concentrator was considered. However, the fact remains that the low average plate count value for the site 1 influent grab sample meant

large variations in the percent recovery values were possible. It is encouraging, though, that the average plate counts for the retentate from the 10 L and 15 L experiments were substantially higher than the grab sample plate count. To some degree, the concentrator was effective.

B. DISCUSSION OF THE RESULTS FROM THE QUESTIONNAIRE

This portion of Chapter 5 deals with a discussion of the questionnaire results. Some of these results, which need little discussion, were presented to develop a framework from which other results can be scrutinized. For example, the fact that ground water was the most frequently reported raw water source does not lend itself to much discussion but may lead one to anticipate that the most frequently reported quality complaint would be ground-water related. On the other hand, given the factual framework developed from the questionnaire results, many subsequent results say something about the preconceptions one has regarding water distribution systems. In other words, some of the results are surprising.

Table VII indicates that most raw water sources were ground waters or rivers. Lakes or impoundments supplied substantially fewer water systems. This result is not unexpected since lakes are rare in Virginia (58) whereas many of the geological formations, such as the Karst topography in the western part of Virginia, lend themselves to ground water formations.

According to Table VIII, taste and odor were the most frequently cited quality complaints. It is interesting to note the difference in the values between the taste complaints and odor complaints. A human's sense of taste is largely dependent upon the sense of odor and yet Table VIII shows a significantly higher percentage of taste than odor complaints. This result is more than likely an artifact of the manner in which consumers complained.

Turbidity complaints were reported by less than half of the plant operators. This result speaks well for the manner in which the operators were running their plants and also indicates that turbidity problems caused by microbial growths in the distribution system, if such was indeed a cause of this problem, were not a widespread occurrence.

Given the prevalence of ground water usage in Virginia, one might have expected more red and black water complaints to have occurred. In fact, black water was the least reported quality complaint. The results of the questionnaire have led the author to wonder how many black water complaints were reported as turbidity complaints. Unfortunately, this question is unanswerable.

"Other" complaints represented a group of nonspecific complaints that plant operators may have received, such as low water pressure. As such, it was an indefinite group about which conclusions cannot be made.

Table IX was composed to determine whether or not complaints about water quality occurred in specific areas of distribution systems. Fifty-one of 62 plants reported that complaints were distributed throughout the system. This is confirmed by the numbers in the "one

area" row in which 50 plants reported that complaints did not stem from one area of the distribution system. However, Table IX also shows that dead-end areas were not more susceptible to water quality problems than other areas. Twenty-four of 62 plants, or only 39 percent, responded that complaints came mostly from dead-end areas. This results contradicts literature statements regarding the relationship between dead-end areas and complaints. It appears that the causes of water quality degradation in water distribution systems were not confined to conditions which existed in specific areas of the distribution system. Explanations profferred for quality degradation problems in Virginia must take this fact into account.

Given the prevalent raw water sources, one might have expected certain types of quality complaints. For example, since ground water was the most prevalent raw water source, one would expect red and black water complaints to have been common. Table X examines what types of water qualtiy complaints plants utilizing a given type of raw water source received. Discussion of this table in the next few paragraphs will proceed by taking each column in the table, i.e., each complaint, at a time.

Taste complaints were reported most frequently by plants which utilized lakes or impoundments. Eighty-five percent of these plants reported taste complaints as opposed to 71 percent for those which utilized rivers and 56 percent for those utilizing ground water. This result can be explained in terms of bacterial and algal food supplies. Lakes, with their relatively quiescent waters and nutrient-bearing

sediments, are more conducive to bacterial and algal growth than rivers or aquifers. These bacteria and algae can produce taste-causing compounds, which is most likely the reason for the high percentage of taste complaints reported by lake water users.

The general result is the same, though the percentages are different, for odor complaints as for taste complaints. This result is not surprising since the same organisms reportedly responsible for taste problems are also often responsible for odor problems and since a human's sense of taste is dependent on the sense of odor.

