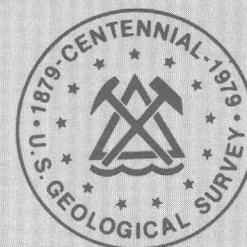


# Microbiological Effects of Recharging the Magothy Aquifer, Bay Park, New York, With Tertiary-Treated Sewage

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GEOLOGICAL SURVEY PROFESSIONAL PAPER 751-E

*Prepared in cooperation with the  
Nassau County Department  
of Public Works*





# Microbiological Effects of Recharging the Magothy Aquifer, Bay Park, New York, With Tertiary-Treated Sewage

By GARRY G. EHRLICH, HENRY F. H. KU, JOHN VECCHIOLI, *and* THEODORE  
A. EHLKE

DEEP-WELL ARTIFICIAL RECHARGE EXPERIMENTS  
AT BAY PARK, LONG ISLAND, NEW YORK

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**UNITED STATES DEPARTMENT OF THE INTERIOR**

**CECIL D. ANDRUS, *Secretary***

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## CONVERSION FACTORS

Metric unit	Inch-Pound equivalent	Metric unit	Inch-Pound equivalent
<b>Length</b>		<b>Specific combinations—Continued</b>	
millimeter (mm)	= 0.03937 inch (in)	liter per second (L/s)	= .0353 cubic foot per second
meter (m)	= 3.28 feet (ft)	cubic meter per second per square kilometer [(m <sup>3</sup> /s)/km <sup>2</sup> ]	= 91.47 cubic feet per second per square mile [(ft <sup>3</sup> /s)/mi <sup>2</sup> ]
kilometer (km)	= .62 mile (mi)	meter per day (m/d)	= 3.28 feet per day (hydraulic conductivity) (ft/d)
<b>Area</b>		meter per kilometer (m/km)	= 5.28 feet per mile (ft/mi)
square meter (m <sup>2</sup> )	= 10.76 square feet (ft <sup>2</sup> )	kilometer per hour (km/h)	= .9113 foot per second (ft/s)
square kilometer (km <sup>2</sup> )	= .386 square mile (mi <sup>2</sup> )	meter per second (m/s)	= 3.28 feet per second
hectare (ha)	= 2.47 acres	meter squared per day (m <sup>2</sup> /d)	= 10.764 feet squared per day (ft <sup>2</sup> /d) (transmissivity)
<b>Volume</b>		cubic meter per second (m <sup>3</sup> /s)	= 22.826 million gallons per day (Mgal/d)
cubic centimeter (cm <sup>3</sup> )	= 0.061 cubic inch (in <sup>3</sup> )	cubic meter per minute (m <sup>3</sup> /min)	= 264.2 gallons per minute (gal/min)
liter (L)	= 61.03 cubic inches	liter per second (L/s)	= 15.85 gallons per minute
cubic meter (m <sup>3</sup> )	= 35.31 cubic feet (ft <sup>3</sup> )	liter per second per meter [(L/s)/m]	= 4.83 gallons per minute per foot [(gal/min)/ft]
cubic meter	= .00081 acre-foot (acre-ft)	kilometer per hour (km/h)	= .62 mile per hour (mi/h)
cubic hectometer (hm <sup>3</sup> )	= 810.7 acre-feet	meter per second (m/s)	= 2.237 miles per hour
liter	= 2.113 pints (pt)	gram per cubic centimeter (g/cm <sup>3</sup> )	= 62.43 pounds per cubic foot (lb/ft <sup>3</sup> )
liter	= 1.06 quarts (qt)	gram per square centimeter (g/cm <sup>2</sup> )	= 2.048 pounds per square foot (lb/ft <sup>2</sup> )
liter	= .26 gallon (gal)	gram per square centimeter	= .0142 pound per square inch (lb/in <sup>2</sup> )
cubic meter	= .00026 million gallons (Mgal or 10 <sup>6</sup> gal)	<b>Temperature</b>	
cubic meter	= 6.290 barrels (bbl) (1 bbl = 42 gal)	degree Celsius (°C)	= 1.8 degrees Fahrenheit (°F)
<b>Weight</b>		degrees Celsius (temperature)	= [(1.8 × °C) + 32] degrees Fahrenheit
gram (g)	= 0.035 ounce, avoirdupois (oz avdp)		
gram	= .0022 pound, avoirdupois (lb avdp)		
metric tons (t)	= 1.102 tons, short (2,000 lb)		
metric tons	= 0.9842 ton, long (2,240 lb)		
<b>Specific combinations</b>			
kilogram per square centimeter (kg/cm <sup>2</sup> )	= 0.96 atmosphere (atm)		
kilogram per square centimeter	= .98 bar (0.9869 atm)		
cubic meter per second (m <sup>3</sup> /s)	= 35.3 cubic feet per second (ft <sup>3</sup> /s)		

