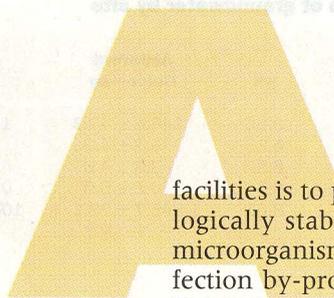# Biological stability
# of groundwater

A conventional treatment train with sand–anthracite columns provided better biological stability to finished water than a conventional train with GAC or membrane filtration.

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An important goal of water treatment facilities is to produce biologically stable water. Biologically stable water minimizes the regrowth of microorganisms and reduces the quantity of disinfection by-products in water distribution systems.[1] Bacterial regrowth is minimized because heterotrophic bacteria do not have a source of biodegradable carbon to oxidize. Disinfection by-products are reduced because there is a paucity of organic compounds to react with the disinfectant(s).[2,3]

Water that is classified as biologically unstable promotes the growth of microorganisms, which in turn affects water quality and causes taste, odor, color, and turbidity problems.[1] Another adverse effect of unstable water is the growth of microorganisms and opportunistic pathogens that increase the heterotrophic plate counts (HPC) and potentially increase the coliform counts, causing noncompliance problems with

Conventional (e.g., coagulation, flocculation, and filtration) or membrane filtration treatment trains were used to remove organic compounds from groundwater. For the conventional train with sand–anthracite columns, the assimilable organic carbon (AOC) of the groundwater was reduced from 349 ± 127 µg/L C to 54 ± 51 µg/L C. For the membrane filtration train, there was no statistical difference between the AOC of the raw water influent (388 ± 126 µg C) and that of the membrane permeate (334 ± 156 µg/L C), suggesting that this treatment produced biologically unstable water. Similar results were obtained using the heterotrophic growth response (HGR) method. Comparison of the biostability methods showed that HGR was positively correlated with AOC ($r = 0.52$; $P < 0.0001$; $n = 156$), indicating that AOC only partially explains the ability of heterotrophic bacteria to grow in water samples.

FIGURE 1 Schematic diagram of treatment trains and sampling sites

FIGURE 1   Schematic diagram of treatment trains and sampling sites
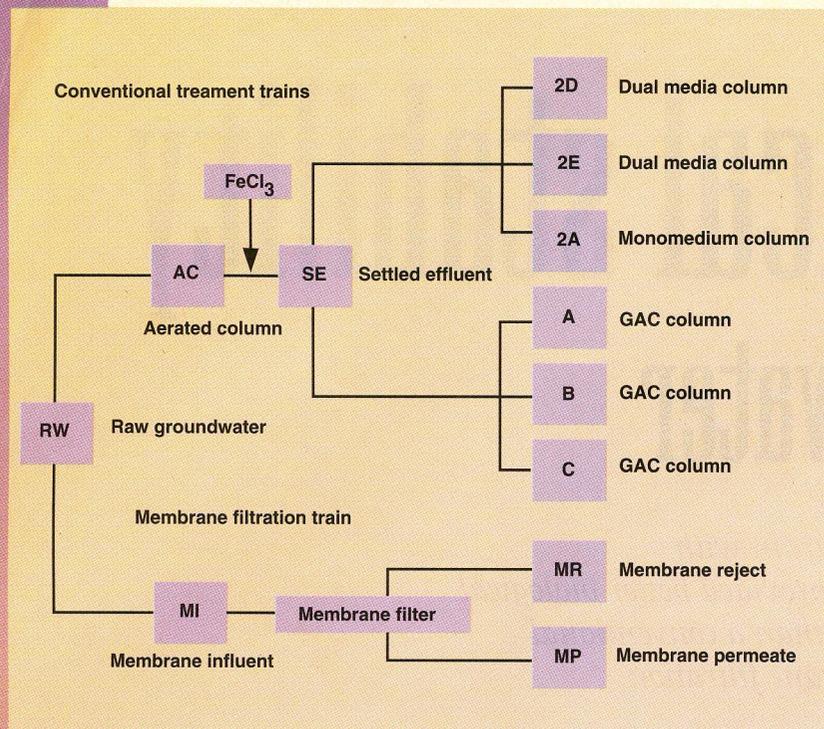
TABLE 1   Physicochemical properties of groundwater by site

Site	Turbidity ntu	pH	Apparent Color—cu	TOC mg/L
Raw groundwater	0.76 ± 0.08	8.85	146.8 ± 40.3	11.0 ± 0.1
Sand–anthracite columns	0.20 ± 0.06	6.16	6.2 ± 4.2	1.6 ± 0.3
GAC columns	0.20 ± 0.17	6.57	3.8 ± 4.6	1.3 ± 0.1
Membrane permeate	0.18 ± 0.05	8.77	0.0 ± 0.0	0.63 ± 0.1
Membrane reject effluent	3.97 ± 0.02	8.66	1,545.7 ± 22.1	104.2 ± 11.1

state regulatory agencies.[2] Water with assimilable organic carbon (AOC) levels exceeding 50 μg/L C can be associated with increased incidence of coliform bacteria[4] and therefore may pose a threat to public health. Bacterial growth that is fostered by the biological instability of water accelerates pipe corrosion,[2] leading to pipe deterioration and metal solubilization. Given the problems associated with biologically unstable water, treatment facilities require dependable methods to evaluate the quality of their finished water.

