AN ASSESSMENT OF THE USE OF THE DIRECT MICROSCOPIC COUNT IN EVALUATING DRINKING WATER TREATMENT PROCESSES

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ABSTRACT: The direct count for the enumeration of microorganisms has been utilized in the field of microbial ecology for decades. As recently as 1977, the direct count method was refined to its current form utilizing epifluorescence microscopy with irgalan black–dyed, track–etched, polycarbonate filters. However, only recently has the direct count been applied in the evaluation of particle removal efficiency by drinking water treatment processes. Due to the reauthorization of the 1986 Amendments to the Safe Drinking Water Act, including the Filtration/Disinfection rule, there is a greatly increased need for the application of a scientifically–defensible methodology for evaluating water treatment plant particle removal performance.

The direct microscopic count may be used in water treatment plants to evaluate total bacterial removals, observe seasonal (temperature) effects on water treatment plant process efficiency and estimate the removal of planktonic versus particle–associated bacteria. It can also be used to evaluate bacterial regrowth (recovery) and aftergrowth (recruitment) in water distribution and household plumbing systems.

The principal weakness in the performance evaluation of water treatment filtration technology for particulate removal to date has been the failure to characterize the particles in suspension in influent and treated water. Because they contribute little to turbidity, large numbers of microorganisms are not detected in filter effluent when turbidity is used to monitor filtration performance.

Based on recent water treatment plant studies, it is evident that the enumeration of the total bacterial population is the most fundamental and basic microbiological measurement that can be used to evaluate water treatment plant performance. In addition, the direct microscopic count is the most rapid, specific, sensitive and accurate method available for observing reductions in a wide range of specific particles during water treatment.

KEY WORDS: Drinking water, aquatic bacteria, epifluorescent enumeration, acridine orange, AODC technique, microbial dynamics, water treatment plant performance evaluation, water filtration, water distribution systems, temperature effects.

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INTRODUCTION

The application of the light microscope has a long history in drinking water treatment going back to Whipple at the start of this century. The earliest edition of Standard Methods for the Examination of Water and Wastewater contained sections on use and application of the microscope (1). In the early development of water treatment technology, the microscope was a major analytical tool. It was used extensively to evaluate treatment processes, including chemical additions, for the removal and destruction of microorganisms (1–3). In the 1950's, a direct microscopic count was developed which facilitated the quantification of organisms and particulate matter recovered on a column of fine sand (4). The counts were expressed as areal standard units per milliliter and were used to evaluate the performance of potable water filtration processes (5,6). However, because the method was relatively lengthy and complex and the microscope had a low working magnification (100x), the procedure was not widely used. It last appeared in the 12th Edition of Standard Methods (4).

The direct microscopic count to enumerate aquatic bacteria evolved along with the development of improved methods for concentrating bacteria. Concentration by filtration (7) or vacuum dessication (8) was followed by counting in minute capillary tubes. Initially, the direct observation of bacteria on the surface of a membrane filter involved rendering the membrane transparent with immersion oil (9). This procedure underwent several improvements involving fixation and staining of the particles retained on the membrane (10,11).

The use of epifluorescent microscopy for the enumeration of bacteria following concentration on membrane filters has been the latest development in the evolution of the direct count. The method of Hobbie, et al. (12) using stained, neutron–track–etched polycarbonate filters is the most current refinement of this method. Following extensive evaluation, this method has been extensively employed by microbiologists from the fields of limnology, oceanography and microbial ecology. Its uses and limitations have been reviewed at a symposium (13) and in a recent book (14).

The suggestion that the epifluorescence direct microscopic count procedure be standardized was first made by Daley in 1977 (13). Since that time, an ASTM Standard Test Method for the enumeration of aquatic bacteria was adopted in 1985 (15). A modification of the direct count to enumerate simultaneously both the total number of bacteria and those which are respiring also was adopted (16). Most recently, a Standard Test Method for enumerating bacteria in electronics–grade water has also appeared (17).

The application of this new methodology to drinking water has been slow. The earliest that a standard method could have appeared in Standard Methods for the Examination of Water and Wastewater was in the 15th Edition in 1980 (18). However, the 16th Edition, 1985, (19), also failed to include the method. Finally, in the 17th Edition, 1989, (20) the "Direct Total Microbial Count" was included to initiate the acceptance procedure for a proposed method.

Previously, the direct count had been used for the enumeration of microorganisms in dairy products (22–24), food (22,23), intravenous fluids (25), wine (23), ultrapure water systems (26,27), beverages (23,28), wine (28), and petroleum (29). Despite its widespread application in other fields and issuance as an ASTM standard method (15), neither the USEPA nor the American Water Works Association

Research Foundation has employed or evaluated the direct count methodology in drinking water treatment. As a result, there have been relatively few studies employing the direct microscopic count to enumerate microorganisms in drinking water. The lack of recognition of the value of the direct microscopic count is pervasive in the drinking water field. This is due primarily to water utilities having had little experience in applying the method, compounded by an entrenched opinion of how to evaluate the performance of drinking water plants. This is illustrated by a panel evaluation of the application of 20 different indicators of microbial quality (30). On a rated scale of (–) to (++++) for the utility as an indicator, the direct count fared poorly receiving only a (+) for evaluating treatment and a (–) for indicating degradation, deterioration and recontamination. Turbidity, total coliform and heterotrophic plate count were recommended for evaluating treatment efficiency (30).