## MICROBIOLOGICAL EFFECTS OF RECHARGING THE MAGOTHY AQUIFER, BAY PARK, NEW YORK, WITH TERTIARY-TREATED SEWAGE

By GARRY G. EHRLICH, HENRY F. H. KU, JOHN VECCHIOLI, and THEODORE A. EHLKE

### ABSTRACT

Injection of highly treated sewage (reclaimed water) into a sand aquifer on Long Island, N.Y., stimulated microbial growth near the well screen. Chlorination of the injectant to 2.5 milligrams per liter suppressed microbial growth to the extent that it did not contribute significantly to head buildup during injection. In the absence of chlorine, microbial growth caused extensive well clogging in a zone immediately adjacent to the well screen.

During a resting period of several days between injection and well redevelopment, the inhibitory effect of chlorine dissipated and microbial growth ensued. The clogging mat at the well/aquifer interface was loosened during this period, probably as a result of microbial activity.

Little microbial activity was noted in the aquifer beyond 20 feet from the well screen; this activity probably resulted from small amounts of biotransformable substances not completely filtered out of the injectant by the aquifer materials.

Movement of bacteria from the injection well into the aquifer was not extensive. In one test, in which injected water had substantial total-coliform, fecal-coliform, and fecal-streptococcal densities, no fecal-coliform or fecal-streptococcal bacteria, and only nominal total-coliform bacteria, were found in water from an observation well 20 feet from the point of injection.

### INTRODUCTION

Continuing population growth in Nassau County, a suburb of New York City, has been accompanied by increased withdrawals of ground water, the only source of public-supply water for the area.

Net withdrawals from the ground-water system have resulted in declining ground-water levels and decreased streamflow (Franke, 1968) and in local landward movement of salty ground water (Luszczynski and Swarzenski, 1966; Cohen and Kimmel, 1970). Net withdrawals are expected to increase with the rising population, per-capita water use, and percentage of population served by sewers. These growth factors indicate a water-supply deficit of between 71.1 and 91.1 Mgal/d by 1990 (Temporary State Commission on Water Supply Needs of Southeastern New York, 1972, p. 142-144).

### PURPOSE AND SCOPE

Artificial recharge of the ground-water reservoir with water reclaimed from sewage is one of several alterna-

tives under examination by Nassau County to meet the anticipated water-supply deficit (Peters and Rose, 1968). From 1968 to 1973, the Nassau County Department of Public Works operated a pilot advanced waste-treatment plant at Bay Park, N.Y., near the south shore of Nassau County (fig. 1). Reclaimed water from this plant was used in a series of 13 deep-well artificial-recharge experiments made by the U.S. Geological Survey, in cooperation with the Nassau County Department of Public Works, to provide some of the scientific and engineering data needed to evaluate (1) the degree and causes of well clogging that resulted from injection of reclaimed water, and remedies for the clogging, and (2) the geochemical compatibility of the reclaimed water with the aquifer.