Several methods have been proposed to determine the biological stability of water.[3] The major philosophical difference among these methods is the inoculum source. For example, whereas the AOC method is based on the growth of *Pseudomonas fluorescens* strain P17[5] and *Spirillum* strain NOX,[6] heterotrophic growth response (HGR)[7] and biodegradable dissolved organic carbon (BDOC)[8] methods are based on the growth of naturally seeded bacteria. The advantage of using specific bacterial strains to determine AOC is that these strains can be used as standards for all types of water samples. It is difficult to compare measurements that depend on the growth of indigenous bacteria because the inoculum differs from one sample to the next.

Another important difference is that the AOC method is based on N_{max} of P17 and NOX in a water sample, whereas the HGR method is based on the difference between N_{max} and $N_{initial}$ of heterotrophic bacteria. The BDOC method provides information on the fraction of organic carbon that can be metabolized by bacteria within a period of a few days to a few months.[8] The AOC method differs from the BDOC method in that easily available organic carbon (>0.1 mg/L) is assayed over a shorter time frame.

This study focused on examining the biological stability of groundwater processed by conventional and membrane filtration methods and investigating the relationship between AOC and HGR. The groundwater source was a deep aquifer (1,640–3,281 ft [500–1000 m]) located in Southern California. Water was pumped from the source to a pilot plant where it was treated by either conventional (aeration, coagulation, and flocculation) or membrane filtration trains. High-molecular-weight humic compounds, which give the water a brown tinge, were removed by these treatments. Because different sites along the treatment trains varied in the amounts and kinds of biodegradable organic carbon as a function of the treatment process, the pilot plant provided a unique opportunity to monitor the biostability of groundwater.

Materials and methods
Bacterial strains and preparation of inocula.
Cultures of P17 and NOX* were streaked on R2A medium to ensure purity. A single colony of P17 and NOX was used to inoculate 100 mL of sodium acetate and sodium oxalate solutions, respectively. Inoculated solutions were incubated at 23°C until the organisms reached a stationary growth phase as determined by viable counts using the spread plate technique.[9] The solutions were then kept in the dark at 4°C. Purity of the stock solutions was determined with every assay.

Media and solutions. The sodium acetate solution consisted of 0.341 g/L anhydrous sodium acetate (100 mg/L acetate-C). The sodium oxalate solution consisted of 0.526 g/L sodium oxalate (100 mg/L oxalate-C). Both solutions were autoclaved at 115°C for 15 min and stored in 1-L Wheaton bottles at 4°C.

Mineral salts stock solution consisted of 0.171 g K_2HPO_4, 0.767 g NH_4Cl, and 1.444 g KNO_3 in 100 mL of AOC-free water. The stock solution was diluted 1:10 and pasteurized at 67°C for 1 h. One mL of the diluent was added to 500 mL of sample.

R2A* plates were prepared following the manufacturer's specifications. Phosphate-buffered water used for diluting P17, NOX, and heterotrophic bacteria was prepared following *Standard Methods*.[9]

All distilled water was deionized and double-distilled using a 6-L automatic distiller.† The distilled water stored in a 20-L glass container was referred to as AOC-free. All stock solutions and dilution buffers were prepared using this water.

Preparation of bioassay vessels and other glassware. Glassware was cleaned using the acid-wash method of van der Kooji et al.[5] Glassware cleaned by this method was referred to as AOC-free glassware. Plastic caps from the collection bottles were washed with laboratory detergent,‡ soaked in distilled water for 1 h, rinsed six times with distilled water, and then dry-autoclaved (121°C for 30 min). The AOC assay was conducted using 1-L Erlenmeyer flasks. The HGR assay was conducted using 2-L sample bottles.

To ensure that the AOC-free glassware was free of organic carbon, the authors evaluated the washing procedures. Six acid-washed flasks were filled with 500 mL of 10 g/L yeast extract and incubated for 12 h at 23°C. The flasks were then rinsed three times with sterile AOC-free water. Two flasks were cleaned by acid-washing followed by baking at 300°C for 8 h, two flasks were just baked for 8 h, and the remaining two flasks served as controls. The different cleaning procedures were evaluated by determining the N_{max} of P17 for each set of flasks. The experiment was replicated three times.

Enumeration of the bioassay organisms. Viable plate counts were determined by serially diluting the sample (if required) with sterile phosphate buffer.[9] One hundred μL of each diluent was spread on an R2A plate using a sterile glass rod. Each series of dilutions was plated in triplicate. The spread plates were inverted and incubated at 23°C for two days for P17, five days for NOX, and seven days for HPC. The dilution containing between 30 and 300 colonies was selected for enumeration.

Operating conditions and water sample collection sites. A schematic diagram of the treatment trains is shown in Figure 1. The coagulant dosage for the conventional treatment train was 120 mg/L $FeCl_3$. The hydraulic loading rate on the filters was 8 gpm/sq ft (5.4 mm/s). A flow feed of 40 gpm (2.5 L/s) was employed. For the membrane treatment train, the recovery rate was set at 90 percent. A flux of 20 gpd/sq ft (0.009 mm/s) and a feed pressure of 90 psi (621 kPa) were used. Approximately 1.5 mg/L of antiscalant foulant was used.