Table X shows that plants which used lakes or impoundments as their raw water sources received the highest percentage of turbidity complaints. It is interesting to note that many plants using groundwater as their raw water also encountered turbidity problems. These result indicate that the turbidity may have been the result of water quality deterioration within the distribution system since all water treatment plants must meet a turbidity standard set by the Department of Health.

Red water and black water appear to have been the most troublesome for lake or impoundment users. At first glance, this result is surprising since red and black water problems are assumed to be primarily in the realm of groundwater users. There are several possible explanations for this result. Plants whose water source was groundwater may have had iron and manganese removal units included in the plant design. These plants would affect continuous iron and manganese removal. Plants whose water source was a lake or impoundment might not

have had iron and manganese removal units included in their design. They may, however, encounter higher iron and manganese levels during certain times of the year. One such scenario would be during lake turnover when anaerobic, reduced waters containing iron and manganese may be mixed with the upper portion of the lake and drawn off. The plants utilizing this water may not have iron and manganese removal capabilities. Red water and black water problems may ensue.

An explanation presented by Ticek (59) also may explain the result. Iron and manganese present in groundwater and surface water can exist in many states, one of which is in organic complexes. Organically-complexed iron and manganese are more difficult to remove than inorganic iron and manganese compounds because the organic molecules surround the iron and manganese, forming a protective layer. Iron and manganese in surface water would have more exposure to organic compounds with which to complex. Ticek further stated that if the soluble iron and manganese are not removed "complaints almost certainly result."

Table XI attempts to correlate the use of certain unit operations in water treatment to the receipt of specific water quality complaints. It was hoped that by producing Table XI, one could establish what effects these unit operations had on receipt of complaints. In some cases, however, this was difficult to do, as will be explained in the following paragraphs.

Of the four utilities that aerated their water, four reported receipt of taste and odor complaints. Aeration did not, therefore,

appear to be an effective means of avoiding taste and odor complaints. Of the 58 plants that did not use aeration, 39 received taste complaints, 32 received odor complaints. These values indicate that aeration had little to do with receipt of taste and odor complaints since not using aeration did not significantly change the chances of receiving either complaint. Although aeration reportedly aids in oxidizing or stripping compounds which can cause tastes and odors, the results of the questionnaire do not confirm the reports.

Use of aeration also did not appear to affect the chance of receiving red or black water complaints. Two of the four plants using aeration received red water complaints while two did not, indicating that the cause of red water was not alleviated by aeration. It is possible that aeration aided oxidation of iron but that oxides were not adequately removed prior to plant discharge to the distribution system. The oxides could then have been the cause of the red water problem. Or, oxidation of the iron during aeration may have been incomplete, with the remaining oxidation occurring in the distribution system. This could also have caused red water problems. Pipeline corrosion also may have played a part in these red water complaints. Whatever the reason, use of aeration did not completely alleviate red water problems.

Aeration may, however, have been more effective in reducing black water problems. Three of the four plants which used aeration did not receive black water complaints. Also, an overwhelming majority of plants that received black water complaints did not aerate. From these

data, it appears aeration was effective in alleviating black water problems.

Assuming that no water quality degradation in water distribution systems occurred, one would expect that plants which utilized coagulation would have received few turbidity complaints. Table XI shows that 38 plants incorporated coagulation in their treatment chain. Sixteen of the 38, or 42 percent, received turbidity complaints. Of the 24 plants that did not use coagulation, the results were split--12 received turbidity complaints and 12 did not. Given these results, it appears that use of coagulation was not a determining factor for receipt of turbidity complaints.

Prechlorination is reportedly effective in alleviating taste and odor problems. Thirty-six utilities responded affirmatively to use of prechlorination. Twenty-six of the 36 received taste complaints and 23 received odor complaints. When one looks at the responses of the 26 plants that did not prechlorinate, it is seen that 17 received taste complaints and 13 received odor complaints. These values imply that prechlorination increased the potential for receipt of both taste and odor complaints. While chlorine oxidizes some compounds which cause taste and odor, it also combines with other compounds to produce taste- and odor-causing substances. For instance, phenols attain their maximum taste-producing level only after chlorination (60). It can be concluded that prechlorination was not the definitive answer to taste and odor problems.