This report, the fifth chapter in Professional Paper 751, "Deep-Well Artificial-Recharge Experiments at Bay Park, Long Island, New York," summarizes the results of microbiological investigations carried out during those tests. The geochemical aspects of injection of reclaimed water are described in the previous chapter (Ragone, 1977).

Microbial activity associated with artificial recharge may have three principal effects: (1) bacteria may grow near the well screen and cause a gradual reduction of aquifer hydraulic conductivity in the immediate vicinity of the well; (2) microbial activity may produce substances that adversely affect the taste and odor of injected water later recovered from the aquifer; and (3) pathogenic organisms in the injected water may travel through the aquifer and cause illness when the water is later recovered for domestic use.

### WATER-RECLAMATION AND ARTIFICIAL-RECHARGE FACILITIES

Reclaimed water for injection was obtained by tertiary-stage treatment of 0.6 Mgal/d of effluent from an activated-sludge-type, 60-Mgal/d sewage-treatment plant (Peters and Rose, 1968; Peters, 1968; Vecchioli, Oliva, Ragone, and Ku, 1975). This treatment consisted of (1) coagulation and sedimentation, (2) primary filtration

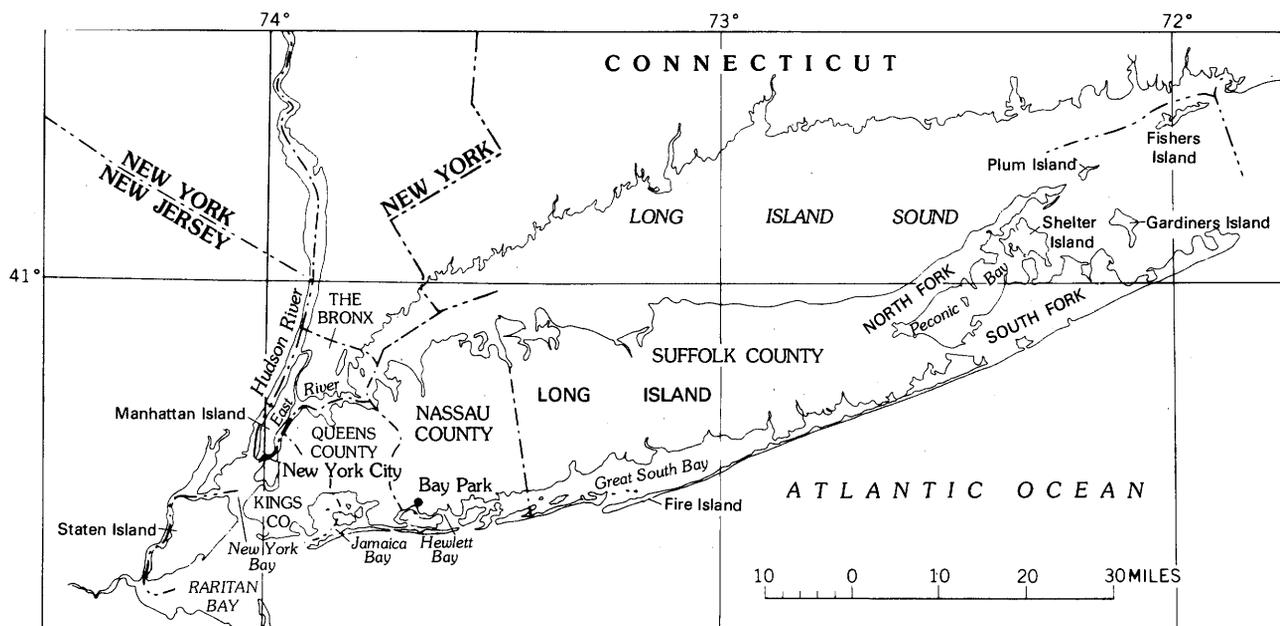


FIGURE 1.—Location of study site, Bay Park, N.Y.

through a dual-media sand-anthracite filter, (3) secondary filtration through one to four activated carbon columns, and (4) chlorination. Additional treatments (degasification, pH adjustment, and dechlorination) were applied for comparison at selected times at the recharge facility 1,000 feet from the sewage-treatment plant.

