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Seasonal Effects on Total Bacterial Removals in a Rapid Sand Filtration Plant

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SUMMARY

The present study is the first comprehensive study of the removal of total bacterial cells from a drinking water supply. Using the direct microscopic count to enumerate the total bacterial population present in raw, settled and filtered water, it was possible to determine bacterial removals by physical processes, such as coagulation, sedimentation and filtration. The 15-month longitudinal study was performed at the Capital City Water Company treatment plant serving Jefferson City, Missouri. The results confirmed earlier survey results indicating that bacterial cell removals by conventional water treatment processes are far lower than turbidity reductions would indicate. Moreover, bacterial removals are significantly impaired when water temperatures are low. Most bacterial removal is accomplished by pre-treatment (coagulation and sedimentation). Filtration, as a single unit operation, was found to be ineffective in achieving significant bacterial removals throughout the entire study period.

Based on the results, it is evident that the enumeration of the total bacterial population is the most fundamental and basic microbiological measurement that can be made to evaluate water treatment plant performance.

HISTORICAL BACKGROUND

Survey of Missouri Water Supply Systems

As part of a systematic survey of microbiological quality of drinking waters in 53 public water utilities in Missouri, under cold weather conditions, an extensive chemical and microbiological survey was conducted in 1984 (1). The survey, which provided the first extensive data base on total bacterial cell counts in raw, finished and distribution system water samples, yielded several unexpected results. Whereas surface water treatment plants achieved excellent reductions in turbidity, bacterial cell removals were far less complete. At one water treatment plant, where the raw water source contained 2.1×10^6 bacterial cells/mL, the filtered, finished water contained 1.5×10^6 cells/mL. Equally surprising, the conventional microbial indicators (total coliform, fecal streptococcus, heterotrophic plate count) did not indicate the inability of the treatment processes to physically remove the bacterial cells. Because these disparate results were not anticipated, intermediate samples had not been taken within the treatment plant to gain insight into the effectiveness of the individual unit processes.

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It was also found that the bacterial populations in individual distribution systems reflected the efficiency of cell removal at the plant. It was important, therefore, to redefine the concepts of regrowth and aftergrowth because the survey of Missouri water systems made it evident that the populations of organisms observed throughout the distribution system were most strongly related to the effectiveness of the treatment plant in removing bacterial cells. Where distribution systems exhibited microbiological problems, it appeared to be related to the failure of the treatment plant to provide cell removal, not to subsequent contamination of the distribution system.

Definition of Regrowth and Aftergrowth

To differentiate between the source of microorganisms found in distribution systems, a clear distinction had to be made between "regrowth" and "aftergrowth." Currently, the water utility industry uses "regrowth" and "aftergrowth" as synonyms to describe the increase in the number of organisms during distribution. On the basis of these early studies, however, it became evident that, in many instances, large numbers (10^4 - 10^6 bacterial cells/mL) of organisms are entering the water distribution system through the well or filtration plant (1,2,3). "Regrowth" was, therefore, defined as the recovery of disinfectant-injured or dormant cells which had passed into the distribution system from the water source or treatment plant. After chlorine dissipation and time for metabolic repair, these cells could regain their ability to reproduce under culture conditions. The subsequent growth of new organisms originating from those passing the treatment processes and surviving disinfection would similarly be classified as "regrowth."

"Aftergrowth" was defined as the subsequent microbial contamination of distributed water by cells from distribution piping surfaces or external sources, such as cross-connections or back siphonage. "Aftergrowth" is most frequently blamed for distribution system contamination problems. As a result, flushing, rechlorination and main disinfection programs are undertaken when, perhaps most often, regrowth may be the root cause of distribution problems. Failure to recognize this distinction has led the AWWA Research Foundation to confine its attention to the control of aftergrowths in distribution systems (4). "Biofilm" formation, rather than treatment plant failure, is viewed as primarily responsible for increased microbial populations during water transmission. Scientific evidence for the contamination of water by periphytic bacteria dislodged from accumulations on pipe surfaces is generally lacking.

Since the adverse effects of microorganisms on drinking water quality due to regrowth can only be controlled at the treatment plant, it is important, for health protection as well as for operational purposes, to be able to distinguish between the two phenomena. Where significant numbers of microorganisms originating in surface waters penetrate and populate the distribution system, greater consideration must be given to potential health effects.

Survey of Missouri Water Supply Systems: Summer Conditions

Based on the first survey results, the University of Missouri-Columbia and the Missouri Department of Natural Resources undertook a second survey to observe the effect of warm temperature on microbial quality in drinking water distribution systems (2). As before, exten-

sive distribution system sampling was conducted to gain insight into the microbial ecology of water distribution systems. This second survey, conducted during the summer of 1985, was expanded to include 83 Missouri water utilities. From sampling during the period of warm water temperature, it was found that bacterial cell removals were improved. In the summer study, as well as in the winter, the data clearly showed that there was no relation between bacterial cell count and turbidity in finished water from various sources. Moreover, turbidity did not reflect increases in microbial growths in water distribution systems.

Since these results created serious questions about the validity of using turbidity as a primary microbiological drinking water standard, reluctant belief and outright skepticism was generated concerning the value and meaning of enumerating the total bacterial cell population. First of all, there was virtually no systematic data available on total bacterial populations in drinking waters except for the data from the Missouri surveys. This is because the direct bacterial count method is unknown to the waterworks profession.

The method for the enumeration of total bacterial cells by a direct count procedure utilizing epifluorescence microscopy and Nucleopore polycarbonate filters was developed and refined by Hobbie, et al. in 1977 (5). Having been extensively evaluated and employed, it has found wide use among microbiologists from the fields of limnology, oceanography, and microbial ecology. Most recently, it has been used for the enumeration of microorganisms in dairy products (6-8), food (6,7), intravenous fluids (9), urine (7), ultrapure water systems (10,11), beverages (7,12), wine (12), and petroleum (13). However, despite its acceptance, wide application in other fields and issuance as an ASTM standard method (14), neither USEPA nor AWWA has employed or evaluated the direct count methodology in drinking water treatment.

Since the survey results were based on grab samples which might have reflected transients of plant upsets (e.g., due to filter backwash), there had been no study of bacterial removals at an individual water treatment plant over a sufficient period of time to determine the effects of seasonal water temperature changes. Consequently, the present study was undertaken to evaluate bacterial removals throughout a calendar year at a well-operated, comprehensive water treatment plant which consistently meets all drinking water standards. In addition, the study sought to determine the temperature ranges at which bacterial removals were impaired. Since turbidity and the heterotrophic plate count (HPC) have been extensively used as microbiological indicators of water treatment plant performance, the present study compared the reduction of both with the removal of bacterial cells.

The study was also undertaken to evaluate assumptions made in the USEPA Turbidity Criteria Document regarding the validity of turbidity measurements and criteria in assuring the microbiological quality of drinking water (15). Although no data analysis was performed, the USEPA Turbidity Criteria Document interpreted existing data as showing "a good correlation between the removal of these organisms (total coliforms, virus and Giardia cysts) and the removal of turbidity." Owing to the fact that the total number of bacterial cells exceeds these other microbial parameters by millions of times, total bacterial cell counts were felt to be "too sensitive as an indicator of removal of health significant microorganisms (16)." This, despite the fact that USEPA had never measured total bacteria.

was raised by the Board of Directors of the AWWA Research Foundation which precluded its use for research on water quality deterioration in distribution systems because "the procedure was too complex and would be too difficult to be practically applied in most of the nation's utility laboratories (17)." Thereafter, the AWWARF Request for Proposal was written to preclude "bacteriological methods which require ultraspecialized analytical equipment not commonly used within the industry (4)." While neither the USEPA nor the water utility industry has reported any use of the total bacterial cell count to date, the Nuclepore Corporation, manufacturer of the polycarbonate membrane filters required for the analysis, has recently marketed an "EpiCount Kit" for the rapid enumeration of microorganisms in liquids (18).

MATERIALS AND METHODS

A 15-month study was undertaken at the Capital City Water Treatment Plant, Jefferson City, Missouri, over the period of March 1985 to May 1986. The Capital City Water Company plant at Jefferson City, Missouri, is a comprehensive, two-stage water treatment facility which is representative of treatment plants along the Missouri River. A simplified schematic flow diagram of the plant is given below (Diagram 1).

Treatment begins with presedimentation (plain sedimentation) in a basin where auxiliary chemicals, such as powdered activated carbon or potassium permanganate, are applied when tastes and odors develop in the river. Subsequently, lime is added in dosages sufficient for softening and ferrous sulfate is added as a coagulant. Chlorine is added to establish a "free" residual. After mixing and flocculation, primary sedimentation removes most of the calcium carbonate and iron coagulant sludge.

Secondary treatment, sometimes aided by the addition of lime, coagulant and activated carbon, follows primary recarbonation. Following secondary sedimentation, the water is recarbonated for a second time to achieve chemical stability. Finally, the water is chlorinated immediately prior to filtration through eight rapid sand filters. Polyphosphate (0.5 mg/L as P_2O_5) is applied to minimize calcium carbonate build-up on filter sand. Ammonium sulfate is applied immediately prior to the clear well to form a chloramine residual.

Filter flows are regulated by rate-of-flow controllers at 0.1 m/minute (2-3 gpm/sf). Two filters are backwashed every night following a four-day filter cycle.

The treatment plant serves a population of approximately 30,000 with an annual average daily flow of 20,000 m³/day (5.2 MGD). Flow averaged 17,000 m³/day (4.4 MGD) from December 1985 to March 1986 and 24,000 m³/day (6.4 MGD) from June to September 1986. This reflects the seasonal variation in water use in Jefferson City and shows that the plant hydraulic loading is markedly lower in the winter. The plant operation is considered excellent and, despite the challenges offered by the Missouri River, the treatment plant often produces finished water with as little as 0.1 NTU, the quality goal adopted by the AWWA Board of Directors in 1968 (19).

On each of 54 sampling dates, samples of raw, settled, filtered and five separate distribution system samples were collected.

Microbiological samples were collected in sterile one-liter polyethylene sample bottles containing 1 mL of a 10% sodium thiosulfate solution. The samples were stored on ice for transport and processed, generally, within 4 hours after collection. In no case were samples analyzed after 8 hours.

The following analyses were performed in accordance with Standard Methods (20): standard plate count by pour plate incubated at 35°C for 96 hours, total coliform by the membrane filter technique, and turbidity with a Hach 2100A turbidimeter. Non-standard methods included a modified standard plate count by spread plate in which 0.1 mL of sample was spread with a glass rod on the surface of dilute media (4.25 g/L M-Standard Methods Broth, BBL Microbiology Systems) and incubated at 18°C for 21 days. Organic carbon was measured using a Dohrmann-Envirotech Model DC-54 Ultra-Low-Level Total Organic Carbon Analyzer. The method used for enumerating the total number of bacteria is a slight modification of the method described by Hobbie, et al. (5) as previously reported (3). Counts included both the total number of cells and potential colony forming units of clumps, chains, and filaments. In the settled water, when possible, depending on floc condition, counts were also made of bacterial cells entrained both in the floc and those free. All counts were performed with a Leitz Ortholux microscope fitted with a Ploem vertical illuminator and 200-w mercury lamp. Micrographs were made with a Leica M1.

Only that portion of the collected data which deals with the plant performance with respect to reductions in turbidity, total coliform, total bacterial populations and heterotrophic plate counts are discussed in this paper. The impact of treatment plant performance on bacterial populations found in the distribution system is reserved for a future presentation.

To reduce the possibility of erratic or spurious data confounding performance evaluations, sampling frequency was increased from weekly during the year to almost daily during the period of low water temperatures. This resulted in roughly equal numbers of samplings during warm and cold weather periods. In addition, the daily sampling during cold weather also confirmed the hypothesis that bacterial cell removals were always poor during cold weather and that this observation was not an artifact due to grab sampling on random days. Filtered water samples were, with three exceptions, taken from filter number 6. Composite finished water samples were taken from the plant clear well. The results obtained from both were generally identical, indicating little fluctuation in filter performance, as compared with the plant averages as indicated by the clear well samples.