Water samples from different sites along the conventional and membrane treatment trains were collected between the months of July 1993 and July 1994. Collection bottles consisted of 2-L heat-resistant glass AOC-free bottles. Water samples were kept in ice until they were processed (usually within 6 h) in the laboratory.

The following sites were sampled along the conventional treatment train with sand–anthracite columns: raw groundwater (RW); aerated water column (AC); settled effluent (SE); dual-media column 2D (0.8-mm-diameter sand, 26.4-cm packing depth and 1.6-mm diameter anthracite, 2.4-m packing depth), column 2E (0.6-mm-diameter sand, 26.4-cm packing depth and 1.2-mm-diameter anthracite, 1.3-m packing depth), and monomedium column 2A (1.2-mm-diameter anthracite, 2.4-m packing depth) (Figure 1). The conventional treatment train with granular activated carbon (GAC) columns had the following sample sites: RW, AC, SE, and three various-sized GAC columns (column A§—0.9-mm

HGR* ($\log_{10}$/mL cfu) by Site						Level of Significance
RW (12)†	AC (13)	SE (12)	DM2D (12)	DM2E (11)	M2A (11)	
3.27†	1.35§	1.01§	0.31**	0.22**	0.55**	$P < 0.0001$

*HGR = $\log_{10}$ (HPC$_{(max)}$) – $\log_{10}$ (HPC$_{(min)}$)
†RW—raw water, AC—aeration column effluent, SE—clarified effluent after coagulation and sedimentation, DM2D—dual-media column D effluent, DM2E—dual-media column E effluent, M2A—monomedium column effluent, number of samples shown in parentheses
†,§,**Values within the same row followed by the same symbol are not statistically different (α=0.01) as determined by SNK test.

diameter media, 1.8-m packing depth; column B*— 0.65-mm-diameter media, 1.4-m packing depth; column C*—0.65-mm-diameter media, 1.5-m packing depth). The membrane filtration treatment train consisted of a membrane filter† (average pore size of 1,000 daltons [D] and a composition of sulfonated polyether sulfide). The following sites were sampled for the membrane filtration train: RW, membrane influent (MI), membrane reject (MR) effluent, and membrane permeate (MP).

HPC determination. HPC was determined by the spread-plate technique.[9] Each water sample was serially diluted tenfold, and 0.1 mL of water sample, the sample diluted with phosphate buffer, or both were plated on R2A plates in triplicate.

AOC assay. Two 1-L AOC-free Erlenmeyer flasks were filled with 500 mL of each water sample. The flasks were pasteurized at 67°C for 1 h using a water bath. After the water sample was cooled to ambient temperature, 200 µL of P17 stock inoculum was added to each flask to a final concentration of 3.8 ± 0.7 × 10^2/mL colony-forming units (cfu) ($\overline{x}$ ± standard deviation, n = 3). The flasks were incubated at 23°C until

maximum density (N_{max}) was achieved. Maximum density was defined as the point at which there was no further increase in the plate count of P17 as determined by two consecutive samplings using the spread-plate method.

Water from each flask was then filter-sterilized into another AOC-free Erlenmeyer flask using a 0.2-µm-pore-size prewashed cellulose acetate filter.‡ The filtered effluent was then inoculated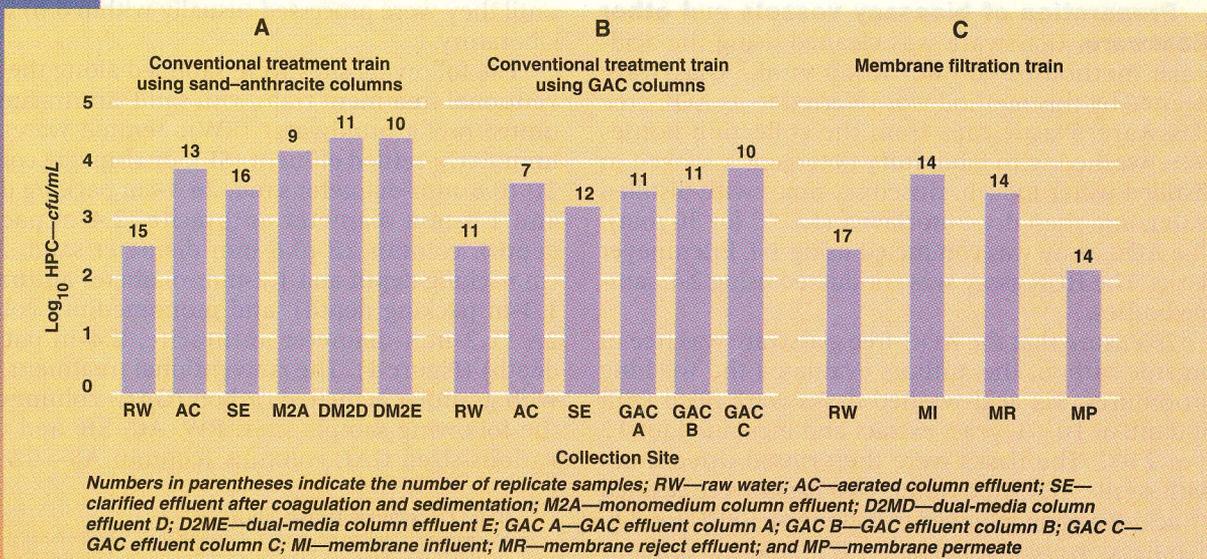 with 200 µL of NOX stock inoculum ((4.6 ± 1.8) × 10^2/mL cfu). Maximum density of NOX was defined as the point at which there was no change or decrease in cfu with incubation time. The mean of each duplicate sample was used to determine the AOC of the sample. The maximum AOC value of the samplings was used for the statistical analyses.