Major components of the recharge facility include (1) a 50,000-gallon storage tank into which either public-supply water or reclaimed water can be delivered; (2) a vacuum-operated degasifier; (3) injection and redevelopment pumps with automatic-flow controls; (4) an injection well consisting of an 18-inch-diameter fiberglass casing above a 16-inch diameter stainless-steel well screen set at 418 to 480 feet below land surface, (5) 18 observation wells, some of which are equipped with water-level-recording equipment, and (6) equipment for monitoring several chemical and physical characteristics of the water. (See fig. 2.)

#### GEOHYDROLOGY OF RECHARGE SITE

The recharge site is in the Atlantic Coastal Plain and is underlain by about 1,250 feet of unconsolidated deposits of Pleistocene and Late Cretaceous age, which, in turn, overlies crystalline bedrock of Precambrian age. The injection well is screened within the lower part of the Magothy aquifer of Late Cretaceous age at a depth of 418 to 480 feet below land surface. The screened interval (injection or receiving zone) consists primarily of stratified fine to medium quartz sand sandwiched between and semiconfined by Magothy beds of lesser hy-

draulic conductivity. At Bay Park, the Magothy aquifer is confined below by the Raritan clay, also of Late Cretaceous age, but is virtually unconfined above owing to the generally coarse-grained character of the overlying Pleistocene deposits. Lateral hydraulic conductivity of the stratified injection zone averages 940 (gal/d)/ft or 126 ft/d, but flowmeter surveys indicate considerable variation within the interval. The hydraulic characteristics of the aquifer system were determined by standard aquifer-test methods and were later simulated by electric analog-model studies.

#### PREVIOUS WORK

The geology and ground-water conditions in southern Nassau and southeastern Queens Counties, which encompass the Bay Park area, were discussed by Perlmutter and Geraghty (1963). Luszczynski and Swarzenski (1966) gave a detailed description of the hydrologic environment in southeastern Nassau County. General discussions of more regional hydrologic conditions were given by Cohen, Franke, and Foxworthy (1968). The geohydrology of the Bay Park site was described in brief by Perlmutter and others (1968) and in detail by Vecchioli, Bennett, Pearson, and Cerillo (1974).

Scope and objectives of the Bay Park wastewater-reclamation and artificial-recharge project have been described by Cohen and Durfor (1967) and by Peters and Rose (1968). These reports also contain general descriptions of the facilities. A detailed description of the injection well was given by Cohen and Durfor (1967). Koch