RESULTS

Reduction in Total Coliform

Total coliform colonies, while measured throughout the study, were so few in number as to be useless in evaluating treatment plant performance. No total coliform organisms were found in the plant effluent over the 15-month sampling period. For that reason, the data were not plotted.

Based on these results and because coliform colony counts often number less than one per million total bacteria, water utility reliance

Reduction in Total Bacterial Cell Counts

Figure 1 shows the total bacterial cell counts in raw, pretreated (softened, coagulated, flocculated, settled) and filtered Missouri River water. The data indicate that the raw water source consistently contains approximately 10^7 bacterial cells/mL throughout the year. Pretreatment (following sedimentation) reduces this number by up to two orders of magnitude (99%; to 10^5 cells/mL) during the summer months. However, in the winter, as the earlier surveys indicated, reductions in total bacterial cell counts average less than an order of magnitude.

From December through March, total bacterial cell populations in finished water generally exceed 10^6 cells/mL. Figure 2 is an expansion of the plant performance data for this critical period of impaired bacterial removal. During this period, a special effort was made to observe the effect of increased plant retention time on bacterial removals. On three separate occasions, plant flows were markedly reduced by turning off service pumps at the river and shutting down filters while maintaining the same coagulant dosages. After a day, markedly improved bacterial removals were observed. These appear as downward "spikes" in Figure 2. Data obtained during each of these three periods of plant slowdown are presented in Table 1. The beneficial effect of reduced hydraulic loading is indicated by both increased bacterial removals and reduced settled water turbidities.

For purposes of discussion and evaluation of plant process performance, bacterial removals less than 90% have been classified as "poor" removals, while up to 99% and 99.9% are rated as "fair" and "good", respectively. By these definitions, overall bacterial removals are seen to be "fair to good" during warm weather and "poor" during the season of impaired bacterial removal.

The ASCE manual on Water Treatment Plant Design confirms the expected performance standards (21). It states that bacterial removal efficiency "with proper pretreatment should exceed 99 percent." It also asserts that "more than 98% of the polio virus is removed by flocculation and filtration..."

Of special note is the importance of pretreatment in removing bacterial cells at Jefferson City. The filtration process had little ability to compensate for ineffective removal by pretreatment. In fact, only when pretreatment was working well did filtration appear to accomplish significant additional bacterial removals. During the period of impaired bacterial removal, when the need was greatest, filtration contributed little to overall plant bacterial removals.

Reduction in Turbidity

Figure 3 shows the turbidity in raw Missouri River water as well as after pretreatment and filtration. Raw water turbidity, while highly variable, fluctuates around 100 NTU. Turbidity tends to decrease during the winter when the flow in the Missouri is diminished. Spring rains bring higher flows while the release of impounded water during the barge navigation season sustains summer flows in the Missouri River.

Overall, turbidity removals are good with finished water turbidity averaging about 0.3 NTU. This is less than the proposed more stringent USEPA criterion of 0.5 NTU which, it is hoped, would provide a greater safeguard against drinking water microorganisms. While consideration is also being given to a turbidity criterion of 0.1 NTU, USEPA has not determined at what turbidity organisms of "health significance" will be removed for all types of source waters.

Figure 3 also shows that, although turbidity reductions are not as complete during the season of impaired bacterial removals, finished water turbidities generally remain below the current 1 NTU USEPA drinking water standard. Filtration, by virtue of the fact that filters remove the larger, light-scattering particles (clumps, clusters, flocs), is effective in further reducing the turbidity found in the pretreated water. However, it is evident that turbidity removals may indicate, but do not clearly define, the period of impaired bacterial removal.

Contrasting the removal of turbidity with the removal of bacterial cells, it is also evident that the effectiveness of filtration for protection against microbial contaminants is overestimated if turbidity is used as the sole index of the presence of organisms of health significance. Moreover, the effect of low temperatures on treatment process efficiency, while evident, is not nearly as sharply defined as when measurements are made of total bacterial cell count reductions. In short, turbidity is not a sensitive indicator of the failure of water treatment processes to achieve effective removal of biotic particles. Most organisms passing the filters are present as single cells which may regain their ability to replicate as plate counts following the dissipation of chlorine residuals (22).

Reduction in Heterotrophic Plate Count (HPC) Organisms

Figure 4 presents the number of heterotrophic plate colony count organisms enumerated in raw (avg. 117,000 CFU/mL), pretreated (avg. 1,300 CFU/mL) and filtered (avg. 53 CFU/mL) Missouri River water. Further reductions of HPC to an average of 30 CFU/mL takes place with storage in the clear well. The reductions in HPC numbers reflect both physical removal and the effect of the added disinfectant, chloramine. By this measurement, physical removal of bacterial cells is confounded with disinfection which may kill or merely injure the organisms enumerated by the HPC. While the observed reductions in HPC are generally excellent (>99.9%), HPC is a poor indicator of the failure of treatment to remove bacterial cells during periods of low water temperature. Only total coliform is of less value as a microbial indicator of water treatment plant performance.

Ratio of HPC to Total Bacterial Population

It is essential to recognize that HPC is not a fixed proportion of the total bacterial population present in water, but varies over orders of magnitude with the antecedent treatment of the water. As can be seen from Figures 5 and 6, estimates of total bacterial cell removal based on HPC data will be incorrect.

The ratio of HPC to total bacterial cell count can be particularly useful in evaluating microbial changes in distribution systems. Figure 7 shows the ratio of HPC to direct count for raw, pretreated and filtered Missouri River water. Contrary to results presented recently

(23), which reports a direct relationship between HPC and total bacterial cell count, no statistically valid relationship is evident which can justify using HPC data to evaluate bacterial removal during water treatment. Moreover, in distribution systems, the ratio of HPC to total bacterial cell count has been found to range widely, from 0.03% to 43% (22). Because it does vary with conditions in the distribution system, this ratio, in fact, may serve as an activity index of progressive changes in microbial activity during distribution.

Percent Removal of Turbidity and Total Bacterial Cell Counts by Pretreatment and Filtration

Figures 8 and 9 show the percent removal of total bacterial cells and turbidity, respectively, by pretreatment and filtration. Applying an acceptable performance standard of 90% removal for each unit process, it is evident that pretreatment is effective for bacterial removals except for the period between December and March. Removal of bacteria by filtration is extremely erratic, averaging no better than 50% of the bacterial cells in the pretreated water throughout the year. Of the total bacteria entering the treatment plant, pretreatment removes an average of approximately 90%, while filtration achieves an additional 5%. The combined operations achieve an average of only 80% removals during cold water periods, however.

Pretreatment is seen to be effective year round for the reduction of turbidity (97%) while filtration, with occasional upsets, is capable of effective (90%) removal except when the water is cold. Total plant turbidity reductions generally approach 99.7%. Even during cold weather, turbidity reductions are around 99%.

Figure 10 compares the percent removals of both turbidity and total bacterial direct cell count by pretreatment and filtration. In each case, it is evident that percent turbidity removal cannot be used to evaluate bacterial cell removals.

Influence of Temperature on Percent Removals of Turbidity and Total Bacterial Cell Counts by Pretreatment and Filtration

The percent removal of turbidity is plotted as a function of temperature in Figure 11. The results show that pretreatment successfully removes turbidity (>90%) at all temperatures, but that filtration begins to fail to meet the performance standard below 10°C.

A similar plot showing the temperature dependence of percent total bacterial cell count removal is given in Figure 12. These results indicate that pretreatment begins to fail to achieve the performance standard when water temperatures decrease below 7°C. Filtration, which fails to achieve effective removals at all temperatures, is seen to steadily deteriorate in performance with decreasing temperature.

Figure 13 shows that temperature has no discernible effect on observed reductions in HPC. This confirms the observation that HPC does not reflect the impaired efficiency of physical water treatment removal processes at low temperatures.

Relation between Total Bacteria and Turbidity in Finished Water

In an earlier study, it was determined that approximately 1.6×10^7 E. coli cells/mL contributed 1 NTU of turbidity to water (1). This

indicated that naturally-occurring bacterial populations contributed little to the total turbidity in natural and treated waters. Only when turbidity was extremely low, would bacterial cells constitute a significant portion of the light-scattering particles present in water.

In the present study, finished water bacterial populations ranged up to 2×10^6 cells/mL. Could the influence of this population be observed in finished water when turbidity due to other particulate matter was at its lowest? Finished water turbidity is plotted versus bacterial cell population in Figure 14. Because the treatment plant consistently produced an effluent with turbidities which ranged between 0.1 and 1 NTU throughout the year, a well-defined relationship between turbidity and total bacteria emerges at turbidities below approximately 0.3 NTU. At 0.3 NTU, total bacterial counts approximate 10^6 cells/mL, while at 0.1 NTU total bacterial counts are in the range of 10^4 to 10^5 cells/mL.

These data would indicate that lowering the turbidity standard from 1 NTU to 0.1 NTU would result in a two order of magnitude decrease in total bacterial population, from 2×10^6 cells/mL to 2×10^4 cells/mL. This would approach the bacterial populations found in well waters and high quality bottled waters. Distribution system data confirm that it would also result in parallel reductions in total bacterial populations in the distribution system.

DISCUSSION OF RESULTS

Rationale for Use of Bacterial Cell Counts to Evaluate Water Treatment Plant Performance

A weakness in the performance evaluation of water treatment technology for particulate removal to date has been the failure to characterize the particles in suspension in influent water. Naturally-occurring particles in drinking water sources are often mixtures of metal oxides, carbonates, silicates, organic debris and microorganisms. Different classes of particles, depending on size distribution, density and surface characteristics, may scatter light differently and impact the measured turbidity. As previously shown, bacterial cells which are abundant in all natural waters and often constitute a majority of particle numbers ($10^6 - 10^7$ cells/mL) in suspension, contribute little to raw water turbidity (1,2). As a result of these differences in light-scattering ability, turbidity is not well-related to the number of particles in suspension and cannot be used to characterize either the nature or the number of particles. Since they contribute little to turbidity, large numbers of microorganisms are not detected in filter effluent when turbidity is used to monitor filtration performance. This accentuates the need for identifying and quantifying the particles in raw, pretreated and filtered water as called for by the National Research Council, Safe Drinking Water Committee (25).

In adjusting solution chemistry to create favorable conditions for removal of particles larger than $1 \mu\text{m}$ which should contribute most significantly to turbidity, it is possible that conditions will be unfavorable for the removal of biotic particles that are smaller than $1 \mu\text{m}$. This would be consistent with the results of the present evaluation of Missouri River water treatment where turbidity reductions were found to be consistently effective while bacterial removals were poor. It would appear that not only should the composition and quantity of the particles present in filter influent be determined for each specific water treatment plant, but treatment conditions should be made most

of greatest health concern, e.g., bacteria and viruses.

Because of time, cost and analytical difficulties with enumeration, biotic particles have been little used in the development of filtration theory. Instead, studies have been conducted on idealized systems employing uniform spherical media coated with polymer to facilitate the attachment of uniformly-sized, synthetic particles (26). The results of these studies indicate 100% removal efficiency for particles 10 μm in radius and larger, 100% removal efficiency for particles with a radius of 0.1 μm and smaller, and minimal removal efficiency for particles with a radius in the vicinity of 1 μm . Unfortunately, there has been no experimental verification of the applicability of these results to water treatment filter media or the specific particles present in natural or coagulated waters. These idealized experiments and subsequent conclusions justifying turbidity as a water treatment plant performance parameter appear to be in serious error based on the results of the present study.

Field studies of filtration performance have employed heterotropic plate colony counts to assess the effectiveness of bacterial removals. Colony counts, however, underestimate bacterial populations by orders of magnitude. In many instances, disinfection practices which kill or merely injure bacterial cells confound estimates of bacterial cell removals because it is impossible to distinguish between cells that are physically removed and cells which have been inactivated by chemical disinfection.