Assuming no water quality degradation in the water distribution system occurred, a strong correlation between use of granular media and recipt of turbidity complaints would be expected. Table XI reveals that of the 38 utilities which used granular media, 17 received turbidity complaints and 21 did not. The results for those plants which did not use granular media are equally as inconclusive. Of the 24 plants which did not perform filtration with granular media, 11 received turbidity complaints while 13 did not. Receipt of turbidity complaints appears to have had little to do with use of granular media filtration. In explaining the cause of turbidity about which consumers complained, one would have to turn to some factor other than use or disuse of granular media.

As explained in Chaper IV - Results, use of powdered activated carbon (PAC) and granular activated carbon (GAC) is often initiated as a remedial measure for taste and odor problems. Twenty-one plants reported use of PAC/GAC. Of these 21, 18 reported receiving taste complaints and 16 reported odor complaints. These values do not reflect whether the use of PAC/GAC was intermittent, whether the taste and odor complaints were intermittent or whether the PAC/GAC was effective in alleviating the taste and odor problem. On the surface, these results appear to imply that PAC and GAC were ineffective against taste and odor problems. But, since PAC and GAC may have been used as a remedial measure for taste and odor problems, it is logical to expect that those using PAC and GAC were those receiving taste and odor complaints. Table

XI merely confirms this and does not provide insight into the effectiveness of the use of PAC/GAC for taste and odor control.

Most of the plant operators who responded to the questionnaire reported use of chlorination. A large majority (74 percent) of these operators also reported receipt of taste complaints. When one compares this 74 percent with the fact that 67 percent of all the plants in the survey reported receipt of taste complaints, it becomes apparent that the use of chlorination was not alleviating taste problems. This information, coupled with the data from the prechlorination row in Table XI, leads one to suspect that the taste and odors about which complaints were made was a chlorine-related taste and odor. Chlorine tastes and odors may have been more of a problem from a consumer standpoint than earthy or musty tastes and odors normally envisioned as the reason for taste and odor complaints.

The final pairings in Table XI relate use of chlorination with receipt of red and black water complaints. Proper chlorination provides residual disinfectant within the distribution system. If red and black water problems are caused by bacteria in the distribution system, chlorination should help alleviate the problem. Of the 57 plants that chlorinated, 15 received red water complaints and 7 received black water complaints. These values appear to imply that chlorination was indeed useful in alleviating red and black water problems. However, when one examines the values for those plants receiving red and black water complaints, the relationship is not at all clear. Of the 15 plants which received red water complaints, 15 chlorinated. Of the 8 plants

which received black water complaints, 7 chlorinated. The problem in evaluating these data is few plants received red or black water complaints while most plants performed chlorination regardless of the likelihood for red or black water problems. Again, conclusions cannot be drawn from these data.

Figure 9 is the first of a series of graphs showing water quality complaints as a function of finished water parameters. The correlation between finished water turbidity and the number of turbidity complaints, as shown in Figure 9, signifies that as the finished water turbidity increased, the number of turbidity complaints increased. The line plotted in the figure is the result of a linear regression analysis performed on the data. The R-square value, which is a measure of how well the line fits the existing data, is 0.35. The smallness of this R-square value reflects the regression line's poorness of fit. However, the general trend of the data is for increased turbidity complaints with increased finished water turbidity. This results could have been caused by two factors. First, the turbidity in evidence at the beginning of the distribution system may have been passed along unchanged to the consumer. Second, the turbidity in the plant discharge may have encouraged microbial growths in the distribution system which were, in turn, causing turbidity problems at the consumer's tap. Turbidity can encourage microbial growths by masking disinfectants and by acting as a food source. Figure 9 suggests that improving effluent turbidity may indeed help to alleviate turbidity complaints.

Figure 10 shows a positive relationship between the finished water iron concentration and the receipt of red water complaints: as finished water iron increased, red water complaints also increased. The R-square value of 0.09 for this relationship makes it evident that, if one speaks in terms of statistically proven correlations, one must conclude that the occurrence of red water complaints was not dependent on finished water iron concentration.