The results of two surveys of drinking water systems in Missouri (31,32) and four year–long surveys to evaluate seasonal effects on particle removals and subsequent passage of those particles through the distribution system has yielded new information on evaluating water treatment plant performance (33–36). Based on these recent water treatment plant studies, it is evident that the enumeration of the total bacterial population by direct count is the most fundamental and basic microbiological measurement that can be used to evaluate water treatment plant performance. In addition, the direct microscopic count is the most rapid, specific, sensitive and accurate method available for observing reductions in particles during water treatment.

APPLICATION OF THE DIRECT MICROSCOPIC COUNT TO THE EVALUATION OF WATER TREATMENT PLANT PERFORMANCE

Two extensive surveys of drinking water systems in Missouri (winter, 1984; summer, 1985) revealed significant systematic differences in the numbers of total bacterial cells enumerated in finished waters from surface water treatment plants (31,32). Based on these observations, a study was undertaken to evaluate bacterial removals throughout the calendar year at a well–operated, comprehensive water treatment plant which consistently meets all drinking water standards (33). Since turbidity and the heterotrophic plate count (HPC) are extensively used as microbiological indicators of water treatment plant performance, the study compared the reduction of both with the removal of the total number 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 (37). 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".

MATERIALS AND METHODS

This initial 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 in Figure 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 first added to establish a hypochlorous acid 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 standard rate rapid sand filters. Polyphosphate (0.5 g/m^3 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 for distribution.

Filter flows are regulated by rate–of–flow controllers at 5–7 m/h. Two filters are backwashed every night following a four–day filter cycle.

The treatment plant served a population of approximately 30,000 with an annual average daily flow of 20,000 m^3/day from December 1985 to March 1986 and 24,000 m^3/day 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 (38).

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 1–liter polyethylene sample bottles containing 1 milliliter 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 (19): 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.

The method used for enumerating the total number of bacteria is a slight modification of the method described by Hobbie, et al. (12). 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 Lietz Ortholux microscope fitted with a Ploem vertical illuminator and 200–W mercury lamp. Micrographs were made with a Leica M1.

REDUCTION IN TOTAL BACTERIAL CELL COUNTS

Figure 2 shows the total number of bacterial cells enumerated in raw, pretreated (softened, coagulated, flocculated, settled) and filtered Missouri River water. The data indicate that the raw water source consistently contained approximately 10^7 bacterial cells/ml throughout the year. Pretreatment (following sedimentation) was found to reduce this number by up to two orders of magnitude (99 percent; to 10^5) during the summer months. However, in the winter, as the earlier surveys had indicated, reductions in total bacterial cell counts averaged less than one order of magnitude. From December through March, total bacterial cell populations in finished water generally exceed 10^6 cells/ml. 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 raw water service pumps at the river and shutting down filters. After a day, markedly improved bacterial removals were observed. They appear as downward "spikes" in Figure 2. The beneficial effect of reduced hydraulic loading was 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 percent have been classified as "poor" removals, while up to 99 percent and 99.9 percent are rated "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 (39). It states that bacterial removal efficiency "with proper pretreatment should exceed 99 percent". It also asserts that "more than 98 percent 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 least to overall plant bacterial removals. The results of this initial study may be summarized, 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 percent in the summer to less than 80 percent in the winter.
3. Turbidity reductions 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 reductions ranged from winter lows of 99 percent to summer highs of 99.9 percent.

4. HPC reductions averaged 99.9 percent through all seasons. HPC values were lowest in the summer when overall bacterial removals were greatest.

5. Pretreatment failed to remove 90 percent of the bacterial cells (performance standard) at temperatures below 7 °C. Filtration failed to achieve the 90 percent performance standard for bacterial removal at all temperatures, averaging only 50 percent removal of filter influent bacterial cells (Figure 3).

6. Turbidity was found to be related to total bacterial population at finished water turbidities less than 0.3 ntu (Figure 4). When finished water turbidity achieves the AWWA goal of 0.1 ntu, total bacterial populations in treated Missouri River water 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 cell removals were found to be poor when water temperatures fell below 7 °C whereas turbidity reductions were consistently good. While also showing the seasonal temperature effect, HPC removals were almost uniformly excellent. The results demonstrate a far poorer performance of physical removal processes for the removal of total bacterial cells than would have been predicted from conventional microbiological indicators.

REMOVAL OF PARTICLE–ASSOCIATED BACTERIA (PAB)

In the initial study of total bacterial removals, particles with attached bacteria were rarely seen in filtered water. Only single, planktonic cells were observed. Therefore, a second seasonal study was undertaken at Jefferson City, Missouri, to determine the relative removal of planktonic versus particle–associated bacteria (PAB) (34). The removal of PAB has been of interest in water treatment because of concern over the protection that larger particles might afford microorganisms from the action of disinfectants.

In this second study, in addition to conventional parameters, five non–standard parameters based on direct microscopic counts were used to evaluate water treatment plant organism and particle removal performance. Total bacterial cell counts were again used to evaluate overall bacterial cell removals. Following 3 μm membrane filtration to separate planktonic cells and recover larger particles, total particles larger than 3 μm, particle–associated bacteria and particles larger than 3 μm which hosted five or more bacterial cells were enumerated. Nematodes were included in the count of particles with five or more particle–associated bacteria because of possible ingestion of bacterial cells by the worms.