Those studies which have employed direct microscopic bacterial counts to characterize biotic particles in filter influent and effluent have shown that large numbers of bacterial cells are not removed by pretreatment or filtration processes (1, 2). The numbers of cells observed in 83 Missouri drinking waters ranged from 10^2 - 10^7 cells/mL. The greatest numbers were found in finished water from plants treating surface water supplies. Despite over four orders of magnitude differences in bacterial populations, finished water turbidities almost always were within the 1 NTU drinking water standard.

Total Coliform for Plant Performance Evaluation

Oddly, the AWWA Research Foundation has proposed and funded a study which would employ coliform organisms to evaluate water treatment plant performance. Calling for the development of rapid detection methods for coliform bacteria, the rationale for the study (specified in the AWWARF Request for Proposals, 1986)(26) appears to be extremely confused. The RFP states that more rapid coliform detection will enable more rapid evaluation of treatment plant performance, thereby making it possible to "optimize bacterial removal efficiencies." In addition, it asserts that coliform speciation is "of interest" to utilities which experience coliform regrowth and biofilm formation. Finally, it was argued that coliform "rapid speciation methodology could possibly differentiate between coliform breakthrough due to treatment deficiencies, a health concern, and the regrowth in the distribution system of environmental coliform isolates which may be significant for other reasons."

The improvement of coliform enumeration techniques to improve detection, enhance speed and achieve economy would have been understandable, but this last objective was specifically denied in the RFP since rapid methods are likely to be more costly.

terial removal efficiency and define microbial changes in the distribution system, there are several very simple and obvious reasons for questioning the use of the coliform group. To begin with, coliform organisms comprise only an extremely small fraction of bacteria in raw or treated drinking waters. They are generally one-millionth or less of the total bacterial population. Improvement in coliform detection by 1000 times would still result in a lack of sensitivity for assessing bacterial removal efficiencies during water treatment. It seems likely that, if the coliform was not a regulated parameter, it would not even be considered for evaluation of treatment plant performance.

Secondly, if the numbers of coliform are insufficient for assessing treatment efficiency, the number of coliform in treated and distributed drinking waters are almost always below the limit of detection. Consequently, it is impossible to assess the extent of microbial activity in distribution systems using coliform measurements, much less differentiate between the sources of coliform. Alternately, intelligible, realistic evidence of the progress and effects of microbial activity in water distribution systems can be readily obtained by enumerating the total bacterial population.

A more subtle reason for questioning the rationale for the study lies in the fact that rapid techniques for coliform enumerations obviate the principal benefit of coliform monitoring, i.e., the assessment of potential system contamination with viable pathogens. Rapid techniques enumerate cells, but do not assess viability. On the other hand, if cell counts are desired for assessments of physical removal processes, then it would be more reasonable to count the total population. At least, there would be enough cells to count.

The improper statement of objectives and rationale for use of the coliform measurement for treatment and distribution system evaluation is an excellent example of why the present method of research management by AWWARF committee is flawed.

As in the past, there continues to be an inverse relation between the abundance of microorganisms in water supplies and the effort made to understand them.

DISCUSSION OF FINISHED WATER ORGANISMS AND POTENTIAL HEALTH IMPLICATIONS

HPC for Plant Performance Evaluation: Removal of Single Cells vs. Aggregations

The observed effectiveness of filtration in reducing HPC highlights several important differences between HPC and the total bacterial cell count. The first difference is that the HPC enumerates, as colonies, both clumps of organisms and selected single cells capable of growing on the medium under the prescribed conditions, e.g., pour plate or spread plate. The reduction in HPC seen on filtration may reflect the selective removal of the clumps of organisms over the individual cells. This results in a decrease in the ratio of HPC to total bacterial cell count following filtration. The microscopic examination which accompanies the direct count demonstrates the numerous single cells in both raw and treated waters as well as those that are particle-bound. No attached cells in either cell aggregates or on particles were observed in the finished water from this plant. The observation that as

... as 5% of cells in natural waters may be particle-bound relative to the traditional belief of water engineers that microorganisms are primarily attached to particles, so that particle removal achieves cell removal (27). In the present year-long survey, the percent bacteria on particles in the raw water ranged from 22% to 70%.

From microscopic observations coupled with plate counts, it appears that the aggregations or agglomerated clumps of organisms are more likely to be enumerated by the HPC than the single cells. However, one should not assume, since they do not replicate on plate count media at the time they are discharged to the distribution system, that the single cells have all been killed. Despite their intimate exposure to the water disinfectant, many of these metabolically inactive cells may have survived exposure to the disinfectant. In fact, the phenomenon of bacterial growth in the distribution system, which has often been described based on increases in HPC, may have simply been the recovery of individual cells which were initially discharged to the distribution system. With dissipation of the effective disinfectant residual in the main and time for recovery, these metabolically inactive cells may regain the ability to grow on plate count media (22).

Similarly, a large portion of the cells enumerated following passage through activated carbon beds may be regrowth organisms rather than periphytic organisms which were dislodged from the carbon surface. Point-of-use carbon adsorbers may promote regrowth to a large extent through the reduction of chlorine. Studies to date have failed to recognize this possibility (28).

Bacterial Dormancy

It has been hypothesized that a significant portion of the bacterial community in most aquatic environments are in exogenous dormancy--a state of neither activity nor death (29,30,31). Exogenous dormancy is defined as a condition in which development is delayed because of unfavorable chemical or physical conditions of the environment. It is an adaptation in which enzyme activity is minimal, thereby contributing to the survival of the organism. Starvation, for example, may cause bacteria to decrease in both size and activity. Release of the stress would permit normal development. Conceivably, disinfectants would have significantly less effect on dormant cells than metabolically active cells whose enzyme systems are more susceptible to inactivation. Since bacteria attached to particles occupy a microenvironment higher in nutrient concentrations than the surrounding water, the epibacteria are more active (27,31-33) but are more susceptible to removal by the physical processes of a filtration plant.

If this hypothesis proves to be true, we may learn that the numerous single cells which penetrate filtration plants are the very cells which have survived disinfection owing to their low level of metabolic activity (dormancy). Evidence that the chlorination of water supplies may select chlorine-resistant organisms has been provided from a study of Legionella pneumophila at a hospital in Pittsburgh, Pa. (34). Experimental results showed that organisms grown in dechlorinated tap water were generally slowly-growing single cells and were far more resistant to destruction than organisms which had been cultured in agar. The more susceptible, agar-cultured organisms were larger, more rapidly-growing aggregates (short filaments). After a 3-week exposure to 2 mg/L chlorine in a plumbing system, a chlorine-resistant strain of L. pneumophila was found to be still present. After culturing on agar,

the chlorine-resistance of the strain was greatly reduced, becoming comparable to those strains not previously exposed to chlorine. This rapid loss of resistance supports the concept of exogenous dormancy and casts doubt on the validity of many previous studies using organisms subcultured from water systems, particularly where these agar-cultured organisms in rapid cell growth phase were used to evaluate disinfection. In addition, major changes in cell morphology are observed following growth in laboratory culture so that the structure of the cultured cells does not resemble that of the organism found in either natural or treated waters.

Very similar results were obtained in studies of Pseudomonas aeruginosa (35). The behavior of naturally-occurring cells was found to be markedly altered on subculturing, rendering them more susceptible to inactivation by chemical agents. Again, the growth phase of the culture at the time of exposure to disinfectants was cited as a significant factor.

Health Effects Implications of Impaired Bacterial Removals

New studies of waterborne bacteria are being undertaken due to increased concern over the presence of opportunistic pathogens which are known to be waterborne. These organisms have been isolated from drinking water faucets, water fountains, ice machines, sink sprays and heated reservoirs as well as from water service connections. In the Boston area, five of twelve hospitals sampled showed the presence of waterborne, chlorine-resistant mycobacteria, M. avium complex (MAC) (36). Medical practitioners are increasingly concerned with these opportunistic pathogens, their environmental ecology and their transmission by water because of the national epidemic of acquired immunodeficiency syndrome (AIDS) which is spreading among more and different human population groups.

Removal of Virus by Water Treatment Processes

If conventional water treatment processes achieve poor removals of bacterial cells when water temperatures are low, how effective are they against the far smaller, naturally-occurring virus particles?

The answer is not known with certainty, partly because virus particle concentrations are very low and their measurement is costly and cumbersome. In the Missouri River, for example, naturally-occurring human enterovirus particles have been found to be in the range of 0 to 25 PFU/m³ (38). The higher virus concentrations were found when water temperatures fell below 10°C, indicating that low temperatures increase virus survival time. In addition, due to flow regulation by the U.S. Army Corps of Engineers, there is far less flow in the Missouri River during the winter months. There is, therefore, less dilution of the comparatively constant flow of virus-bearing effluent from wastewater treatment plants along the Missouri River. As a result, viruses pose a far greater challenge to Missouri River water supplies during cold weather. Unfortunately, this is also when particle removal processes are markedly impaired.

Other results from the two-year longitudinal survey of naturally-occurring human enterovirus in the Missouri River confirmed the fact that most of the virus recovered were not attached to the suspended matter which is monitored as turbidity (38). Less than 10% of the

total number of virus particles recovered from the Missouri River over the two-year survey were found on the 5 μ m prefilter which retained most of the suspended solids in the water. The remaining 90% were recovered from the filtrate by adsorption on aluminum hydroxide floc.

Since it is evident that most virus particles in the Missouri River are not attached to, or entrained in, suspended solids and since Missouri River virus concentrations are highest during winter months when both pretreatment and filtration are least effective, the likelihood of the passage of virus into the distribution system may be very great. Distribution system virus sampling programs, if initiated, should be conducted primarily during these periods of low raw water temperature and process failure.

As a result of a number of recent studies, concern over the passage of virus through treatment plants is increasing. Viruses have been isolated from drinking waters receiving conventional treatment including coagulation, sedimentation, filtration and post-chlorination. These chlorinated waters met standards for total coliform and turbidity (38-41). Two of the studies which evaluated the removal of microorganisms at each step in the treatment process concluded that total coliform, turbidity and HPC bacteria were more effectively removed than enteric viruses (40,41).

The health significance of those low numbers of virus in drinking water has been debated for years. The "Low Level Transmission Theory" of Berg (42), speculates that low numbers of viruses in water may infect susceptible individuals which later spread the virus in the community by person-to-person contact. The merits and problems of this hypothesis were recently debated as to whether it is a realistic consideration in estimating health risk of viruses in water (43). Most recently, a review of the literature on viruses in drinking water concluded that the question of the health significance of low concentrations of virus has not been answered (44).

Validity of Turbidity as a Primary Microbiological Drinking Water Standard

There has been considerable discussion of the use of turbidity as one of only two primary microbiological standards for drinking water. An extensive USEPA Turbidity Criteria Document was developed in 1985 to provide the justification for continued use of the measurement both as a standard and for the evaluation of water treatment plant performance (15). Even while USEPA held workshops and hearings to develop a consensus for its continued use, scientific evidence continued to accumulate which undermined the rationale for using the turbidity measurement as a primary drinking water standard.

As previously noted, it had become increasingly clear that turbidity levels in raw and finished waters did not reflect the numbers of organisms present (1,2). Nor did treatment provide parallel removals of turbidity and more direct microbial indicators, such as total coliform, HPC, Giardia cysts, virus or total bacteria (15).

In addition, a study which evaluated the chlorine demand in 160 raw waters determined that only 10% of the chlorine demand was associated with the suspended solids (45). A recurrent argument for the health implication of the reduction in turbidity had been that turbidity interfered with disinfection by creating a significant disinfectant demand (15).

...is also credited with protecting bacteria and viruses held within or on particle clumps from inactivation by chlorine residuals. However, there is no evidence for such particles contributing to the turbidity of finished, filtered waters.