Figures 11, 12 and 13 examine taste, odor and turbidity complaints as a function of finished water total organic carbon. Figure 11, which pairs finished water total organic carbon and taste complaints, is distinctly noncommital. The horizontal regression line can be interpreted to mean TOC had nothing to do with receipt of taste complaints. The R-square value, barely distinguishable at 0.01, confirms this interpretation.

Figure 12, on the other hand, shows a slight positive relationship between finished water total organic carbon and receipt of odor complaints. However, the R-square value of 0.03 implies that the relationship is not significant.

The last finished water TOC/complaint pair involves receipt of turbidity complaints as is shown in Figure 13. The graph reveals an inverse relationship between the two variables: as finished water TOC increased, turbidity complaints decreased. Again, the R-square value, equal to 0.03, indicates that no relationship is evident. A conclusion which can be drawn from these Figures 11, 12, and 13 is that receipt of water quality complaints bears no relationship of finished water ROC.

To summarize the discussion of the questionnarie results, the relation of unit operations to receipt of water quality complaints was examined. In all cases, no clear-cut relationship between the use of an operation and the receipt of a complaint could be found.

Also examined in the questionnaire analysis were relationships between certain finished water parameters and the receipt of water quality complaints. No significant correlations were found for any of these pairings except finished water turbidity and receipt of turbidity complaints. These results show that finished water parameters were not indicative of the quality of water at the user's tap.

VI. CONCLUSIONS

From the results of the concentrator experiments, the following conclusions can be made regarding the use of the Pellicon concentrator.

1. The concentrator's performance varies with the type of bacteria being recovered.
2. Thirty to sixty percent recovery of bacteria in the retentate can be expected when a single-pass operation is used.
3. A multiple-pass procedure, or some other type of process modification must be instituted if higher recoveries are desired.
4. Recovery of bacteria in the filtrate will be zero or negligible.
5. The influent microbial concentration will have a minor effect on the percent recovery. As the influent microbial concentration increases, the recovery in the retentate can be expected to decrease.
6. Depending on the type of bacteria to be recovered, the percent reduction in volume may have a more significant affect on the percent recovery in the retentate. For P. cepacia, as the percent reduction in volume increased, the recovery in the retentate decreased. For E. coli, the effect of the volume reduction on the percent recovery was indeterminate.

From the use of the Pellicon concentrator in the laboratory and in the field, advantages and disadvantages of the Pellicon concentrator became evident. These are listed below.

Advantages

1. The time required for the concentration procedure is small.
2. When operated in the recirculating flow mode, the concentrator can greatly reduce the sample volume, facilitating sample transportation and testing.
3. The filter membrane is reusable, which is advantageous from two standpoints. The first is a cost standpoint since a one-time investment in a filter membrane is all that is necessary. The second standpoint is one of time. Because the filter membrane is reusable, set-up and dismantling of the concentrator between samples or experiments is unnecessary.
4. Although the filter membrane itself is fragile, once the concentrator has been assembled, the apparatus is rugged.

Disadvantages

1. Without use of the multiple-pass procedure, high percent recoveries of bacteria in the retentate cannot be expected.
2. The concentrator must be chlorinated and then dechlorinated between uses; with conventional filters, one simply starts with a fresh filter for each use.
3. The concentrator requires use of a pump. A power source then becomes necessary.

In examining the results of the questionnaire, it was hoped that a conclusive statement could be made about whether the water quality complaints received by water treatment plant operators in Virginia were caused by microbially-caused degradation within the water distribution

system or by poor water quality leaving the treatment plant. The most easily-reached conclusion on this matter is that the causes of water quality complaints are complex. Both the manner in which information about this issue can be obtained and the manner in which it can be interpreted are indirect and imprecise. Nevertheless, the fact of consumer complaints is one which water treatment plant designers and operators must deal with. The constraints within which information about these complaints are obtained, interpreted and disseminated must be accepted.