To ensure extraneous AOC was not added to the water samples by the cellulose acetate filters, the authors determined the volume of AOC-free water needed to rinse the organic compounds off the cellulose acetate filter. This was accomplished by testing the filter rinses with AOC-free water over the range of 500 to 2,000 mL and processing the filtrates by the AOC method. This experiment was conducted with duplicate flasks and replicated four times over the course of the study.

Yield experiments for P17 and NOX were conducted using RW and SE water samples. These experiments were conducted in the following manner: dupli-

FIGURE 2 HPC of water samples by collection site

Numbers in parentheses indicate the number of replicate samples; RW—raw water; AC—aerated column effluent; SE—clarified effluent after coagulation and sedimentation; M2A—monomedium column effluent; D2MD—dual-media column effluent D; D2ME—dual-media column effluent E; GAC A—GAC effluent column A; GAC B—GAC effluent column B; GAC C—GAC effluent column C; MI—membrane influent; MR—membrane reject effluent; and MP—membrane permeate

cate flasks were inoculated with 0, 100, 200, 400, and 800 µg/L acetate-C, pasteurized, and then inoculated with P17 following the procedure described earlier. The yield was determined by a regression plot between cfu and amount of acetate-C added to the sample. Yield for NOX was determined in the same manner except oxalate-C was used instead of acetate-C. Total AOC was defined as the sum of $AOC_{(P17)}$ and $AOC_{(NOX)}$ and was referred to as AOC.

To determine whether nitrogen, phosphate, or any other mineral limited the growth of P17, the authors conducted AOC determinations using two sets of flasks containing RW; one set was supplemented with mineral salts solution, and the other set was not. The same experiment was conducted using flasks containing high-performance liquid chromatography (HPLC) water supplement with 100 µg acetate-C with and without mineral salts solution.

HGR assay. HGR was defined as the mean logarithmic increase in HPC of a 1-L water sample after a predetermined incubation time at 23°C.[7] The equation describing this relationship is $HGR = \log_{10}(HPC_{(max)}) - \log_{10}(HPC_{(min)})$. The amount of incubation time needed to obtain maximum HPC was determined by monitoring the HPC of water samples on a weekly basis for eight weeks.

Data analysis. Correlation analysis (Pearson product-moment) was used to determine the degree of association between variables. Linear regressions were used to estimate the relationship of one variable to another.[10] An analysis of variance (ANOVA) was used to determine the source of variability in the experimental data. The Student-Newman-Keuls (SNK) test was employed to determine whether the difference between any two means in a set of means was significant.[11] All analyses were conducted using the Statistical Analysis System* on a VAX/VMS computer.

Results and discussion

The physicochemical properties of the RW and the finished products of the conventional and membrane filtration trains are shown in Table 1. A significant change in turbidity, apparent color, and total organic carbon (TOC) ($P < 0.0001$) of the groundwater as a function of both treatment trains suggests that the organic carbon load of the groundwater was reduced.

There was no difference in the N_{max} of P17 grown in flasks cleaned by acid-washing and baking at

TABLE 3   **HGR as a function of conventional treatment of groundwater using GAC columns**

HGR* ($\log_{10}$/mL cfu) by Site						Level of Significance
RW (12)†	AC (13)	SE (11)	GAC A (11)	GAC B (11)	GAC C (8)	
3.34‡	1.71§	1.55§	0.90**	0.94**	0.71**	$P < 0.0001$

*HGR = $\log_{10}$ ($HPC_{(max)}$) − $\log_{10}$ ($HPC_{(min)}$)
†RW—raw water; AC—aeration column effluent, SE—clarified effluent after coagulation and sedimentation, GAC A—GAC column A effluent, GAC B—GAC column B effluent, GAC C—GAC column C effluent, number of samples shown in parentheses
‡,§,**Values within the same row followed by the same symbol are not statistically different (α = 0.01) as determined by SNK test.

TABLE 4   **HGR as a function of membrane treatment of groundwater**

HGR* ($\log_{10}$/mL cfu by Site				Level of Significance
RW (16)†	MI (12)	MR (11)	MP (13)	
3.35‡	1.73§	2.12§	3.04‡	$P < 0.0001$

*HGR = $\log_{10}$ ($HPC_{(max)}$) − $\log_{10}$ ($HPC_{(min)}$)
†RW—raw water, MI—membrane influent, MR—membrane reject effluent, MP—membrane permeate effluent, number of samples shown in parentheses.
‡,§Values within the same row followed by the same symbol are not statistically different (α = 0.01) as determined by SNK test.

300°C for 8 h and the N_{max} of P17 grown in flasks baked at the same temperature and time ($P < 0.1159$, $n = 3$). In general, the positive control flasks had 2 logs more cfu per mL ($\bar{x} = 3.82$, $n = 9$) than either the acid-washed ($\bar{x} = 0.62$, $n = 9$) or baked ($\bar{x} = 1.20$, $n = 9$) flasks ($P < 0.0009$). Presumably, residual organic carbon attached to the surface of the glass of the positive control flask provided a carbon source for P17. Flasks cleaned by acid-washing, baking at 300°C, or both, effectively removed organic carbon that could possibly interfere with the AOC assay. Bacause of these results, glassware for the HGR and AOC assays was prepared by baking at 300°C for 8 h.