While high turbidity does interfere with coliform detection by the membrane filter procedure (46), that is a minor consideration in establishing a turbidity standard for low turbidity finished water. The interference might be best addressed by using alternate analytical procedures.

Perhaps the major flaw in the rationale for the use of turbidity as a microbiological surrogate lies in the widely held concept that bacteria and virus in natural waters are generally attached or adsorbed to the surface of suspended solids. Consequently, the near-complete removal of the suspended solids are believed to ensure an equally-complete reduction in the naturally-occurring population of microorganisms.

This hopeful assertion is made repeatedly in the 1977 National Academy of Sciences report on Drinking Water and Health (24).

The report states: "The tendency of microorganisms to form aggregates and to become concentrated at the surfaces of solid particles, rather than to be uniformly and individually dispersed, may have important consequences for their survival and for their reactions to the various processes of water treatment. It is doubtful that many of these microbial agglomerates will pass through an efficiently operating water-treatment process..."

"Studies of microbial aggregates in terrestrial environments . . . demonstrated that the most extensive microbial growth takes place in nature on the surfaces of particles and inside loose flocs of solid particles. This occurs because the nutrients required for microbial growth are also adsorbed at the surfaces of these particles. Only a few microorganisms are found free in the soil solution or in raw water because of the lack of dissolved nutrients."

"River silt adsorbs viruses with moderate efficiency and does not relinquish them very easily..." "...studies on viral adsorption to sand, silt, clays and organics (feces) to form particulates are consistent with what is known for bacterial aggregates."

They conclude that: "Investigations are required of the physical-chemical attachment of microorganisms to sand, silt, clays, and organic particles, and disaggregation of these particulate complexes."

One might question why it was so widely assumed that microorganisms were predominately attached to the surfaces of suspended particles. Aside from being an optimistic observation of convenience, the answer might be in the interpretation of the measurement of the HPC as "total bacterial" count. As evidenced by the present study, HPC reductions routinely exceed 99%. Since single, unattached bacteria might be expected to pass through the interstices of sand filters, it would logically follow, from 99% removal of "total bacteria," that the cells must be attached to larger, more readily removable particles. Once the assumption had been made that microorganisms were attached to the solid particles in suspension, then a turbidity criterion seemed to be an appropriate surrogate for evaluation of treatment plant performance.

since microscopic observations were not made, the fallacy of the assumption never became apparent.

In the present study, direct microscopic examination continually reveals that bacterial cells in source and finished waters are not all attached to the solids present. The subsequent removal of these solids does not correspond to the reduction of cells. It is primarily for this reason that there are marked differences in turbidity and microbial reductions during treatment. Moreover, it is for this reason that turbidity removal is inadequate for microbial evaluations of water treatment plant performance. Finally, it should now be clear that turbidity should be replaced by direct microscopic examination for the removal of microorganisms.

Turbidity is even more inappropriate for use as an indicator of microbial regrowth or aftergrowth in water distribution systems (2). Turbidity is relatively insensitive to changes in organism population during distribution but is greatly affected by the sloughing of accumulated corrosion products from pipe walls. In addition, the post-precipitation of coagulant, calcium carbonate or phosphates may have a far greater influence on turbidity in distributed waters than changes in organism populations.

Evaluation of Effectiveness of Pretreatment

There has been a notable difference in thought regarding the evaluation of "coagulation." Traditionally, coagulation has been evaluated through use of the jar test in which the solids separation occurs as a result of sedimentation. The jar represents the treatment plant sedimentation tank prior to filtration. The optimum coagulant dosage is measured as that which yields the lowest settled water turbidity.

More recently, authors have advocated the use of filtered water turbidity to evaluate optimum coagulant dosage (47,48). Poor settling, it is argued, is not necessarily a symptom of poor coagulation (48). Instead, high filter effluent turbidity is evidence that coagulation is poor or that the floc formed is weak.

The results of the present study provide yet another criterion for evaluating pretreatment (coagulation, flocculation and settling). The coagulation process can be evaluated with far greater accuracy and precision by enumeration of the large population of bacterial cells than through turbidity measurements or particle counting.

For the specific plant studied, it is evident that it is the pretreatment, and not the filtration, process that is most important to the removal of biotic particles. The sensitivity of plant performance to low water temperatures is demonstrated most dramatically by the enumeration of total bacterial counts in pretreated and filtered water.

Optimization of treatment plant performance for the removal of the most numerous particles in natural waters, microorganisms, represent an important additional step in the development of methodology for more scientifically evaluating the efficacy of each individual water treatment unit operation.

Effect of Temperature on Pretreatment

While temperature has been acknowledged to be an important factor

in water treatment, remarkably little study has been made of the adverse influence of low temperatures. An early study concluded that "there is no preventative or retarding effect on alum floc formation with low raw water temperatures (49)."

This conclusion prompted Camp, et al., to further evaluate the effect of temperature on the rate of floc formation (50). It is noteworthy that Camp discarded the measurement of turbidity in favor of the direct measurement of iron or aluminum. He concluded that temperature did not have a measurable effect on the time of floc formation.

However, pilot plant studies of aluminum sulfate-coagulated river water demonstrated the overall adverse effect of low temperatures on sedimentation and filtration in 1967 (47). The authors simply advised that, "where raw water temperature is low, the jar test must be run on samples held at the same temperature, if results are to be used in plant control."

It was not until 1984 that a systematic, fundamental evaluation of the adverse effects of low temperature on water treatment plant performance was undertaken (51). The investigators reported significant temperature effects on coagulation with sharp decreases in turbidity removal efficiency, particularly when aluminum sulfate was used. Using decreases in alkalinity and measurements of the metal coagulant in solution, Morris and Knocke determined that the reduction in turbidity removal efficiency was not due to reduced metal hydroxide precipitation rate, but to retardation of floc growth. Under equivalent conditions, iron salts produced larger flocs than aluminum salts and resulted in lower residual turbidity values.

These results are particularly significant in light of the major temperature effects observed in the present study. Using total bacterial counts in lieu of turbidity and particle size distribution, the present study confirms and extends the results of Morris and Knocke.

Since low temperatures seriously impair total bacterial removals by pretreatment, what remedial measures might be effective in restoring treatment plant performance? Although the benefits are speculative, the use of liquid, rather than solid forms, might result in more rapid and complete solution of the coagulants used. Modified mixing and flocculation energy inputs may similarly have a beneficial effect on bacterial removals. In the present study, however, when plant flows were reduced to increase settling tank retention times (Table 1), more complete bacterial removals were obtained. Bacterial removals improved immediately, although not to levels observed during the summer when plant flows were highest.

If, because of reduced floc size and floc settling velocity, sedimentation is the treatment step most adversely affected by low temperature, water treatment plants may require far larger settling tanks than are currently being utilized. Assuming the present indication is valid, sedimentation basins would seem to be less than 50% of the size required for good bacterial removal. The undersizing of settling tanks may be a further consequence of the profession's failure to recognize the adverse effect of low temperature on conventional water treatment plant microbial removal performance.

SUMMARY

A 15-month study was conducted to evaluate water treatment plant

performance with respect to the removal of total coliform, total bacterial cell counts, turbidity and HPC from Missouri River Water.

The results were, as follows:

1. No total coliform organisms were found in the plant effluent over the 15-month sampling period (54 samples). Only one of 318 distribution system samples contained a coliform colony.
2. Total bacterial cell counts averaged approximately 10^7 cells/mL in raw, Missouri River water and 10^5 cells/mL in finished water. However, during the period of cold water temperatures (December-April), finished water contained in excess of 10^6 bacterial cells/mL. Overall, bacterial removals ranged from over 95% in the summer to less than 80% in the winter.
3. Turbidity removals were far greater than bacterial removals. Raw Missouri River water turbidities generally exceeded 100 NTU. Finished waters rarely exceeded 1 NTU and often were as low as 0.1 NTU. Overall, turbidity removals ranged from winter lows of 99% to summer highs of 99.9%.
4. HPC reductions consistently averaged 99.9% through all seasons, irrespective of temperature.
5. There was no relationship between turbidity or HPC removals and total bacterial cell removals by pretreatment and filtration. Moreover, there was no direct relationship between HPC and total bacterial cell counts in raw, settled or filtered water.
6. Pretreatment fails to remove 90% of the bacterial cells (performance standard) at temperatures below 7°C . Filtration fails to achieve the 90% performance standard for bacterial removal at all temperatures.
7. Turbidity was found to be related to total bacterial population at finished water turbidities less than 0.3 NTU. When finished water turbidity achieves the AWWA goal of 0.1 NTU, total bacterial populations approach those found in groundwater supplies.

CONCLUSIONS

The evaluation of the bacterial removal performance of a well-functioning water treatment plant over more than a calendar year has shown that total bacterial removals are not predicted by reductions in turbidity, HPC or total coliform colony counts. Total bacterial removals were found to be poor when water temperatures fell below 7°C , whereas turbidity removals were consistently good, and HPC removals were almost uniformly excellent. The results demonstrate a far poorer performance of physical removal processes for the removal of total microorganisms than would have been predicted from the microbiological indicators.

To increase the removal of bacteria, including pathogens, from raw drinking water supplies, efforts must be made to monitor and increase bacterial cell removals through improved pretreatment, particularly when water temperatures are low. The extent to which decreases in rates of coagulant dissolution, precipitation, cell enmeshment, floc formation, or sedimentation impairs bacterial cell removal is unknown.

however, more intensive studies employing varied coagulant additions, mixing protocols and sedimentation periods may indicate which modifications may result in good bacterial removals.

Recognition of the large populations of bacteria in raw and finished drinking waters should, ultimately, attract attention to questions regarding the fate of those organisms penetrating the treatment system and entering the distribution system. Their viability, their microbial ecology in the distribution system and the cycling of the microbial nutrients which they contain in their cells may be key elements in understanding the deterioration of water quality in distribution systems.

In the future, when large numbers of organisms are found in any water distribution system, consideration must be given to the water plant as their origin. It can no longer be assumed that they grew in the distribution system or were dislodged from the interior surfaces of distribution mains

EPILOGUE

Currently, there is a vigorous dispute in progress between USEPA and AWWA over proposed more stringent turbidity standards directed at providing additional safeguards to consumers from microorganisms such as Giardia cysts and virus. AWWA is opposing the more rigid standards. Neither contender has characterized or quantitated turbidity and both lack scientific data directly linking turbidity to microorganisms. Thus, neither side can justify a position advocating a specific drinking water turbidity criterion. In their most recent rebuttal, industry spokesmen have reported that they will argue, since the water industry does not have the technology to routinely meet a 0.1 NTU turbidity standard, that the MCL for turbidity remain at 1.0 NTU and that 0.5 NTU be used as an "operational goal (52)." Despite their long history of using turbidity as a primary drinking water standard, they now state that "turbidity is an 'operational parameter,' and no direct adverse health effects are linked to it nor are turbidity levels a direct measure of the microbiological safety of the water (52)." Failure to conduct the appropriate microbiological analyses and plant performance evaluations have led to the present state of contentious confusion over regulating the microbiological quality of water. Turbidity can not be used as either an operational parameter or drinking water standard unless the turbidity is characterized or demonstrated to correlate with microorganism removal under all conditions.

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Recognition is also due each of those water consumers who allowed the research team to enter their homes and work places for sampling on numerous occasions.

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Figure 1.