METHOD FOR ENUMERATION OF PARTICLE–ASSOCIATED BACTERIA

The enumeration of PAB in the filter effluent was accomplished by epifluorescence microscopy after filtering 1 to 6 liters of finished water at approximately 13 kPA (100 mm mercury) through a 3 μm Poretics polycarbonate filter. The filters were stained with irgalan black as described by Hobbie, et al. (12). Neutron track–etched polycarbonate filters have been shown to function as screens which quantitatively separate size fractions of particles from water samples. In the field of aquatic microbiology, 3 μm is the consensus filter size utilized for the separation of unattached planktonic bacteria from PAB. The PAB are reported as the total number of bacteria colonizing particles. While not enumerated in the source water, the total number of particles larger than 3 μm as well as nematodes were enumerated in the finished water. Generally, two liters of filter effluent were filtered during the cold–weather period while six liters were processed during warm weather.

RESULTS

Again, seasonal temperature effects were very evident from all measures of plant performance. The principal results were:

1. Turbidity reductions were between two and three orders of magnitude year–round. Finished water turbidities averaged 0.26 ntu in the winter and 0.07 ntu in the summer.

2. Finished water HPC averaged 64 cfu/ml in the winter and 8.5 cfu/ml in the summer when reductions averaged 99.96 percent.

3. Total bacterial removals averaged 92.3 percent in the winter and 99.85 percent in the summer so that finished water total bacterial counts averaged 1.0 x 10^6/ml in the winter and 21 x 10^3/ml in the summer. Total bacterial removals were highly sensitive to the adverse effect of low temperature on water treatment plant performance.

4. Bacteria on particles larger than 3 μm (PAB) constituted approximately two–thirds of the total bacterial count in the Missouri River in the winter and one–third in the summer. These particle–associated bacteria were consistently reduced by more than 99.99 percent.

5. Planktonic bacteria were reduced by 78 percent in the winter and 99.8 percent in the summer. Winter populations of planktonic bacteria were approximately two orders of magnitude greater than summer populations.

6. The removal of particles with five or more associated bacteria exceeded 99.999 percent both in the winter and summer indicating that few particles of potential health significance enter the distribution system. The numbers found in the finished water averaged 2.5 and 1.1 per milliliter in the winter and summer, respectively.

7. Of the particles with five or more associated bacteria, 79 percent had between five and ten associated cells. Only one particle out of 383 had more than 31 associated bacteria.

8. The total number of particles larger than 3 μm in the filtered water averaged 1167 per milliliter in the winter and 28 per milliliter in the summer. Long, thin bacterial rods, which were present only during the winter, contributed an average of 725 particles per milliliter to the winter totals.

9. Nematode concentrations averaged 0.36 per liter in the winter and 0.09 per liter in the summer.

10. HPC organisms were six times more likely to be associated with bacteria on particles larger than 3 μm than with planktonic bacteria.

11. The effect of particles larger than 3 μm on finished water turbidity was studied during a five–day period in March. Membrane filtration for total removal of those particles had no effect on the measured turbidity.

12. One–half to two–thirds of the particles associated with bacteria found in the finished water appeared to be formed within the plant.

CONCLUSIONS

Again, all parameters used in the seasonal evaluation of water treatment plant performance indicated the adverse effect of low temperature on particle removal efficiency. The largest adverse effect was observed with respect to the micrometer–sized planktonic bacteria. Since, in general, the smaller particles were most poorly removed, the question of the removal of still smaller virus particles was again highlighted.

A comparison of turbidity versus particle removals confirms the inconsistency and insensitivity of using turbidity as an index of plant performance. Since finished water turbidity was no further reduced by 3 μm membrane filtration, it appears that filtered water turbidity is primarily caused by the planktonic bacteria and other smaller particles.

Removals of particle–associated bacteria were found to be extensive (>99.99 percent) both in winter and summer. In addition, the removals of particles with five or more attached bacteria were consistently greater than 99.999 percent during both seasons. These results indicate that rapid sand filtration plants are extremely effective in reducing the challenge posed by bacteria which may be protected from disinfection through particle association. Conversely, it was the planktonic bacteria which penetrated the filtration plant, particularly during the winter. Based on the extensive removal of particle–associated bacteria during treatment, the HPC in filtered water appears to be primarily related to the planktonic bacteria which penetrate the plant. Further reduction in HPC would therefore require improved efficiency of removal of planktonic bacteria.

The particles found in Missouri River water were found to vary seasonally with the proportion of attached bacteria ranging from roughly two–thirds in the winter to one–third in the summer. While almost all of the particles larger than 3 μm were removed during treatment, other particles were formed or recruited which were not in the original source. Only direct microscopic observation permitted the discrimination of these particles by origin.

REMOVAL OF CYST–SIZED BIOTIC PARTICLES

A third study of the Jefferson City water system was conducted in 1989 (35). In addition to the measurements made in the previous studies, additional direct microscopic measures were made of algal cells and colonies, long bacterial rods and carbon fines. The principal objective of this study was to observe the removal of algal cells which, because of their size and surface properties, might indicate the degree of removal of similar–sized pathogenic protozoan cysts. A summary of the numerical results of the study, averaged for the winter and summer sampling periods, are given in Table 1. As in the previous studies, seasonal effects were evident for reductions in turbidity, HPC, total bacteria, particle–associated bacteria and total particles. Long bacterial rods retained on 3 μm membranes were found to be major contributors to the total number of particles enumerated.

Particles larger than 3 μm with five or more attached bacteria, while more abundant in the winter, were found to be removed with great efficiency throughout the year.

The average removal of algal cells exceeded three orders of magnitude when water temperatures were high. At low temperatures, algal cell removals declined to 95 percent. As a result, despite initially lower winter raw water populations, algal cell counts in the finished water increased 26–fold.