The questionnaire results indicated that only one consumer complaint, that of turbidity, can be reasonably linked to a treatment process utilized or a finished water parameter. The cause of consumer complaints, therefore, cannot be explained in terms of the quality of the water leaving a treatment plant.

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APPENDIX A. CULTURE MEDIA SPECIFICATIONS

1. Standard plate count agar

Tryptone	5.0 g
Yeast extract	2.5 g
Glucose	1.0 g
Agar	15.0 g
Distilled water	1.0 L

2. Tryptone glucose yeast broth

Same ingredients as used in the standard plate count agar except no agar was added.

3. Ferric ammonium citrate agar (FAC agar)

Ammonium nitrate, NH_4NO_3	0.5 g
Dipotassium hydrogen phosphate	0.5 g
Magnesium sulfate	0.5 g
Ferric ammonium citrate	5.0 g
Agar (Bactoagar)	15.0 g
Distilled water	1.0 L

4. Nutrient agar (as manufactured by BBL™)

Gelysate™ peptone	5.0 g
Beef extractives	3.0 g
Agar	15.0 g
Distilled water	1.0 L

5. Lactose broth (as produced by Difco Laboratories)

Beef extract	3.0 g
Peptone	5.0 g
Lactose	5.0 g
Distilled water	1.0 L

6. Lauryl tryptose broth (as produced by Difco Laboratories)

Tryptose	20.0 g
Lactose	5.0 g
Dipotassium hydrogen phosphate	2.75 g
Potassium dihydrogen phosphate	2.75 g
Sodium chloride	5.0 g
Sodium lauryl sulfate	0.1 g
Distilled water	1.0 L

When dehydrated media was available (in all cases except the ferric ammonium citrate agar), it was used rather than preparing the media from basic ingredients. Broth tubes were prepared in the following manner:

1. Broth prepared according to manufacturer's directions.

2. Broth dispensed into test tubes using a Becton, Dickinson and Co. syringe-type automatic pipet; tubes stoppered.

3. Broth tubes autoclaved for 15 minutes at 15 psi (Barnstead autoclave).

4. Broth tubes used immediately or refrigerated until needed.

FAC agar for pour plates was prepared and stored in the following manner:

1. Agar ingredients prepared as indicated above; heated until boiling.

2. Ten ml portions of agar dispensed into test tubes using a syringe-type automatic pipet; tubes stoppered.

3. Agar tubes autoclaved for 15 minutes at 15 psi.

4. If agar to be used immediately for pour plates, tubes put into 45.5°C water bath (GCA/Precision Scientific) to cool agar and to keep it in liquid state. If agar to be used at a later date, tubes stored in refrigerator and, when needed, autoclaved for a short time to melt the agar and then placed in 45.5°C water bath to maintain agar in liquid state. Each tube supplied enough agar for one pour plate. Plates used were Fisherbrand 100 x 15 millimeter (mm) pre-sterilized plastic petri dishes.

COLLEGE OF ENGINEERING

VIRGINIA POLYTECHNIC INSTITUTE AND STATE UNIVERSITY

Blacksburg, Virginia 24061

DEPARTMENT OF CIVIL ENGINEERING

APPENDIX B

To: Water Utility Engineers and Operators

From: Drs. William R. Knocke and Gregory D. Boardman

Re: Water Utility Questionnaire Related to Water Quality Problems in Distribution Systems

Dear Engineer/Operator,

Enclosed please find one copy of a questionnaire related to water quality problems in distribution systems. On the surface, this may appear to be just another piece of "junk mail". However, it is our hope that, as you read this letter, you will perceive the need for your responding to the questionnaire. At the present time, we are initiating a research study here at Virginia Tech related to water quality problems which develop once the treated water has left the plant and entered the distribution system. This research study, which is being funded by the Office of Water Research and Technology, will involve the participation of water utilities throughout the Commonwealth of Virginia over the next two years. The major objective of the study is to develop a useful protocol which could be utilized by water utility engineers and/or operators to assess, document and remedy problems related to the quality of potable waters. One phase of the study involves the distribution and analysis of a state-wide questionnaire related to water quality problems in the distribution system. Our goal is to assess the current "state-of-the-art" that is utilized to handle consumer complaints related to water quality.