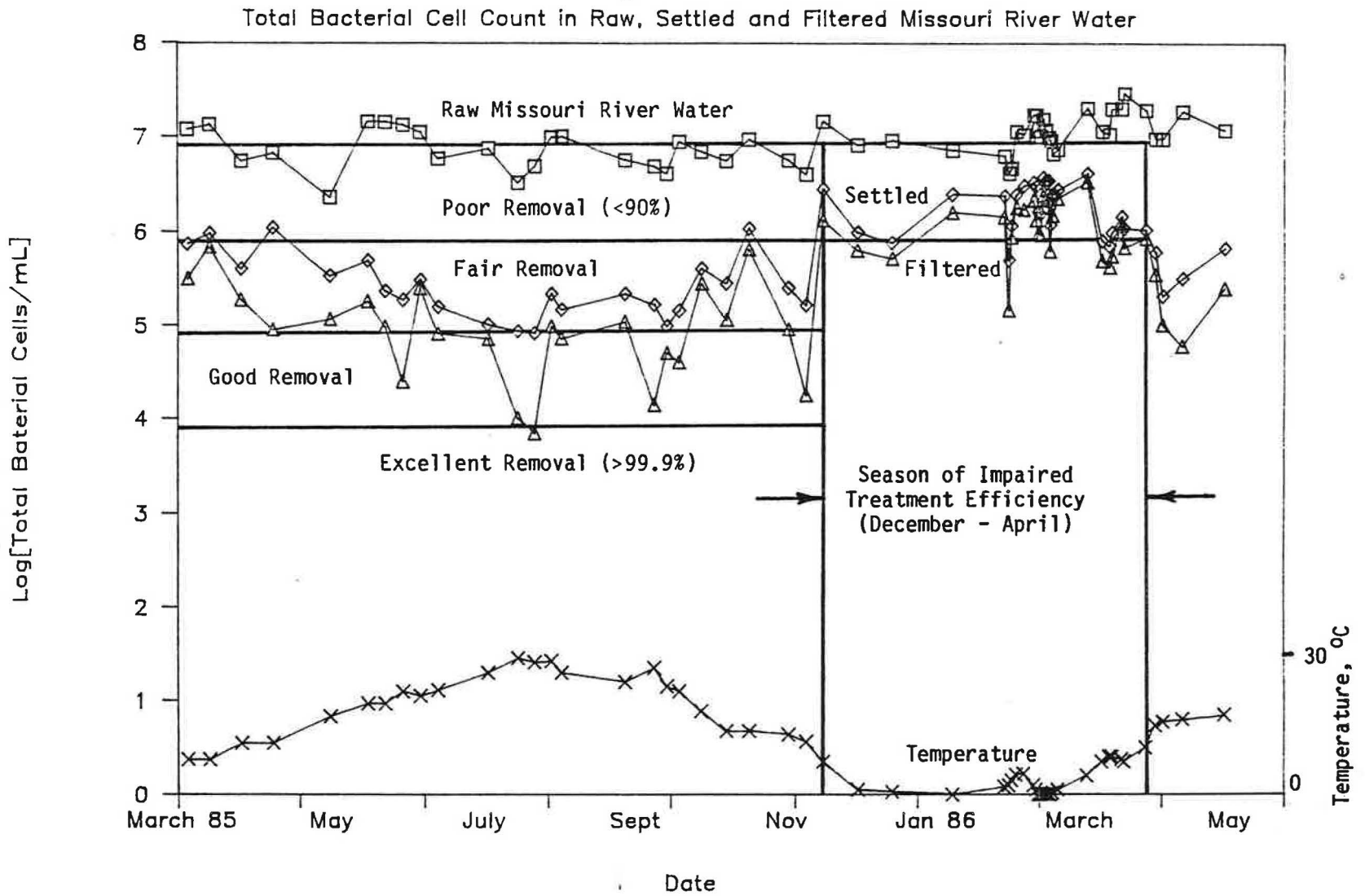


Figure 2.

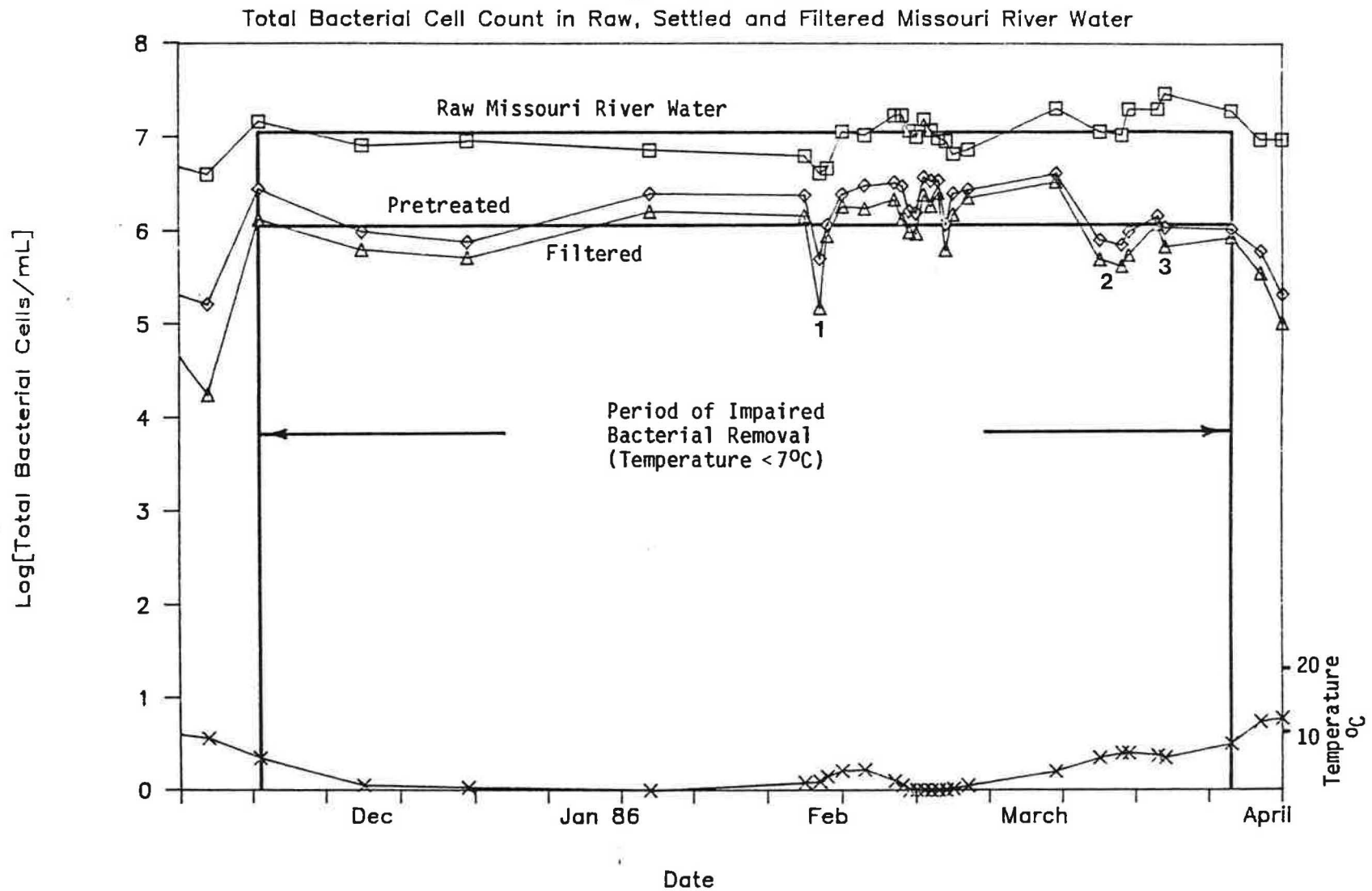


Figure 3.