Since the results obtained have been consistent and reproducible through the three separate evaluations, it is evident that various groups of particles respond very differently to water treatment plant particle removal processes. Moreover, water temperature is a dominant factor influencing all particle removal efficiencies.

DISCUSSION

Conventional and Direct Microscopic Methods for Evaluation of Water Treatment Plant Performance

The conventional measures used for assessing the microbiological efficiency of water treatment plants are the regulated parameters, turbidity and total coliform. Although not required, some water utilities have also compiled supplementary data on HPC organisms. Recently, a number of water treatment plants have started to utilize electronic particle counters in an effort to quantify the particle removals achieved as a function of particle size. Alternately, the studies reported here have employed direct microscopic techniques to observe and compare the removals of various groups of particles and to observe seasonal variations in water treatment plant particle removal efficiency.

New federal regulations, recent waterborne disease outbreaks and advancing technology are now forcing a reappraisal of the methods by which water treatment plant microbiological efficiency is evaluated. The following discussion is directed toward an assessment of the appropriate technology for engineering evaluation and control of water treatment processes. Appropriate technology would permit evaluation of the two–barrier protection (physical removal; disinfection) on which regulation of drinking water quality for the protection of public health is predicated. Such technology would assist regulatory authorities in evaluating compliance with the filtration/disinfection rule and provide a basis for allowing additional "credit" for treatment which goes beyond conventional treatment (e.g., two–stage treatment and lime softening).

More scientific methods of process evaluation should also facilitate a re–evaluation of filtration theory and make it possible to optimize water treatment plant performance for particles most directly related to health concerns.

Appropriate technology should also lead to improved plant design with respect to flocculation energy input, sedimentation requirements and filter rate and media selection. This is especially critical at a time when the engineering profession is attempting to reduce treatment plant construction and operational costs by eliminating large sedimentation tanks, reducing energy inputs and increasing filter flow rates. Finally, appropriate technology should enable plant operators to monitor plant performance and maintain daily operational control.

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. The 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 (37). 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 (31,32). Nor did treatment provide parallel removals of turbidity and microbial indicators, such as total coliform, HPC, Giardia cysts, virus or total bacteria (37).

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 (40). Consequently, the near–complete removal of the suspended solids is believed to ensure an equally–complete reduction in the naturally–occurring population of microorganisms.

Total Coliform for Evaluation of Water Treatment Plant Performance

In studies conducted to assess treatment plant bacterial 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 often 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 public health parameter, it would not be considered for evaluation of treatment plant performance.

Monitoring of coliform organisms does play a major role in the protection of drinking water supplies from wastewater discharges. However, because coliform organisms are readily inactivated so that coliform are generally absent in treated waters, they have no value for the monitoring of water treatment plant particle removal performance. No coliform were found in the finished water at Jefferson City, Missouri. For all practical purposes, coliform was undetectable in treated or distributed water.

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 (35).

In addition, observed plant reductions in HPC (>99.9 percent) were considerably higher than total cell removals because physical removal is confounded with chemical inactivation which may kill or merely injure the organisms.

Alternate Parameters for Evaluation of Water Treatment Plant Performance with Respect to the Removal of Microbial Particles of Potential Health Significance

Total Bacterial Cells: Bacteria are generally the most numerous particles larger than 0.2 μm in water supply sources, often ranging from 10^6 to 10^7 particles per milliliter. Direct enumeration gives a measure of the removal of all particles larger than 0.2 μm. While coagulated, settled and filtered waters may contain 10^5 to 10^6 cells per milliliter, comparatively few particles larger than 3 μm are found (10^3 per milliliter). Therefore, the effective removal of total bacteria is a strong indication of the effective removal of all particles larger than 0.2 μm. However, achieving a goal of 99 percent bacterial removal might require significant treatment modifications during winter months.

Planktonic and Particle–Associated Bacteria: Of the total bacterial population in drinking water sources, a fraction are attached to particles and a fraction are planktonic. The fraction attached to particles appears to vary with season in the Missouri River, averaging 39 percent in the winter and 71percent in the summer. The fraction of bacteria attached to larger particles is as well removed as the larger solids, validating the long–held contention that surface attachment to particles in natural water provides a major advantage for the removal of bacteria. What remains unknown are the reasons for seasonal variations in the degree of microbial attachment.

Alternately, the planktonic bacteria tend to remain highly dispersed. They appear to be less readily coagulated than silt and clay particles which appear to have a tendency to aggregate spontaneously. As a result, planktonic bacteria offer an outstanding challenge to the coagulation process. If such bacteria are not entrained in precipitates or coagulant floc during pretreatment of water prior to filtration, they appear to penetrate filters readily. Because of the comparatively poor efficiency of physical removal of planktonic bacteria, particularly during periods of low temperature and high hydraulic loads, this measure may be taken as the most critical test presently available of the removal of sub–micrometer and larger particles during water treatment.

Removal of Bacteria on Particles of Potential Health Significance: To further refine the measurement of the effectiveness of water treatment physical removal processes relative to the protection of public health, a fraction of bacteria was evaluated with respect to the degree of protection that they received from the particles to which they were attached. Recognizing the difficulty in establishing a scientific rationale for this selection,the distribution of the number of bacteria on particles was carefully assessed (35). In most instances, only a few bacteria were attached per particle. The selection of five or more attached bacteria, therefore, identified only a fraction of the total bacterial population which colonized larger particles. In the winter of the present study, the number of bacteria on particles having five or more attached cells was 11 percent of the total in the raw water. In the filtered water, these bacteria were essentially absent. If such bacteria are, indeed, an index of the particles which are most likely to transmit disease, then their consistent removals to the extent of greater than 99 percent in the winter and greater than 99.9 percent in the summer is reassuring.