It is our hope that you will find the time to respond to the questionnaire. We have attempted to produce a questionnaire which is straight forward and one that does not involve a great deal of operator time to complete. You will find that a vast majority of the questions simply involve a numerical or yes/no response. Few questions will involve a lengthy response on your part. Upon receipt of your responses, they will be entered into a data bank, allowing us to perform a thorough analysis of the prevalent problems and practices utilized in the state. Also, one of the major functions of the questionnaire is to locate water utilities that would be willing to work with our research team in a series of field monitoring studies. This program of field testing will involve minimal effort on the part of the water utility personnel, requiring only that they

periodically collect water samples from various sections of their distribution system. All of the necessary physical, chemical and microbiological testing will be performed by the Virginia Tech research team. The expected benefit to any water utility participating in the field studies would be obtaining additional data related to their water distribution system. Also, the water utility may gain some insights into methods that would be appropriate for assessing conditions related to water quality problems within their own distribution system.

In summary, we wish to thank you for taking the time to fill out the water quality questionnaire. Your effort, along with the efforts of operators and engineers around the state, will aid our research studies greatly. Again, our goal is to develop methods which will improve your ability to respond to water quality problems in your system. If you have any questions about any phase of our proposed research study, please contact either of us by phone (703) 961-6132 or 6021.

Sincerely,

William R. Knocke, Ph.D.
Assistant Professor of
Civil Engineering

Gregory D. Boardman, Ph.D.
Assistant Professor of
Civil Engineering

WRK/dkm

Enclosure

QUESTIONNAIRE FOR WATER UTILITY MANAGERS:
WATER QUALITY PROBLEMS IN DISTRIBUTION SYSTEMS

A. Utility Information:

1. Please state the complete name and location of water utility

_____ (city) _____ (county).

2. Is the water utility

a. Privately owned _____
b. City or County owned _____
c. State or Federally owned _____
d. Other (please specify) _____

3. The approximate average flow to the water distribution system is ___ gpm (_____mgd), but the rated capacity of the treatment facility is ___ gpm (_____mgd).

4. Approximately what population is served by your water utility? _____.

B. Raw & Finished Water Information

1. Raw water source is

well or ground water _____
surface: river water _____
surface: lake or impoundment _____
other (please specify) _____

2. List the average raw and finished water quality and indicate levels, if available, in mg/l (except pH, turbidity and bacterial counts). If the parameter is not measured, please indicate by marking "NM".

	Raw	Finisned
Turbidity	_____	_____
pH	_____	_____
Iron	_____	_____
Manganese	_____	_____
Calcium	_____	_____
Magnesium	_____	_____
Sodium	_____	_____
Ammonia-N	_____	_____
Hardness, as $CaCO_3$	_____	_____
Alkalinity, as $CaCO_3$	_____	_____
Sulfate	_____	_____
Chloride	_____	_____
Hydrogen Sulfide	_____	_____
Nitrate-N	_____	_____
Phosphate	_____	_____
Dissolved Oxygen	_____	_____

	Raw	Finished
Temperature	_____	_____
Total Solids	_____	_____
Total Organic Carbon	_____	_____
Total Coliform	_____	_____
Fecal Coliform	_____	_____
Total Plate Count	_____	_____

C. Water Treatment Process Information

1. Please check the unit processes employed in your water treatment plant.

Pretreatment

Aeration _____
Presedimentation _____
Coagulation/Flocculation _____
Sedimentation _____
Pre-Chlorination _____
Permanganate Oxidation _____

Softening

Lime _____
Excess Lime _____
Split Treatment _____
Lime-Soda Ash _____
Ion Exchange _____
Recarbonation _____

Filtration

Sand (Granular media) _____
Diatomaceous Earth _____
Direct Filtration _____

Carbon Treatment

Powdered _____
Granular _____

Disinfection

Chlorine _____
Chloramine _____
Ozone _____

Post Treatment

pH Adjustment _____
Fluoridation _____
Phosphate Addition _____
Ammonia Addition _____

None _____

Others (please specify)
_____

2. Please list the average chlorine residuals generally maintained:

	Plant Effluent	Distribution System
a. Free chlorine, mg/l	_____	_____
b. Combined Chlorine, mg/l	_____	_____
c. Total Chlorine, mg/l	_____	_____

D. Distribution System Water Quality Problems

1. Does the water utility maintain records of consumer complaints regarding water quality? Yes _____ No _____. If yes, approximately how many years have such records been kept?