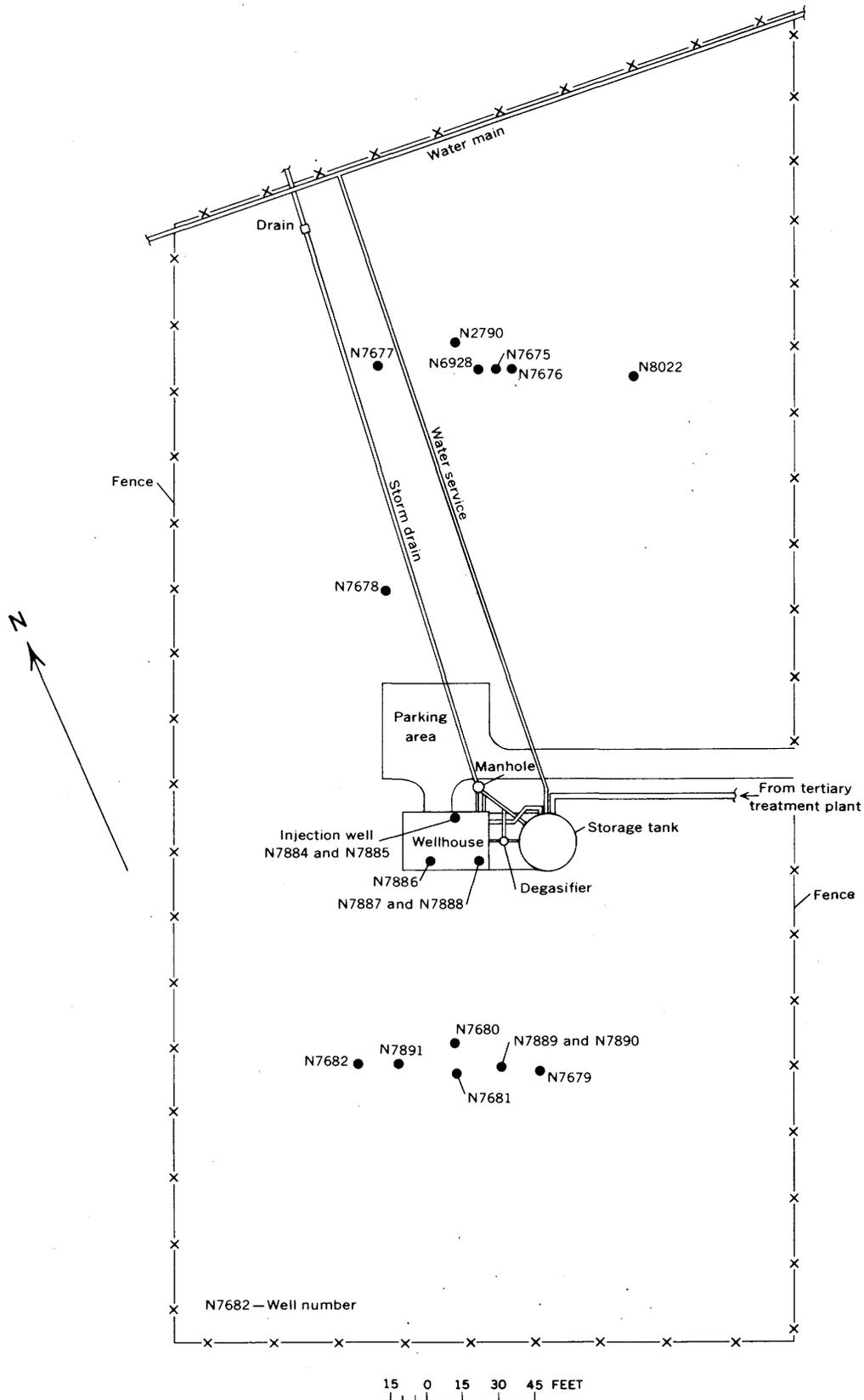


FIGURE 2.—Location of observation wells, structures, and external piping at artificial-recharge site. (Modified from Koch and others, 1973.)

and others (1973) described in detail the design and operation of the entire recharge facilities, including the injection well.

Many progress reports have dealt with all or most aspects of the project in varying detail. Peters (1968) commented on the project overall, including some discussion of early operations of the treatment and injection plants. Vecchioli and Ku (1972) presented preliminary results of early injection experiments. These early results were later updated and summarized by Vecchioli (1972). Principal conclusions drawn from all tests were discussed in brief by Vecchioli and others (1975). Significant features of the longest recharge experiment (41.7 Mgal) were reported on by Sulam (1973).

Reports dealing specifically with geochemical aspects of the recharge experiment include Vecchioli and Giaimo (1972) on potential corrosion of metals; Faust and Vecchioli (1974) on several problems; Ragone, Vecchioli, and Ku (1973), Ragone and Vecchioli (1975), and Ragone, Ku, and Vecchioli (1975) on dissolution of iron from the aquifer; and Ku, Vecchioli, and Ragone (1975) on movement of sewage-related substances through the aquifer. Ragone (1977) presented a comprehensive discussion of all geochemical problems studied.

Bacterial growth around the injection well and the significance thereof was reported on by Vecchioli (1970) and Ehrlich, Ehlke, and Vecchioli (1972, 1973). Movement of bacteria through the aquifer along with the injected water was discussed by Vecchioli, Ehrlich, and Ehlke (1972).