Turbidity in Raw, Settled and Filtered Missouri River Water

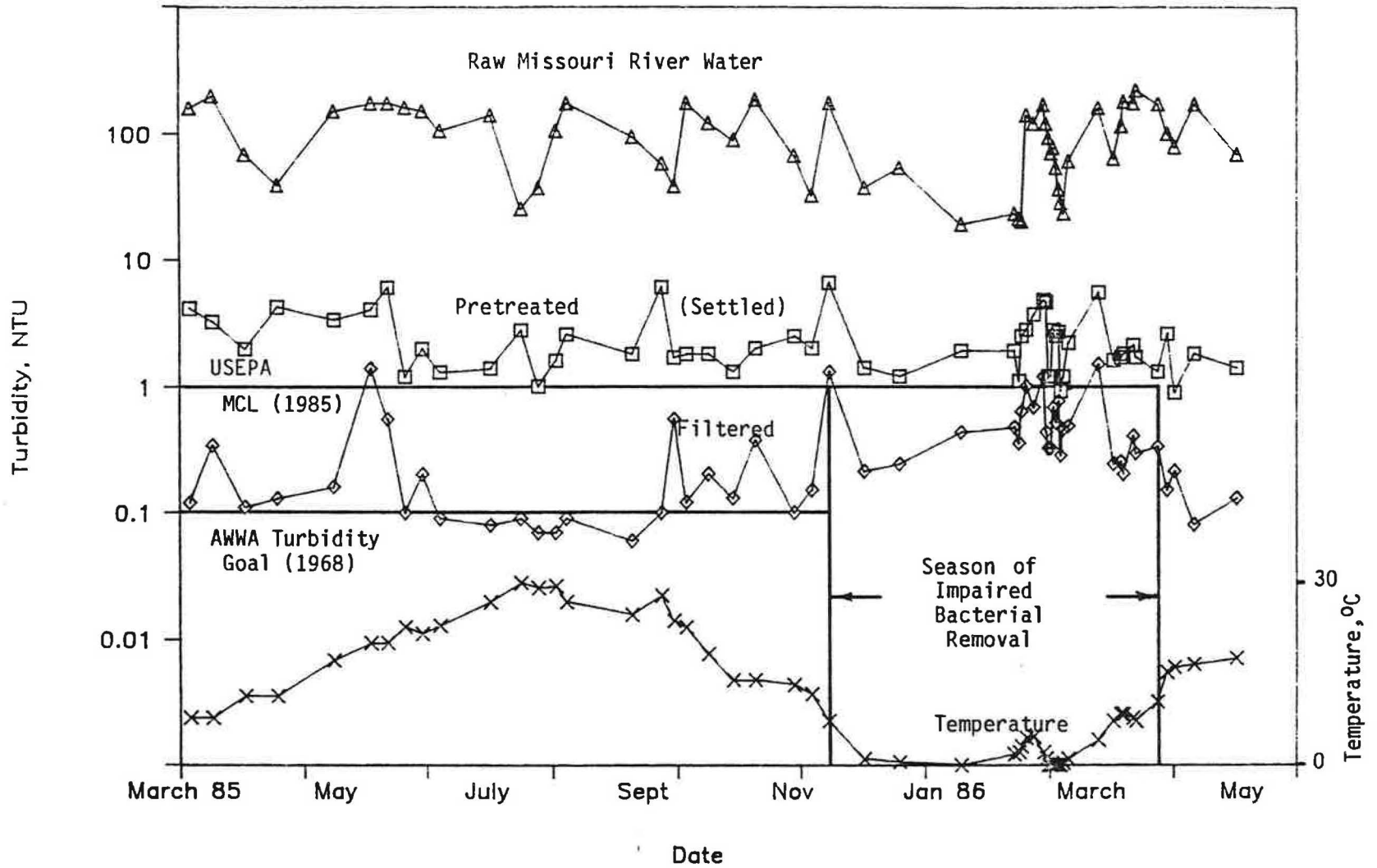


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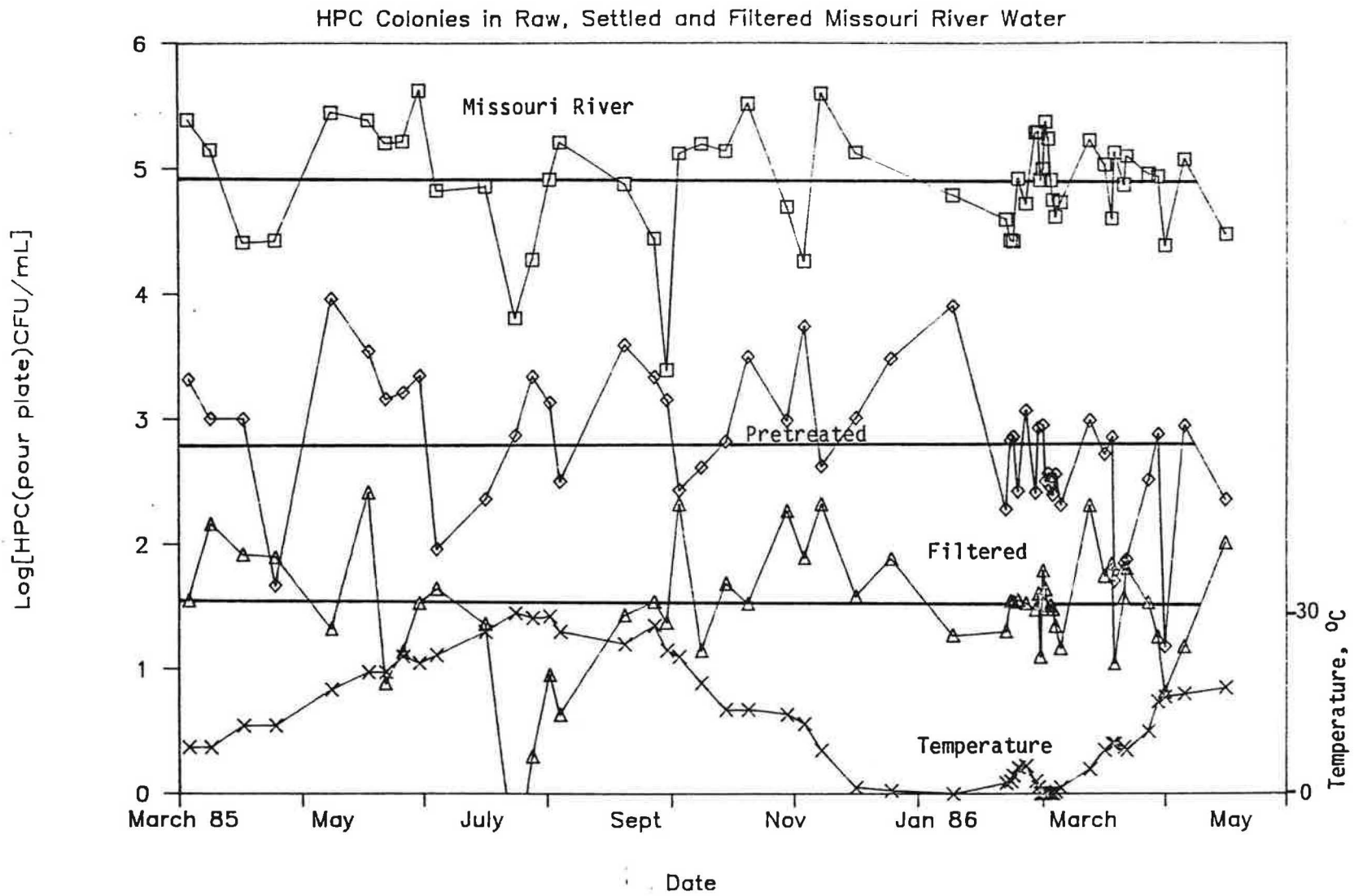


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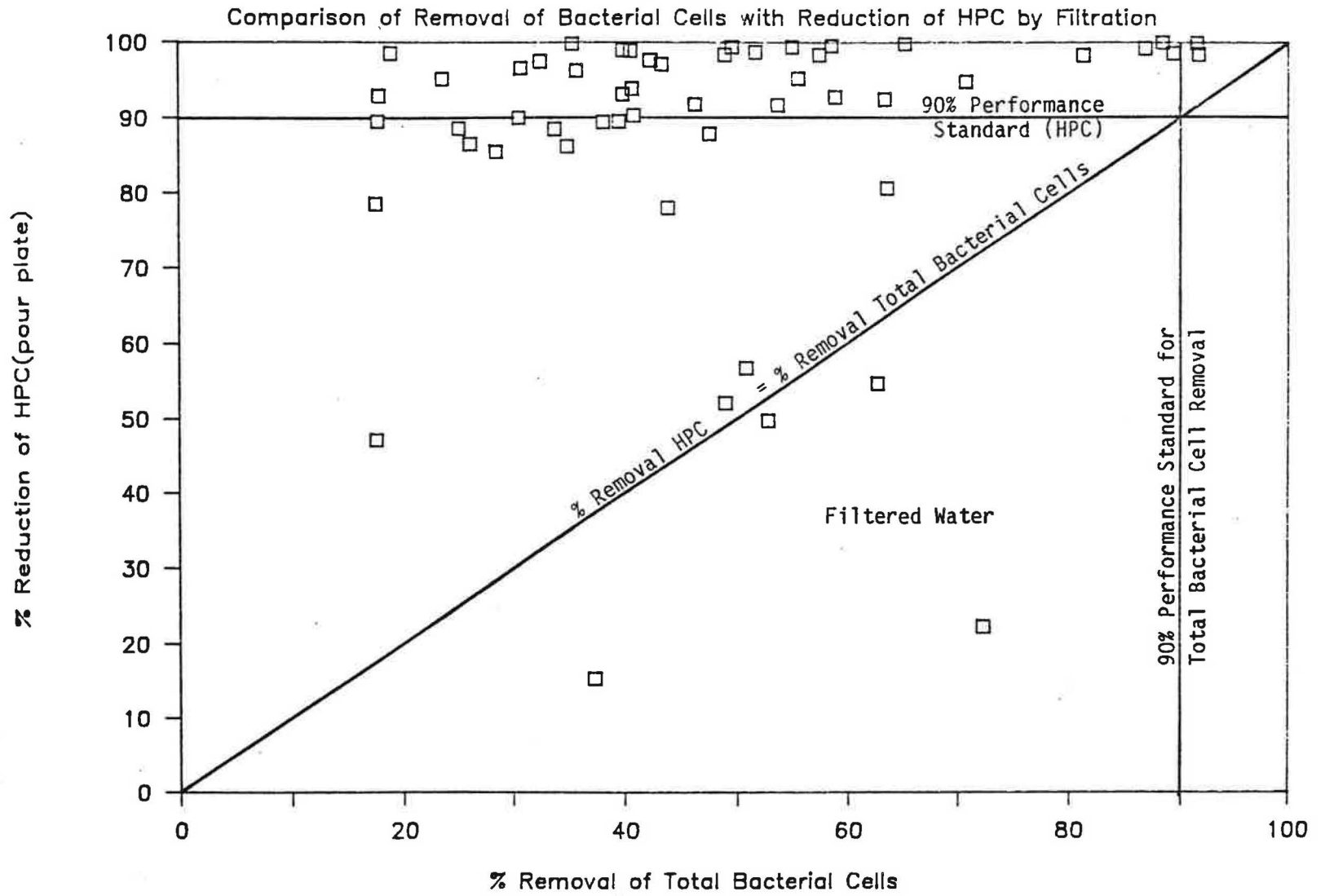


Figure 8.

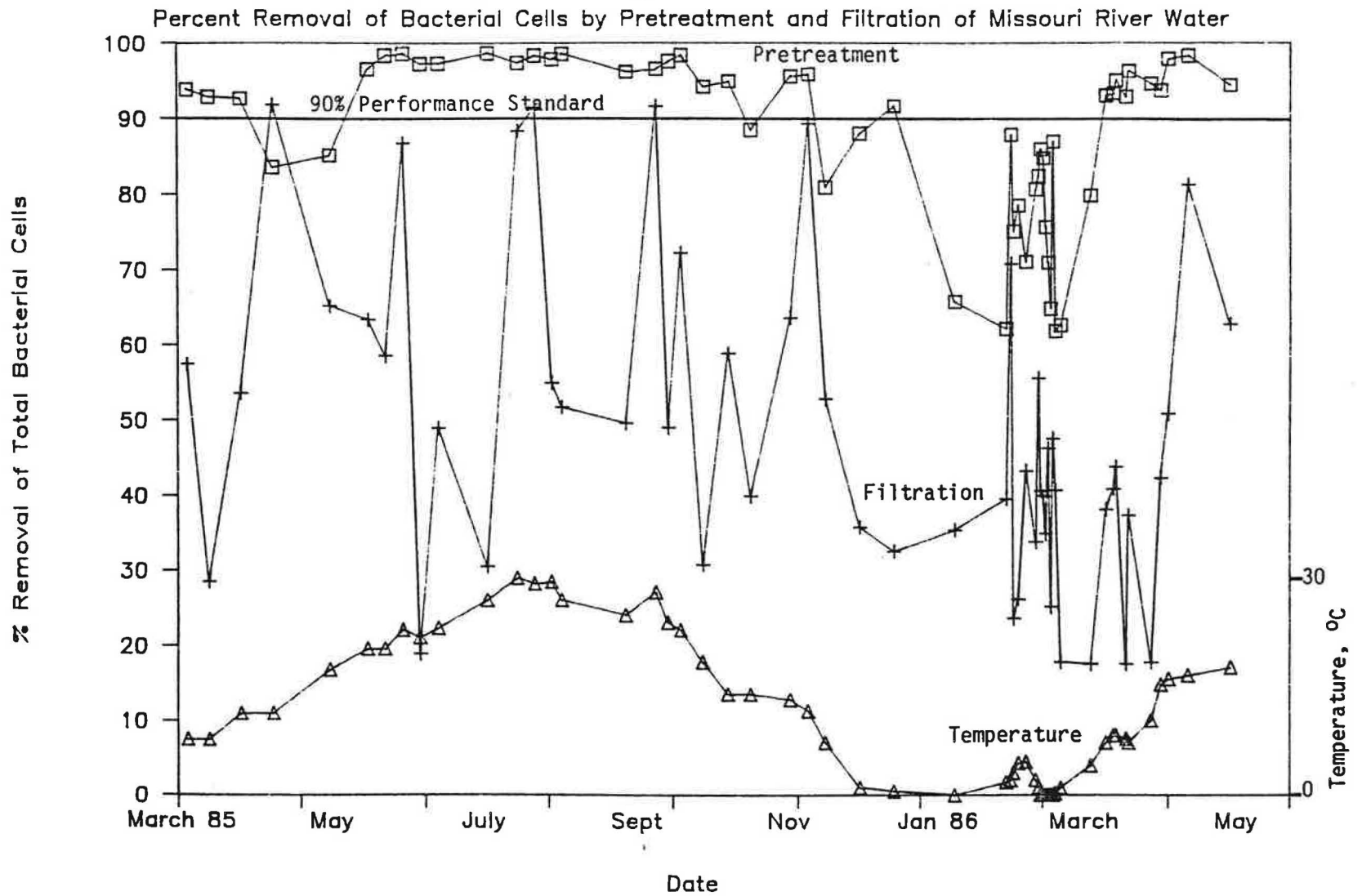


Figure 9.