2. Approximately how many consumer complaints are received monthly? _____

3. Are the consumer complaints seasonal? Yes _____ No _____. If yes, which season(s) _____.

4. Please indicate which of the following consumer complaints are received, as well as the relative percentage of the total number of complaints:

		Occur?	% of Total
a.	Taste	_____	_____
b.	Odor	_____	_____
c.	Cloudiness or Turbidity	_____	_____
d.	"Red" water or plumbing	_____	_____
e.	"Black" water or plumbing	_____	_____
f.	Other, please specify	_____	_____

5. Are most of the consumer complaints located in one area of the municipality or distributed throughout the system?

One area _____
Distributed _____

6. Are most of the water quality problems reported in "dead end" areas of the distribution system? Yes _____ No _____

7. Please check which of the following bacteriological tests are undertaken in examining water quality problem areas.

	Measured
Total plate count	_____
Total coliform	_____
Fecal coliform	_____
Iron bacteria	_____
Actinomycetes	_____
Others, please specify	_____

8. Are records maintained of the bacteriological tests performed? Yes _____ No _____. If yes, please indicate the number of years of records available _____.

9. Please check any of the following parameters which are monitored in areas of water quality problems:

a.	turbidity	_____
b.	pH	_____
c.	Cl_2 Residual	_____
d.	Fe^2	_____
e.	Mn	_____
f.	H_2S	_____
g.	Taste and Odor	_____
h.	Dissolved Oxygen	_____
i.	SO_4 or S	_____
j.	Organic N	_____
k.	Organic C	_____
l.	Others, please specify	_____

10. Are the Cl_2 residuals being maintained in water quality problem areas? Yes _____ No _____. If no, what is the Cl_2 residual in these trouble areas? _____ mg/l.

11. Please check which of the following remedial measures are used to control water quality problems.

    a. Main flushing _____
    b. Increased Cl_2 residual in trouble areas after complaint _____
    c. Cl_2 residual reinforcement throughout the system _____
    d. Sacrificial anodes _____
    e. Other, please specify _____

12. Of the above mentioned remedial measures, which do you find most useful in combating water quality problems in the distribution system? _____

13. Please relate any other specialized distribution system problems which occur in your distribution system. _____

14. Would your water utility be willing to cooperate in a research program directed at remedying water quality problems in the distribution system? Yes _____ No _____

15. Name and address of person(s) responding to questionnaire:

    _____
    Name

    _____
    Street

    _____
    City State Zip Code

    _____
    Phone Number

The members of the VPI & SU water quality research team wish to thank you for taking the time and effort to respond to this questionnaire. We realize that it has taken a certain amount of time to answer many of the questions, but it is our feeling that the anticipated results of this study will provide the water utilities of Virginia additional knowledge for combating water quality problems in distribution systems.

APPENDIX C. RESULTS OF SINGLE-PASS CONCENTRATOR EXPERIMENTS USING *P. cepacia*

Experiment Number	Concentrations #P. cepacia/ml			Sample Volume L	Retentate Volume ml	Reduction in Volume %	Recovery %
	Influent	Retentate	Filtrate				
1	1500	2000	0	15	1820	88	16
2	2600	3900	0	20	3420	83	26
3	4800	5900	0	20	3230	84	20
4	4800	7600	0	20	3450	83	27
5	49	84	0	20	3550	82	30
6	99	125	0	20	2550	87	16
7	169	92	0	20	2615	87	7
8	400	1300	61	20	2475	88	40
9	1500	2300	38	20	2820	86	22
10	6900	6900	49	20	2830	86	14
11	2165	3650	0	16	2550	84	27
12	655000	635000	1	5.1	690	86	13
13	8800	17300	0.1	10.1	3025	70	59
14	4833	4433	253	15	9290	38	57
15	4533	4067	0	15	7765	48	46
16	5400	5500	48	15	8935	40	60
17	547	433	693	10	6080	39	48
18	3550	3467	0	14.5	9650	33	65
19	2600	4033	0	15	8235	45	85