Direct microscopic particle counting, while requiring the human ability to recognize particles, is very precise and offers unparalleled scientific information. In the most recent study, since microscopic examination had shown that most particles smaller than 3 μm were, indeed, planktonic microbial cells, treated water samples were passed through a 3 μm membrane filter prior to direct particle counting. The number of particles recovered on the 3 μm membrane averaged 1116 per milliliter in the winter and 293 per milliliter in the summer. This seasonal difference again sharply delineated the increased effectiveness of treatment under warm water temperature conditions.

More important, the direct microscopic count allowed the observer to identify the major particles penetrating the water treatment plant. In this instance, about one–third of the particles present were long, almost needle–like, bacterial rods. This percentage was the same, both summer and winter. Because of the unusual shape of the cells, which may be actinomycetes, the percentage recovery of cells from the sample was found to vary with the flow rate through the membrane filter. One can also envision the removal of these rather large, flexible cells varying with flow through a full–scale water filter.

Overall, algal cells and colonies were second in abundance, ranging from 49 percent in the winter to 9 percent of the total in the summer. Both these results contrast with the consistently effective removal of the most abundant silt and clay particles in the raw water.

Electronic Particle Count for Evaluation of Water Treatment Plant Performance

In a study of the performance of high–rate filtration plants, Cleasby, et al., reported a relationship between turbidity and particle count (as measured using a 60 μm HIAC PC320 sensor) in both raw and filtered water (41). The "total particle count" reported in the filter effluents of 21 water treatment plants ranged from 41 to 2200 per milliliter. From data presented, the particle counter failed to detect the majority of particles less than 5 μm.

Assuming that the particles larger than 5 μm detected by the electronic particle counter are of special importance because of their potential for protecting microorganisms from the action of disinfectants, the indicated degree of their removal by pretreatment and filtration seems disappointing. If it is not unreasonable to expect that each physical water treatment process (sedimentation, filtration) reduces influent large particle concentrations by a minimum of one order of magnitude (90 percent), then, the performance of many of the water plants surveyed failed to meet minimal expectations.

The data reported by Cleasby, summarized in Table 2, confirms the importance of pretreatment to overall filtration plant particle removal performance. Conventional water treatment plants which employ sedimentation and filtration were found to average 99 percent overall particle removal. However, the four conventional plants which achieved over an order of magnitude particle removal by pretreatment averaged 99.6 percent reduction in particle counts. The eight other conventional plants where pretreatment was less effective averaged 98.8 percent. Where sedimentation was not provided, average particle count reductions declined to 97.7 percent.

All four of the direct filtration plants failed to achieve 99 percent removal of influent particles and averaged 96.2 percent overall reduction. Three of these plants, however, showed significant (70–80 percent) reductions in particle counts prior to filtration. The exception exhibited a minimum number of particles in the raw water to begin with. Similarly, the in–line filtration plant had less than 10,000 particles per milliliter in the influent water.

Lime softening plants serve as notable exceptions to plant performance evaluation schemes relying on turbidity or electronic particle counting. The softening plant studied utilizes coagulation during pretreatment plus two–stage clarification in addition to dual media filtration. The apparent low particle reduction (52 percent) during pretreatment is misleading since softening plants produce a far greater number of particles within the plant than are present in the source water. The reported 97 percent overall particle removal may have been orders of magnitude greater if the calcium carbonate crystals formed during treatment had been redissolved. Removals of particle–associated bacteria in a similar softening plant were routinely found to exceed 99.9 percent (35). Moreover, particles larger than 3 μm having 5 or more bacteria attached (potentially health–significant particles) were found to be reduced by 99.997 percent in the winter and 99.99994 percent in the summer. In virtually all water treatment plants where particles are generated or modified during treatment, the inability of the electronic particle counter to discriminate native from plant–generated particles may result in a gross underestimation of water treatment plant efficiency.

The data of Cleasby, et al., clearly demonstrate that it will be to the disadvantage of most water utilities which generate particles during treatment (e.g., by coagulation or precipitation) to utilize either turbidity or particle counting to demonstrate the efficiency with which microorganisms are removed by physical treatment processes. Eleven of the 21 plants evaluated failed to achieve 99 percent (two–log) reductions of particles as determined by electronic particle counter. Evidence based on direct microscopic count indicates the actual removal of "native" particles 3 μm and larger by surface water treatment plants markedly exceed these values.

Another major difference between electronic particle counting and microscopic counting is that electronic particle counts indicate a near–uniform reduction in particles irrespective of particle size. Direct microscopic counts clearly reveal a far greater removal of large particles and far smaller removals of small particles. Electronic particle count data for the City of Corvallis, Oregon (Table 3) illustrate the uniformity of apparent particle reduction. The results would imply that particles ranging in size from 1 to 50 μm are removed with equal efficiency.

Implications of Particle Morphology for the Theory of Filtration

Whereas the particles used to establish theoretical filtration models were uniformly charged, rigid latex spheres, the particles observed in natural water sources are incredibly varied. Some scatter light well, others are translucent. Some are dense and settle readily, others approach the density of water. Many biotic particles are flexible and deformable, able to squeeze through pores and narrow passages. Still others are motile. Particle surfaces may have a high charge density or virtually none. In the case of microorganisms, surface properties and their tendency to attach may even vary with metabolic activity. In the present series of studies, particle attachment was found to vary markedly with seasonal temperature changes.