#### ACKNOWLEDGMENTS

Individuals who had a major role in the design and construction of the injection facilities include John H. Peters, former Commissioner of the Nassau County Department of Public Works; John L. Rose, formerly of Burns and Roe, Inc.; Philip Cohen and Bruce L. Foxworthy, Geological Survey; and Charles N. Durfor, formerly with the Geological Survey. Particular recognition is given Messrs. Cohen and Durfor, whose efforts early in the project made possible the detailed testing of the hydraulics and hydrochemistry of the aquifer system that followed.

Installation and early testing of the wells was done under the joint direction of Messrs. Cohen and Durfor. Most of the credit for the design of the operating controls and monitoring instrumentation of the injection facilities belongs to Mr. Durfor. In preparation for the injection of reclaimed water Gordon D. Bennett of the Geological Survey directed extensive hydraulic testing of the injection facilities, the injection well, and the aquifer; many of the test procedures developed by him were followed with little modification throughout the project. Bennett and

F. J. Pearson, Jr., also directed the early recharge tests with public-supply water; some of the data from those tests are included in this report.

The authors are especially grateful to former Commissioner Peters and Deputy Commissioner James S. Gillen, Nassau County Department of Public Works, for making available the physical and human resources of the department throughout the project. Particular thanks are given also to Francis J. Flood, former Superintendent of Operations and Maintenance, James A. Oliva, Sanitary Engineer, and to many others in the department for coordinating water-treatment operations with the injection experiments, for providing assistance in making modifications to the injection equipment, and in helping to make various measurements during the experiments. Louis S. Guaracini of the Department of Public Works provided numerous chemical analyses throughout the course of the project.

Several members of the Geological Survey assisted in making test measurements and in reducing and compiling the data acquired. Particular recognition in this respect are given Anthony A. Giaimo, Dennis J. Sulam, and Lillian B. Maclin for their assistance throughout the project. The authors are grateful to all who assisted.

## METHODS OF INVESTIGATION

### INJECTION PROCEDURES

Reclaimed water from the tertiary-treatment plant was injected into the well under positive head at a rate of about 350 gal/min, except in the first and part of the seventh tests (tests RW1 and RW7), when the injection rate was 200 gal/min. Pertinent information about the 13 recharge tests is given in tables 1 and 2.

Chemical and physical properties of typical reclaimed water, and their values recorded during the study, are listed in table 3; except where otherwise stated, reclaimed water used in the tests conformed closely to these values. Chemical composition of water from observation wells at the site before injection began is presented in table 4.

In all tests except RW8, RW10A, and RW11, reclaimed water used for injection was chlorinated. In tests RW8 and RW10A, chlorination was omitted; in test RW11, chlorinated water from the treatment plant was treated with sodium thiosulfate to remove all traces of free and available combined chlorine before injection. In test RW10, the secondary filtration through activated carbon was omitted to test the aquifer response to the inclusion of phosphate, chemical oxygen demand (COD), and methylene blue active substances (MBAS) in injection water. In test RW12, sodium hydroxide was added