The cellulose acetate membrane filters were rinsed with AOC-free water before they were used to filter out P17 from the water samples. AOC-free water removed organic carbon compounds in the membrane that could be rinsed into the water sample and interfere with $AOC_{(NOX)}$. For example, when the filter was rinsed with 500 mL, the AOC of the effluent water was 46 ± 10 µg/L C. An additional rinsing with 500 mL of AOC-free water reduced the $AOC_{(NOX)}$ of the filter to less than 2 ± 3 µg/L C. As a result, all cellulose-acetate filters were rinsed with 1,000 mL of AOC-free water before they were used to filter water samples containing P17.

Yield experiments for P17 and NOX were similar to those obtained by van der Kooij et al.[5,6] These findings suggest that the concentration of coagulant (FeCl$_3$) used to clarify the water had no effect on the growth of P17. It is important to consider the effects of coagulates on the growth of P17, because

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*SAS Institute Inc., Cary, N.C.

FIGURE 3 Growth of heterotrophic bacteria in 1 L of colored groundwater

Number of samples—9
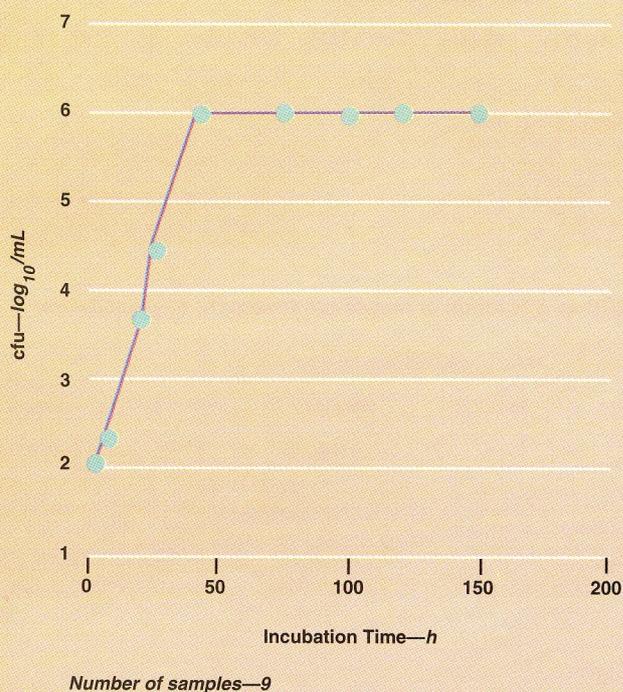
elevated levels of coagulates in a water sample can inhibit the growth of P17.[12]

There were no significant differences in N_{max} of P17 grown in RW supplemented with and without mineral salts solution (1.76×10^5 and 1.92×10^5/mL cfu ($n = 3$), respectively) suggesting that nitrogen, phosphate, and other minerals were not limiting.

HPC assay. Samples collected from RW and MP had much lower HPC values than samples collected from other locations (Figure 2). There was a significant increase in HPC for the aerated groundwater (AC) samples. Presumably, aerating the water changes the redox potential of the water, making the organic carbon available to heterotrophic bacteria.

Low HPCs were obtained from the MP, presumably because the pores of the membrane retained particles that were >1,000 D, preventing heterotrophic bacteria from passing through the membrane.

To determine whether differences in HPC by site were a function of the actual collection sites, the collection times of each treatment train, or both, the authors analyzed the data using a linear model that considered the effect of site and time on the observed variance. For the conventional train with sand–anthracite columns, the ANOVA showed that variance in the model was explained by site ($P < 0.0001$) and by time ($P < 0.0007$). Similarly, for the conventional train with GAC columns, variance in the model was attributed to site ($P < 0.0001$) and by

time ($P < 0.0073$). Given the experimental design of this project, it might be expected that most of the variability was due to collection site. The finding that some variability in the conventional treatment train was explained by time suggests that the treatment train was at dynamic rather than static equilibrium. Presumably, the source of this variability is the operating conditions of the pilot plant.

For the membrane train, variance in the model was explained by site ($P < 0.0001$) but not by time ($P < 0.0881$). Variance by time, although not statistically significant, could be observed and was likely attributable to operating conditions such as length of time the filter was in service or when it was last backflushed.

HGR assay. To determine the amount of time required for water samples to reach $HPC_{(max)}$, HPC was conducted on nine water samples on a weekly basis for seven weeks. An ANOVA showed the HPC for all samples increased with incubation time ($P < 0.0001$); however, there was no statistical difference in HPC after seven days of incubation ($\alpha = 0.01$) (Figure 3). Because of this result, HPC was determined immediately after sample collection and after seven and fourteen days of incubation.

An ANOVA revealed that for all treatments there was a significant difference in HGR by collection site. For the conventional treatment train with sand–anthracite columns, HGR decreased as the groundwater was processed, particularly after the water was aerated, clarified, and passed through the columns (Table 2). There was apparently no difference in the HGR of water effluents collected from the different sand–anthracite columns. This result is particularly interesting, given that the media of the columns differed in particle size, composition, and packing depth.