The most obvious difference in waterborne particles to the observer is their shape. The long, thin flexible (needle–like) rods commonly observed in the finished water in the present study would clearly be expected to have different filtration properties than their equivalent spheres.

A willingness to observe and characterize the particles present in natural waters, with a special emphasis on the biotic particles of potential health significance, is a prime prerequisite to the establishment of a rational filtration theory. This is particularly important if reliance for particle removal is to be shifted from conventional coagulation and sedimentation to polymer–assisted direct filtration. Since turbidity and electronic particle counting are inadequate for quantitating or characterizing the particles in treated water, it is especially important that direct observations be made when traditional particle removal technologies are not utilized.

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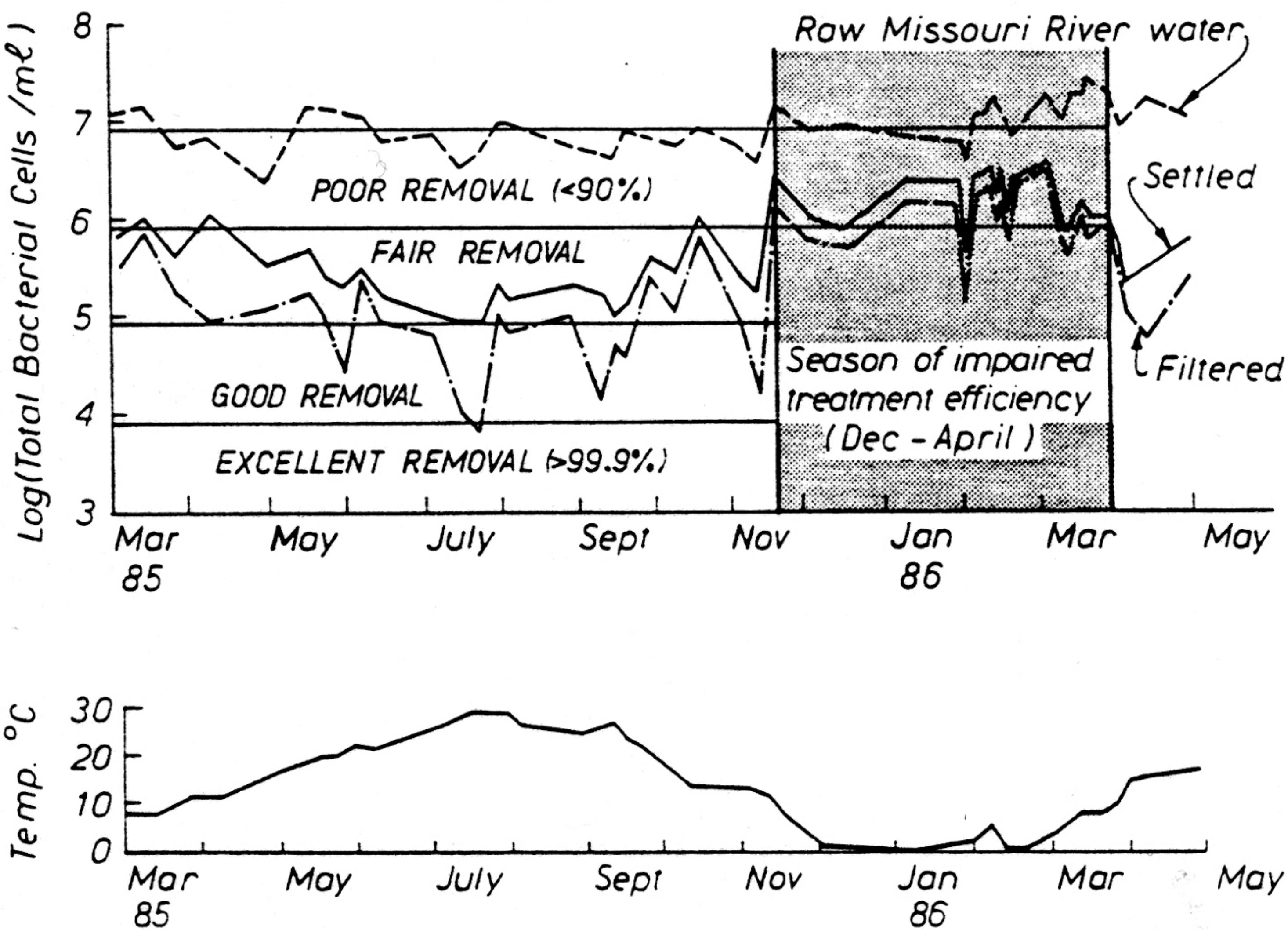

FIG. 1 -- Total bacterial cell count in raw, settled and filtered Missouri River water