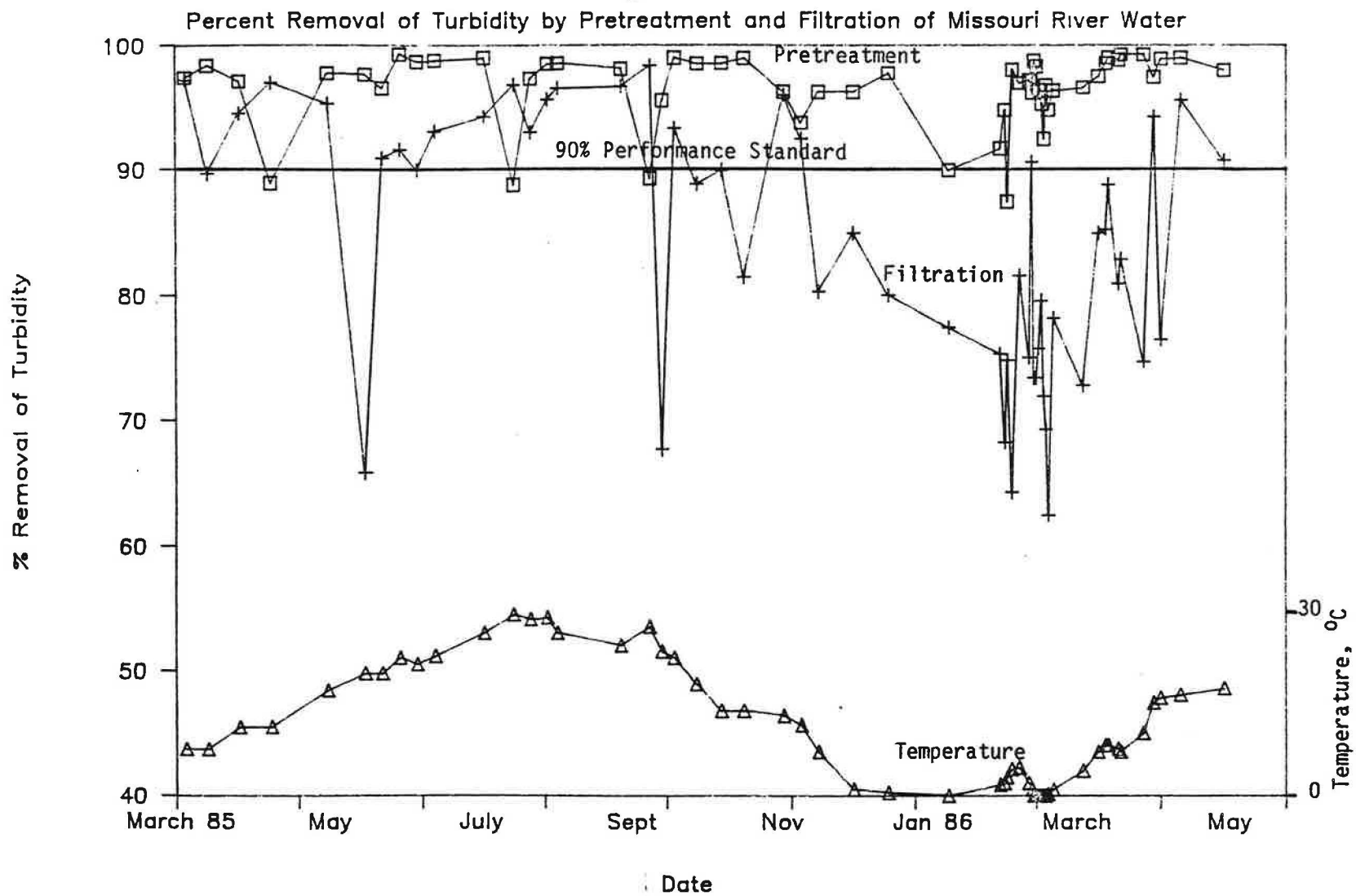


Figure 10.

Comparison of Removal of Bacterial Cells with Removal of Turbidity by Pretreatment and Filtration

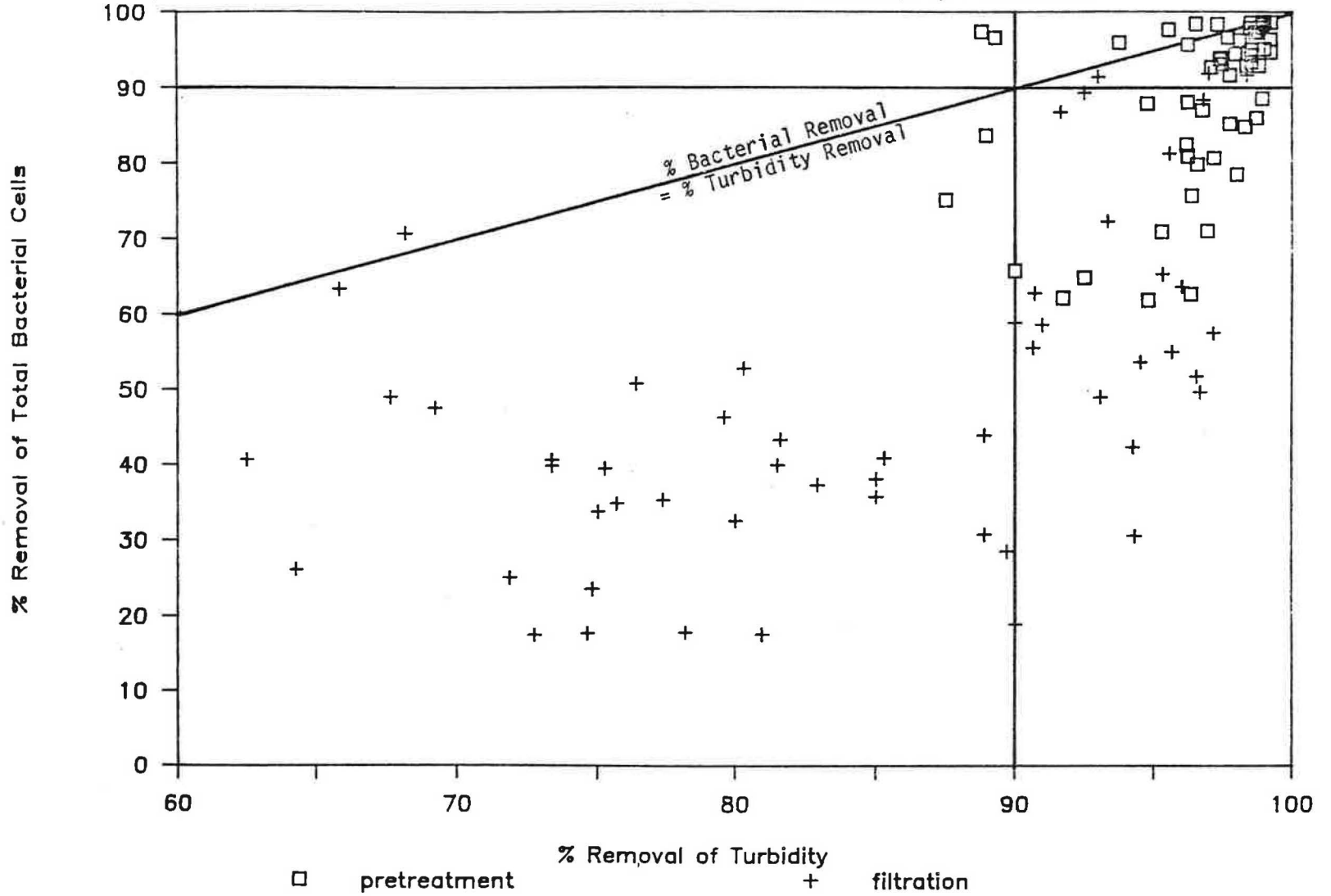


Figure 11.

Effect of Temperature on Removal of Turbidity by Pretreatment and Filtration of Missouri River Water