The conventional treatment train with GAC columns (Table 3) produced similar results, i.e., there was a reduction in HGR as the water was processed through the columns. Again, the HGR of effluents collected from the different GAC columns did not differ significantly, even though the columns differed in particle size, composition, and packing depth.

For the membrane treatment train, HGR of the RW was not significantly different from MP, indicating that biodegradable carbon was not sufficiently removed from groundwater (Table 4).

Analysis of the model used for the ANOVA showed that variance in the model for the conventional train with sand–anthracite columns was explained by site ($P < 0.0001$) and time ($P < 0.0007$). For the conventional train with GAC columns, most of the variance in the model was attributed to site ($P < 0.0001$) rather than time ($P < 0.0537$). Similarly, for the membrane

AOC as Determined By:	AOC ± SD (µg/L C) by Site						Level of Significance
	RW (10)*	AC (11)	SE (12)	DM2D (12)	DM2E (12)	M2A (11)	
P17	214 ± 129†	245 ± 195†	42 ± 40‡	35 ± 43‡	33 ± 28‡	39 ± 47‡	$P < 0.0001$
NOX	135 ± 42.1†	131 ± 60†	23 ± 16‡	24 ± 19‡	2 ± 16‡	15 ± 10‡	$P < 0.0001$
P17 + NOX	349 ± 127†	375 ± 225†	64 ± 40‡	51 ± 34‡	53 ± 40‡	54 ± 50‡	$P < 0.0001$

*RW—raw water, AC—aeration column effluent, SE—clarified effluent after coagulation and sedimentation, DM2D—dual-media column D effluent, DM2E—dual-media column E effluent, M2A—monomedium column effluent, number of samples shown in parentheses
†,‡Values within the same row followed by the same symbol are not statistically different ($\alpha = 0.01$) as determined by SNK test.

AOC as Determined By:	AOC ± SD (µg/L C) by Site						Level of Significance
	RW (11)*	AC (4)	SE (9)	GAG A (11)	GAC B (11)	GAC C (8)	
P17	304 ± 177†	294 ± 162†	89 ± 96‡	132 ± 67†,‡	205 ± 152†,‡	155 ± 131†,‡	$P < 0.336$
NOX	91 ± 49†	76 ± 56†	36 ± 16†	39 ± 25†	41 ± 20†	27 ± 11†	$P < 0.0002$
P17 + NOX	396 ± 201†,‡	437 ± 154†	147 ± 99‡	177 ± 67‡	246 ± 166‡	182 ± 128‡	$P < 0.0053$

*RW—raw water, AC—aeration column effluent, SE—clarified effluent after coagulation and sedimentation, GAC A—GAC column A effluent, GAC B—GAC column B effluent, GAC C—GAC column C effluent, number of samples shown in parentheses
†,‡Values within the same row followed by the same symbol are not statistically different ($\alpha = 0.01$) as determined by SNK test.

train, variance in the model was attributed to site ($P < 0.0001$) and time ($P < 0.0786$). These findings agree with the HPC analysis in that variability in the data was attributable to not only site differences but also sampling time differences.

AOC assay. *Modifications to the van der Kooij method.* It is important to recognize that the AOC method proposed by van der Kooij et al[5] was modified to provide accurate results for this study. The van der Kooij et al method[5] requires that the N_{max} of P17 be determined on days 3, 4, and 5. In this study, the growth of P17 in RW samples was monitored using the recommended inoculum concentration;

The lower correlation between HGR and AOC suggests that AOC only partially describes the ability of heterotrophic bacteria to use the available carbon in water samples.

viable counts on days 3, 4, and 5 were below the detectable limit of the spread-plate method, suggesting that the groundwater samples contained low AOC values. However, when the water samples were incubated for 15 to 25 days, the viable count of P17 increased to approximately 10^6/mL cfu (AOC = 250–300 µg/L). This can be explained by the fact that the high pH (8.3) of the groundwater affected the growth of P17. Even when the pH of the groundwater was adjusted to pH 7.0, however, N_{max} was not obtained until 15 to 20 days of incubation,

which suggests that other factors are influencing the growth of P17.

The slow growth of P17 in RW samples demands further examination, given the fact that heterotrophic bacteria reach N_{max} in less than two days (Figure 3). Presumably, P17 requires time to adapt to carbon present in the groundwater before it can utilize it. The importance of this finding is that previous AOC determinations by other investigators may have significantly underestimated the amount of biodegradable carbon in groundwater by not allowing P17 to reach N_{max} if a five-day maximum was chosen without testing to obtain the true N_{max}. The accuracy of new, rapid AOC methods, such as those of LeChevallier et al,[13] may be compromised if the growth of P17 in groundwater is not accounted for.

Comparison of AOC among sample sites. An ANOVA revealed that for all treatments, AOC differed significantly by collection site. For the conventional train with sand–anthracite columns, AOC of the water decreased after the groundwater was aerated; presumably, this decrease came about because indigenous bacteria utilized the readily degradable carbon, resulting in an overall increase in HPC. There was no significant reduction in AOC as the water was passed through the sand–anthracite columns (Table 5). These findings were consistent for AOC calculated using P17 alone ($AOC_{(P17)}$) and NOX alone ($AOC_{(NOX)}$).