APPENDIX D. RESULTS OF SINGLE-PASS CONCENTRATOR EXPERIMENTS USING *E. coli*

Experiment Number	Concentrations #E. coli/ml			Sample Volume L	Retentate Volume ml	Reduction in Volume %	Recovery %
	Influent	Retentate	Filtrate				
1	54,000	24,000	470	15	6210	59	17
2	24,000	79,000	0	15	5750	62	126
3	79,000	79,000	0	15	5890	61	39
4	11,000	7,900	0	15	5975	60	29
5	1,400	2,700	0	15	6440	57	83
6	33,000	46,000	0	15	6850	54	64
7	17,000	13,000	0	15	6950	54	35
8	49,000	70,000	0	15	5340	64	51
9	13,000	49,000	0	15	4745	68	119
10	79,000	33,000	0	15	5570	63	16
11	35,000	13,000	0	15	5800	61	14

APPENDIX E. RESULTS OF MULTIPLE-PASS CONCENTRATOR EXPERIMENTS USING *P. cepacia*

Pass	# Microbes in Sample	# Microbes in Retentate	# Microbes in Filtrate	Cumulative % Recovery in Retentate	Cumulative % Recovery in Filtrate
Experiment No. 1:					
1	8.9×10^7	5.23×10^7	3,538	59	0.004
2	--	2.26×10^7	20,000	81	0.024
3	--	3.83×10^7	54,188	124	0.084
Experiment No. 2:					
1	7.25×10^7	4.12×10^7	1.44×10^6	57	2
2	--	3.20×10^7	--	101	--
3	--	2.14×10^7	--	131	--
Experiment No. 3:					
1	8.1×10^7	4.9×10^7	2.9×10^6	60	4
2	--	4.6×10^7	--	117	--
3	--	4.3×10^7	--	170	--

APPENDIX F. RESULTS OF MULTIPLE-PASS CONCENTRATOR EXPERIMENTS USING *E. coli*

Pass	# Microbes in Sample	# Microbes in Retentate	# Microbes in Filtrate	Cumulative % Recovery in Retentate	Cumulative % Recovery in Filtrate
Experiment No. 1:					
1	1.95×10^8	2.33×10^8	0	119	0
2	--	3.43×10^7	0	137	0
3	--	9.75×10^6	0	142	0
4	--	2.33×10^6	0	143	0
Experiment No. 2:					
1	5.25×10^8	7.54×10^7	0	14	0
2	--	2.67×10^7	--	19	-
3	--	1.65×10^6	--	19.3	-

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A QUESTIONNAIRE AND A CONCENTRATOR SAMPLING TECHNIQUE USED TO EVALUATE WATER QUALITY DEGRADATION IN DISTRIBUTION SYSTEMS

by

Deborah Kathleen Manning

(ABSTRACT)

Two approaches for the examination of microbially-caused degradation of water quality within water distribution systems were considered: the results of a questionnaire were analyzed and a sampling technique utilizing a concentrator was evaluated.

A questionnaire, completed by water treatment plant operators in Virginia, was analyzed to ascertain what complaints regarding water quality were being received and what the causes of the complaints were. The most frequently reported complaints were those of taste and odor. Although they were not the sole factor, it appears nuisance bacteria were causative agents in degradation problems related to turbidity and red water.

The Pellicon cassette system concentrator was evaluated for use during sampling in water distribution systems. The evaluation included determing the range of percent recovery in the retentate to be expected and the factors affecting this recovery. Thirty to sixty percent recovery of bacteria (*Pseudomonas cepacia* or *Escherichia coli*) in the retentate can be expected. The percent reduction in volume was the system parameter which most affected the percent recovery.