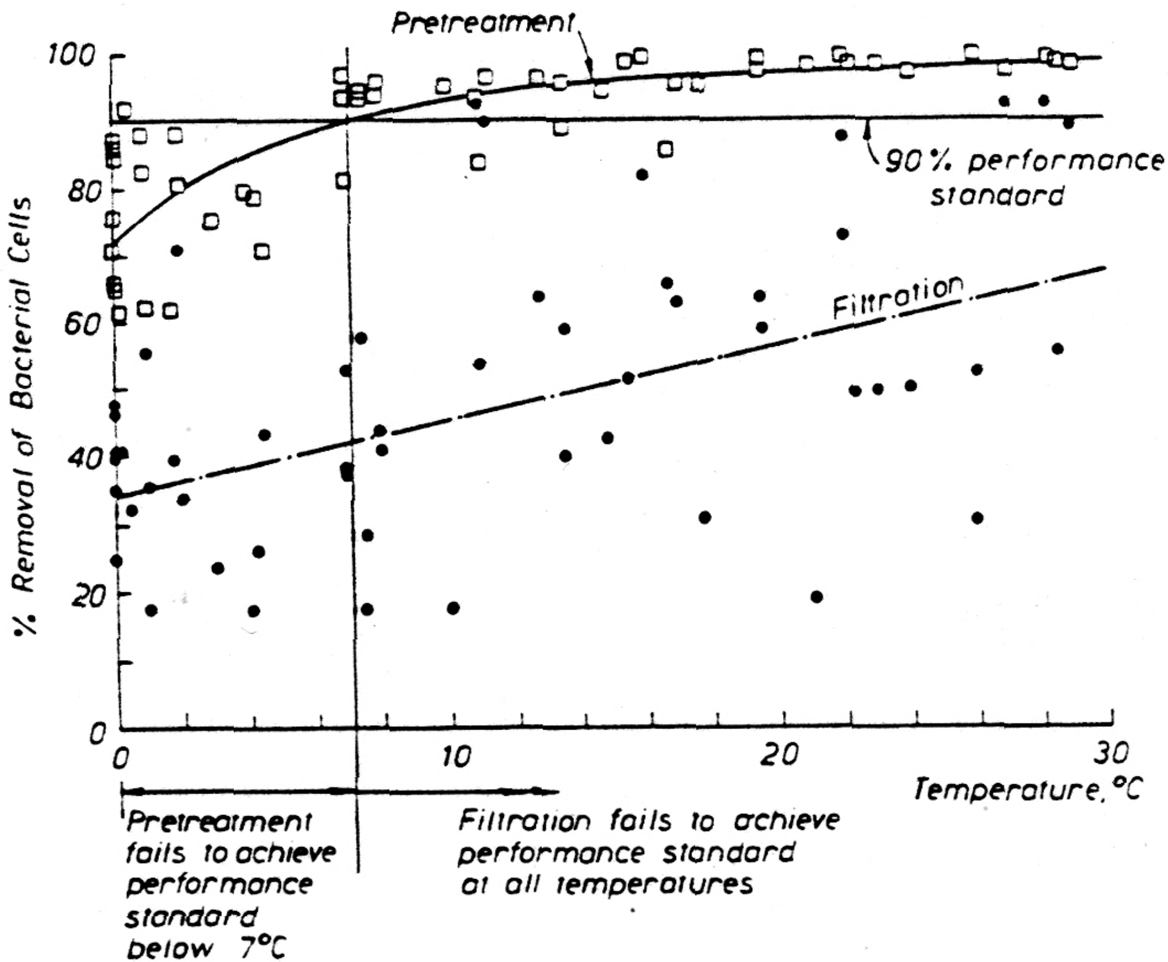

FIG. 2 -- Effect of temperature on removal of bacterial cells by pretreatment and filtration