TABLE 7 AOC as a function of membrane filtration treatment of groundwater

AOC as Determined By:	AOC ± SD (μg/L C) by Site				Level of Significance
	RW (15)*	MI (14)	MR (15)	MP (14)	
P17	254 ± 129	212 ± 100	289 ± 129	288 ± 145	$P < 0.3363$
NOX	134 ± 80‡	129 ± 77‡	300 ± 121†	45 ± 30‡	$P < 0.0001$
P17 + NOX	388 ± 126‡	342 ± 97‡	591 ± 191†	334 ± 156‡	$P < 0.0001$

*RW—raw water, MI—membrane influent, MR—membrane reject effluent, MP—membrane permeate effluent, number of samples shown in parentheses.
†,‡Values within the same row followed by the same symbol are not statistically different ($\alpha = 0.01$) as determined by SNK test.

The conventional train with GAC columns showed a significant difference in AOC following clarification and sedimentation (Table 6). When the clarified water passed through the GAC columns, however, $AOC_{(P17)}$ increased, presumably because biodegradable organic carbon leached into the effluent in the form of small particles.[14,15] Although an ANOVA showed that $AOC_{(NOX)}$ values differed significantly by collection site, it was difficult to determine the source of this variation. The SNK analysis showed that the $AOC_{(NOX)}$ of water samples decreased slightly (but not significantly) following clarification.

For the membrane train, AOC differed significantly by collection site (Table 7). This difference was attributed to $AOC_{(NOX)}$, however, because $AOC_{(P17)}$ did not differ by collection site. Presumably, the membrane selectively removes organic carbon compounds that can be utilized by NOX but not P17. These findings were supported by the fact that NOX utilized three times more AOC in the MR than in the RW.

The ANOVA for AOC showed that variance in the model for the conventional train with sand–anthracite columns was explained by site ($P < 0.0001$) and time ($P < 0.0002$). Again, these findings suggest that this treatment train was at dynamic rather than static equilibrium. For the conventional train with GAC columns, variance in the model was attributed to site ($P < 0.0007$) rather than time ($P < 0.1195$). Similarly, for the membrane train, variance in the model was attributed to site ($P < 0.0001$) rather than time ($P < 0.1935$).

The ANOVA for $AOC_{(P17)}$ showed that variance in the model for the conventional train with sand–anthracite columns was explained by site ($P < 0.0001$) and time ($P < 0.0011$). For the conventional train with GAC columns, variance in the model was attributed to site ($P < 0.0007$) rather than time ($P < 0.1195$). For the membrane train, variance in the model was attributed to time ($P < 0.0279$) rather than site ($P < 0.1675$), suggesting that there was no change in

$AOC_{(P17)}$ as the water passed through the filter.

The ANOVA for $AOC_{(NOX)}$ showed that variance in the model for the conventional train with sand–anthracite columns was explained by site ($P < 0.0001$) and time ($P < 0.0031$). The conventional train with GAC columns yielded similar results, i.e., variance in the model was attributed to site ($P < 0.0001$) and time ($P < 0.0029$). For the membrane train, variance in the model was attributed to site ($P < 0.0001$) and time ($P < 0.0003$).

Relationships among the biostability measurements. Pearson correlation analysis revealed an inverse correlation between HGR and $\log_{10}$ HPC (Table 8). This was attributed to the fact that samples having low HPC values tended to produce higher HGR values than samples having high HPC values. The lower correlation between HGR and AOC suggests that AOC only partially describes the ability of heterotrophic bacteria to use the available carbon in water samples. The authors attribute this finding to the fact that the HGR is based on the maximum growth of many types of heterotrophic bacteria, whereas AOC determination is based on the maximum growth of P17 and NOX, which are surrogates for the presence of amino acids, carboxylic acids, carbohydrates, and polyalcohols and aromatic acids.[5,16] Moreover, these findings suggest that HPC is a good representation of those bacteria that are able to utilize the available carbon in water samples.

Relationships between biostability measurements and abiotic parameters. The pH of water samples was correlated with HPC, HGR, and AOC

Water that is classified as biologically unstable promotes the growth of microorganisms, which in turn affects water quality and causes taste, odor, color, and turbidity problems.

measurements. With the exception of HPC, these correlations can be explained by the fact that RW, AC, MI, MR, and MP water samples have high pH, HGR, and AOC values, whereas clarified (SE) samples have low pH, HGR, and AOC values. Presumably, the pH of the water samples was reduced because the addition of $FeCl_3$ caused the formation of $Fe(OH)_3$ flocculant, which coprecipitates organic carbon compounds. This is supported by the fact that the TOC of the RW was reduced by 87 percent after it was clarified (Table 1).