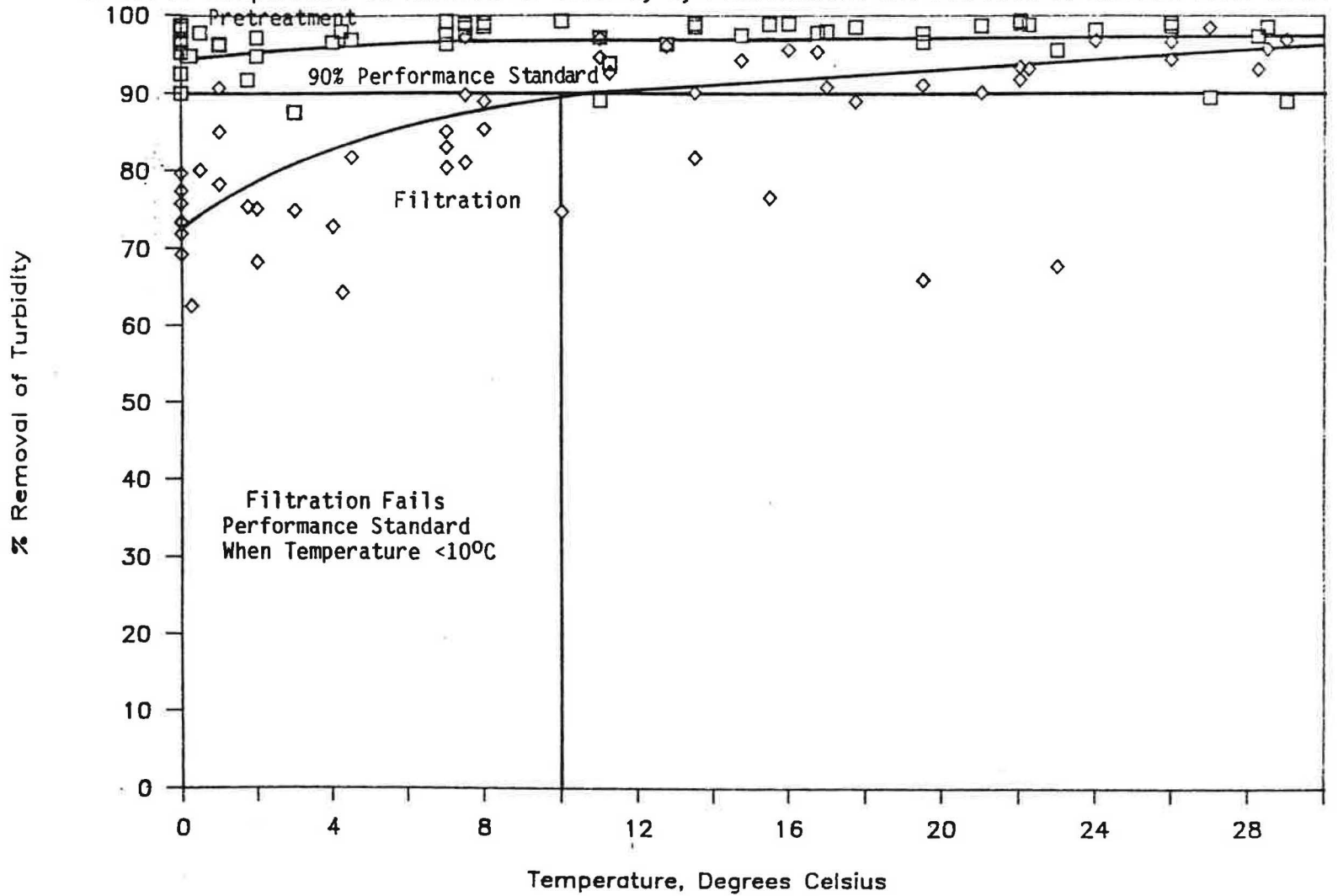


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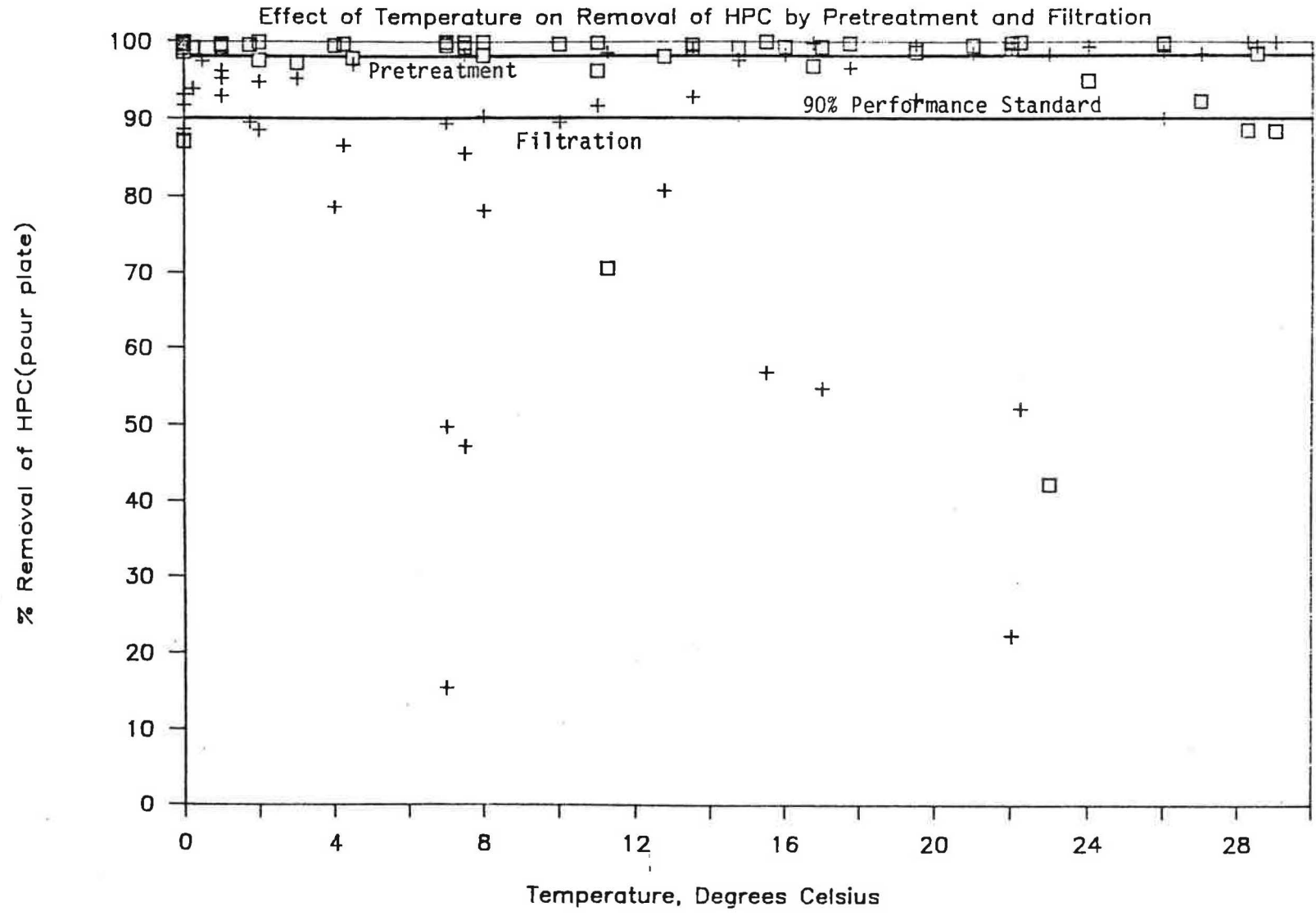
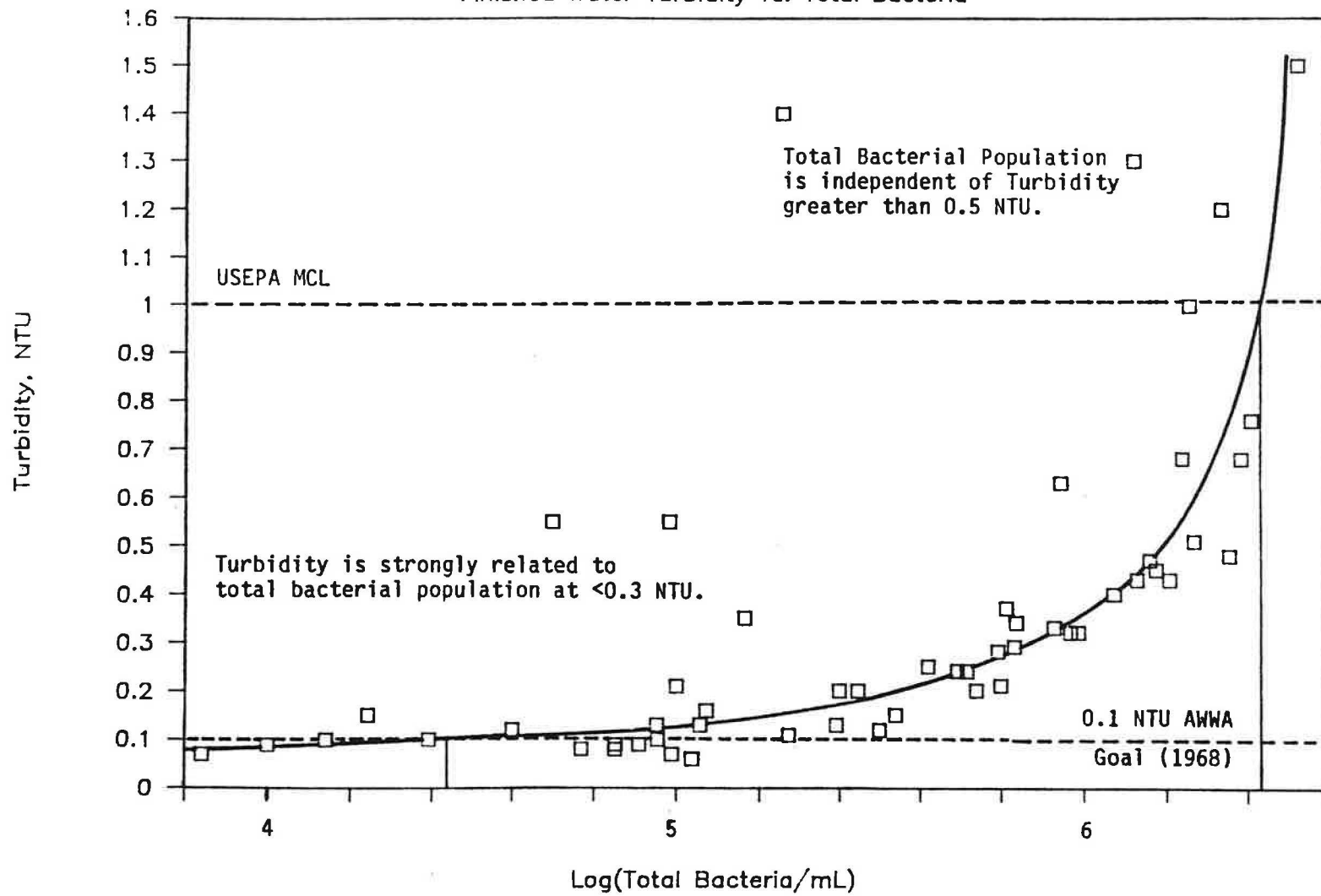
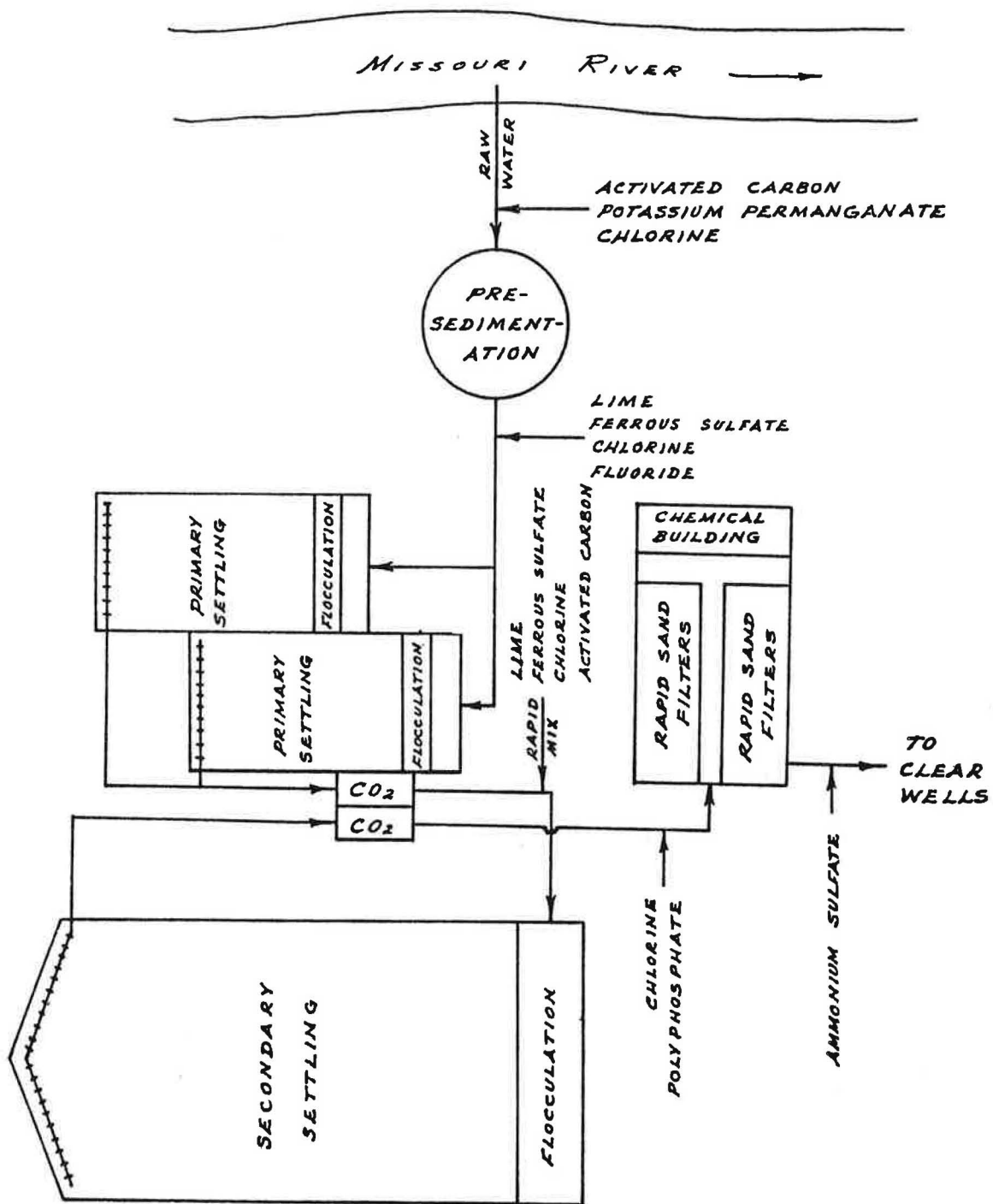


Figure 14.

Finished Water Turbidity vs. Total Bacteria





Schematic Flow Diagram of Capital City Water Treatment Plant
Jefferson City, Missouri

Table 1

Effect of Changes in Flow on Turbidity and
Bacterial Removals on Successive Days

| Date | Total Bacteria, Cells/mL | Turbidity, NTU | Temp, °C |
|-------------------------------|-----------------------------|----------------------|----------|
| 1. 31 Jan 86 (Slowed Flow) | Raw | 4.11x10 ⁶ | 21.00 |
| | Sett. | 4.99x10 ⁵ | 1.10 |
| | Filt. | 1.46x10 ⁵ | 0.35 |
| | Raw | 4.61x10 ⁶ | 20.00 |
| | Sett. | 1.15x10 ⁵ | 2.50 |
| | Filt. | 8.79x10 ⁵ | 0.63 |
| 2. 13 Mar 86 (Slowed Flow) | Raw | 1.05x10 ⁷ | 115.00 |
| | Sett. | 7.03x10 ⁵ | 1.70 |
| | Filt. | 4.16x10 ⁵ | 0.25 |
| | Raw | 1.97x10 ⁷ | 180.00 |
| | Sett. | 9.75x10 ⁵ | 1.80 |
| | Filt. | 5.48x10 ⁵ | 0.20 |
| 3. 18 Mar 86 (Normal Flow) | Raw | 2.00x10 ⁷ | 175.00 |
| | Sett. | 1.43x10 ⁶ | 2.10 |
| | Filt. | 1.18x10 ⁶ | 0.40 |
| | Raw | 2.90x10 ⁷ | 220.00 |
| | Sett. | 1.08x10 ⁵ | 1.70 |
| | Filt. | 6.77x10 ⁵ | 0.29 |
| 19 Mar 86 (Slowed Flow) | Raw | 2.90x10 ⁷ | 7.00 |
| Sett. | 1.08x10 ⁵ | 7.25 | |
| Filt. | 6.77x10 ⁵ | 7.25 | |