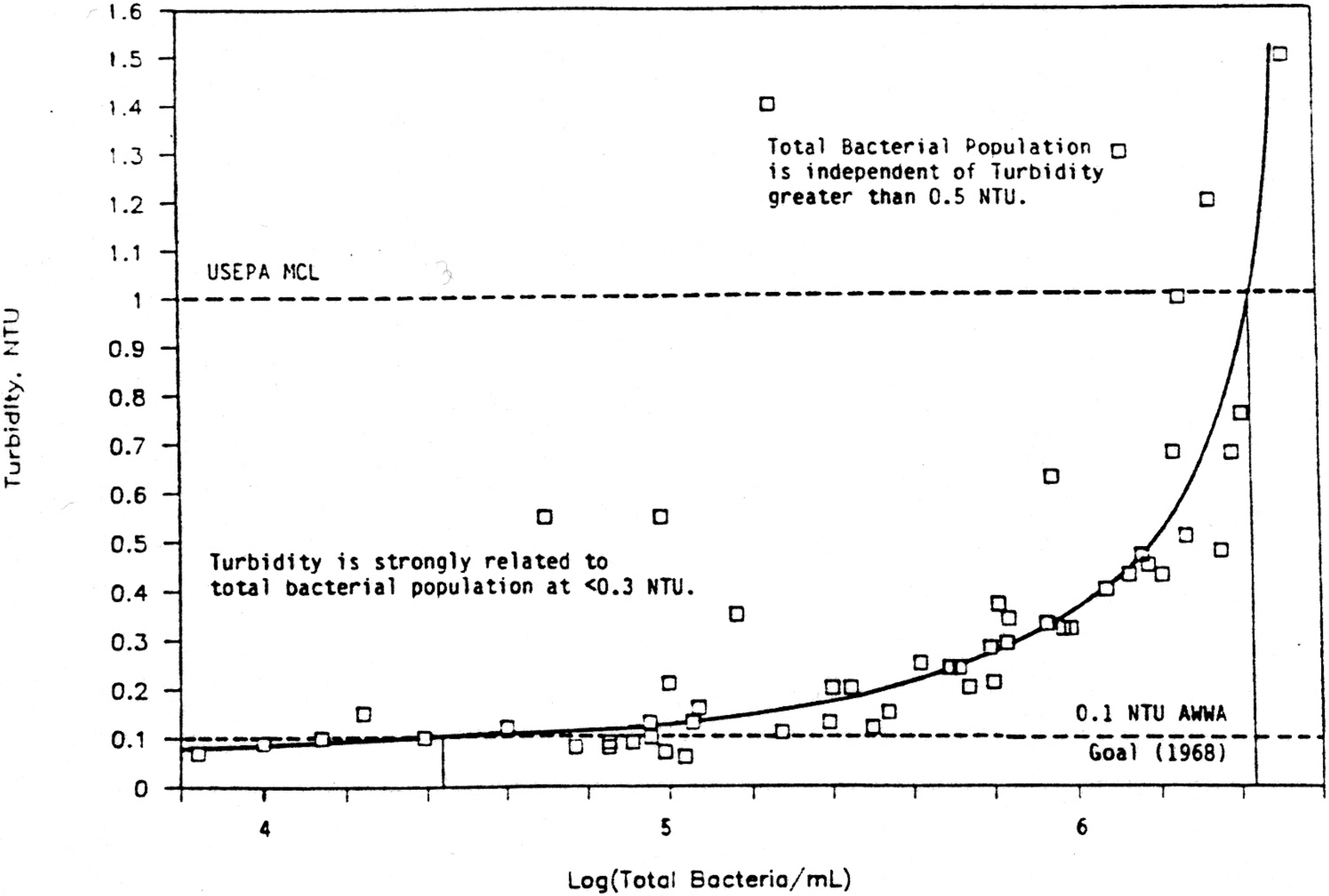

FIG. 3 -- Finished water turbidity versus total bacterial cell count

Table 1 -- Winter and summer average values for conventional and direct microscopic measures of water treatment plant performance

				Bacteria by Direct Count, 10^6 Cells/mL				Particles >3 μm,** Number/mL			
DATE/ SAMPLE (1)	Temperature °C (2)	Turbidity NTU (3)	HPC, CFU/mL* (4)	Total Bacteria (5)	Planktonic Bacteria (6)	Particle-Associated Bacteria (% of Total) (7)	Bacteria on Particles host to ≥5 Cells (% of Total) (8)	Total Particles (9)	Long Bacterial Rods (10)	Particles with ≥5 Attached Bacteria (11)	Algal Cells (12)
Averages for 16-day period: 12 FEB - 27 FEB 89											
Raw	1.0	18.8	37400	5.90	3.58	2.32(39.3)	0.67(11.4)	–	–	74400	14466
Settled	1.0	–	–	–	–	0.00	0.00	–	–	–	–
Filtered	1.0	0.22	144	0.95	0.95	0.00	0.00	1229	380	1.93	659
Clear Well	2.6	–	–	–	–	0.00	0.00	–	–	–	–
% Reduction		98.8	99.6	83.9	73.5	>99.9	>99	–	–	99.997	95.2
Dist. System	6.0	0.34(0.2-0.6)	63	0.91	0.91	0.00	0.00	1116	405	1.87	542
(Δ)		(+0.12)	(-81)	(-0.04)	(-0.04)	(0.00)	(0.00)	(-225)	(-20)	(-0.85)	(-213)
Averages for 17-day period: 31 AUG-16 SEPT 1989											
Raw	23.4	1200	629000	34.6	10.1	24.5(70.8)	7.38(21.3)	–	–	610000	28700
Settled	23.7	–	–	–	–	0.00	0.00	–	–	–	–
Filtered	23.7	0.15	170	0.24	0.24	0.00	0.00	186	113	0.31	25
Clear Well	24.4	–	–	–	–	0.00	0.00	–	–	–	–
% Reduction		99.99	99.97	99.3	97.6	>99.99	>99.9	–	–	99.99994	99.91
Dist. System	24.6	0.32	882	0.19	0.19	0.00	0.00	293	97	3.51	25
(Δ)		(+0.17)	(+712)	(-0.05)	(-0.05)	(0.00)	(0.00)	(+107)	(-16)	(+3.20)	(0)

Table 2 -- Percentage reduction in raw water particle counts (after Cleasby [47])

Type of Plant	Pretreatment	Filtration	Total Plant
Conventional	88	74	96.8
	88	93	99.1
	94	94	99.6
	70	99.4	99.8
	92	96	99.6
	84	93	98.9
	80	99.5	99.9
	(45)	99.9	98.9
	55	94	97.4
	87	92	98.9
	91	93	99.3
	95	92	99.6
		Mean	98.9
Rapid mix, detention, filtration	22	96	96.9
	10	99	99.1
	35	95	96.6
		Mean	97.6
Direct filtration	75	85	96.2
	80	94	98.8
	71	96	98.8
	(78)	95	90.2
		Mean	96.2
In-line filtration	24	89	91.8
Lime softening	52	95	97.0
	( ) Indicates increase		
		Grand Mean	97.8

Table 3 -- Percent reductions in particle numbers as a function of particle size at H.D. Taylor Water Treatment Plant, Corvallis, Oregon (after Cleasby, et al. [47])

	Particles/ml		
Geometric Mean Size, μm	Raw	Filtered	Percent Reduction
1.20	4,200	42	99.0
1.75	30,000	340	98.9
2.54	13,000	140	98.9
3.68	11,000	110	99.0
5.34	6,600	56	99.2
7.75	3,400	23	99.3
11.24	1,400	10	99.3
16.31	620	5.0	99.0
23.66	240	2.9	98.8
34.33	59	0.58	99.0
49.81	19	0.12	99.4
Total	71,000	740	99.0
Total >2.5 μm	12,000	98	99.2