TABLE 8   Correlation coefficients among AOC, HGR, and $\log_{10}$ HPC

Measure	$\log_{10}$HPC	HGR	AOC$_{(P17)}$	AOC$_{(NOX)}$
			*r**	
HGR	−0.89 (199; $P < 0.0001$)			
AOC$_{(P17)}$	−0.35 (182; $P < 0.0001$)	0.43 (179; $P < 0.0001$)		
AOC$_{(NOX)}$	−0.15 (159; $P < 0.0513$)	0.37 (156; $P < 0.0001$)	0.36 (159; $P < 0.0001$)	
AOC$_{(total)}$	−0.34 (159; $P < 0.0001$)	0.52 (156; $P < 0.0001$)	0.88 (159; $P < 0.0001$)	0.76 (159; $P < 0.0001$)

*Number of samples, level of significance shown in parentheses

TABLE 9   Correlation coefficients between biostability measurements and abiotic parameters

Measure	$\log_{10}$HPC$_{(t = 0)}$	HGR	AOC$_{(P17)}$	AOC$_{(NOX)}$	AOC$_{(total)}$
			*r**		
pH	−0.73 (74; $P < 0.0001$)	0.85 (72; $P < 0.0001$)	0.56 (74; $P < 0.0001$)	0.56 (59; $P < 0.0001$)	0.63 (59; $P < 0.0001$)
Turbidity	−0.39 (80; $P < 0.0004$)	0.51 (79; $P < 0.0001$)	0.14 (80; $P < 0.2132$)	0.79 (65; $P < 0.0001$)	0.42 (65; $P < 0.0004$)
Apparent color	−0.47 (69; $P < 0.0001$)	0.55 (67; $P < 0.0001$)	0.40 (69; $P < 0.0006$)	0.93 (57; $P < 0.0001$)	0.71 (57; $P < 0.0001$)
TOC	−0.42 (71; $P < 0.0003$)	0.47 (69; $P < 0.0001$)	0.33 (71; $P < 0.0049$)	0.96 (61; $P < 0.0001$)	0.68 (61; $P < 0.0001$)

*Number of samples, level of significance shown in parentheses

As shown in Table 9, turbidity, collapsed by site and time, was positively correlated with HGR and AOC$_{(NOX)}$. Presumably, turbid water contains more particulate matter, and organic matter adsorbed to particulate matter provides higher HGR and AOC$_{(NOX)}$ values than water having low amounts of turbidity. It is difficult to explain why AOC$_{(P17)}$ did not increase with turbidity.

Apparent color and TOC of the water samples were positively correlated with HGR and AOC measurements, implying that organic matter associated with color and TOC provides carbon sources for microorganisms. Strong positive correlations existed among turbidity, apparent color, and TOC and AOC$_{(NOX)}$. It may be possible that low-molecular-weight substrates such as carboxylic acids were associated with turbidity and apparent color of the water. Perhaps low-molecular-weight substrates provide a carbon source for NOX that could not be utilized by P17. More experiments are needed to clarify these findings.

One of the problems with monitoring the biological stability of water is that currently, no standard method exists for measuring low concentrations of biodegradable organic carbon.[2] In this study, the authors compared AOC and HGR methods to determine which method(s) could be used as an index to the biostability of groundwater containing low amounts of biodegradable carbon. The results showed that AOC and HGR methods could be used as indicators of the biostability of treated groundwater and that these measures were positively correlated with one another.

Biostability of treated groundwater. *Conventional treatment.* Treatment of groundwater by conventional methods—coagulation, sedimentation with sand–anthracite, and GAC columns—significantly decreased AOC and HGR values of the groundwater. Presumably, coagulation and sedimentation decreased AOC in the SE because biomass components were removed with the coagulant.[17] Although the influent of sand–anthracite columns had higher AOC values than the effluent, these differences were not significant.

For the GAC columns, AOC of the influent water was much less than the effluent. Filtration velocity of the GAC columns,[8] other factors such as maintenance of the GAC columns, or a combination of these factors may be responsible for differences between influent and effluent AOC values. The most likely explanation for the increase in AOC of the effluent samples is that carbon fines from the GAC columns were present in the water samples.[15,16] Presumably, carbon fines laden with adsorbed carbon provided a nutrient source for P17 and NOX. The fact that the HGRs of the GAC columns were higher than those of the sand–anthracite columns also supports this hypothesis. Pasteurization of the water sample may also release more carbon. Further studies are needed to support this hypothesis.

Membrane filtration treatment. There was no difference between AOC values of the influent and the effluent for the membrane filtration train. Similar results were obtained for HGR values. These findings imply that the membrane filtration treatment train does not produce biologically stable water. Because AOC values of the membrane filtration effluent were in excess of 300 µg/L C, bacterial regrowth would likely occur in the water distribution system. Undoubtedly, water quality will be affected, given that AOC values as low as 50–100 µg/L C can support 10^8 to 10^9/L cfu of heterotrophic bacteria[14] and an excess AOC$_{(P17)}$ of 50 µg/L C may promote the

growth of coliform bacteria.[4] Because many of these bacteria are known opportunistic pathogens, elevated HPC and coliform counts pose a threat to public health. Furthermore, heterotrophic bacteria provide a carbon source for undesirable organisms such as amoebas, nematodes, and crustaceans,[18] and some of these organisms can harbor bacterial pathogens such as *Legionella*.[19] Clearly, implementating a membrane filtration train as the treatment process without considering the biostability of the product water could have potentially harmful effects on water quality and public health, creating a greater reliance on disinfection to control bacterial regrowth in water distribution systems.

Summary

Conventional treatment train with sand–anthracite columns provided better biological stability to finished water than the conventional treatment train with GAC columns. The membrane filtration train produced biologically unstable water. AOC and HGR provided adequate methods for evaluating the biostability of groundwater.

Acknowledgment

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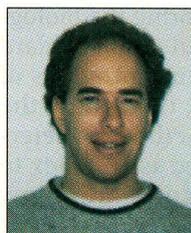
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