TABLE 1.—Data on injection tests at Bay Park, N. Y., 1968–1972

Test <sup>1</sup>	Date	Approximate length of test (days)	Injection rate (gal/min)	Gallons injected (thousands)	Special treatment of water
RW1-----	10-8-68 to 10-10-68	2	200	616	Degasified.
RW2-----	12-10-68 to 12-10-68	2	350	1,063	Do.
RW3-----	2-25-69 to 2-27-69	2	350	1,066	None.
RW4-----	5-6-69 to 5-16-69	10	360	5,247	Do.
RW5-----	9-15-69 to 9-25-69	10	350	5,117	Degasified.
RW6-----	3-17-70 to 3-19-70	2	340	1,133	None.
RW7 <sup>2</sup> ----	4-14-70 to 5-17-70	33	350	13,970	Do.
			and 200		
RW8-----	11-30-70 to 12-10-70	10	340	4,930	Unchlorinated.
RW9-----	4-27-71 to 5-7-71	10	355	5,156	None.
RW10----	11-1-71 to 11-11-71	10	350	5,180	Bypassed carbon adsorbers.
RW10A---	1-10-72 to 1-14-72	4	350	2,000	Unchlorinated.
RW11----	3-6-72 to 3-16-72	10	350	5,124	Dosed with Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> ·5H <sub>2</sub> O
RW12----	6-5-72 to 6-15-72	10	355	5,050	Dosed with NaOH.
RW13 <sup>3</sup> ---	10-24-72 to 5-11-73	84.5	355	41,695	None.

<sup>1</sup>RW, reclaimed water.

<sup>2</sup>Injection rate reduced after 19 days because output from treatment plant had been reduced.

<sup>3</sup>Injection done intermittently. See table 2.

to the reclaimed water to increase pH. In test RW13 (the longest test), the injection was not continuous as a result of weekend shutdowns, equipment breakdowns, and short periods of backflushing for well redevelopment. The injection period in test RW13 was divided into 18 separate segments. (See table 2.)

Samples for chemical and microbiological testing were taken at frequent intervals. Injection water was sampled

TABLE 2.—Injection data from test RW13, October 1972 to May 1973

[From Sulam, 1973. Injection rate 350 gal/min unless otherwise noted]

Segment No.	Date started	Date ended	Total number of days	Total gallons injected	Cumulative gallons injected
1-----	10-24-72	10-27-72	3	1,598,400	1,598,400
2-----	11- 7-72	11-17-72	10.5	5,192,400	6,790,800
3-----	11-20-72	11-22-72	2.5	1,213,600	8,004,400
4-----	11-27-72	12- 8-72	11.5	5,657,300	13,661,700
5-----	12-13-72	12-15-72	2.5	1,246,800	14,908,500
6-----	12-19-72	12-21-72	2.5	1,255,400	16,163,900
7-----	1- 3-73	1-12-73	9.5	4,706,700	20,870,600
8-----	1-15-73	1-19-73	4.5	2,246,600	23,117,200
9-----	1-29-73	2- 4-73	6.5	3,206,300	26,323,500
10-----	2- 6-73	2- 9-73	3	1,470,600	27,794,100
11-----	2-10-73	2-10-73	.33	149,400	27,943,500
12 <sup>1</sup> -----	2-12-73	2-13-73	.67	324,700	28,268,200
13-----	3-12-73	3-16-73	4.5	2,207,700	30,475,900
14-----	3-19-73	3-30-73	11.5	5,678,200	36,154,100
15-----	4- 2-73	4- 6-73	4	1,964,000	48,117,100
16-----	4- 9-73	4- 9-73	.5	233,500	48,350,600
17-----	5- 2-73	5- 4-73	2.5	1,177,400	49,528,000
18-----	5- 7-73	5-11-73	4.5	2,166,700	41,694,700

<sup>1</sup>Injection rate reduced to 200 gal/min.

at least daily, with few exceptions, for microbial analysis during all tests except RW12. Water samples were also taken from observation wells at appropriate times. Samples were collected in sterile containers from flowing streams of water pumped from the observation wells.

Bacterial population of the aquifer near the well screen during tests RW7, RW8, RW11, and RW13 was determined by evaluating the contents of a specially designed probe that was lowered into the gravel pack during injection. The probe was built from a piece of stainless-steel well screen 2 inches in diameter and 27 inches long. Before each test, it was filled with sand from the aquifer and autoclaved for 30 min at 121°C. To assure sterility of sand in the probe after autoclaving, samples of its contents were inoculated into tubes of sterile nutrient broth. Absence of microbial growth in the broth after 48 hours indicated the absence of viable microorganisms in the probe. If growth developed, the autoclaving and testing cycle was repeated until growth did not appear in the broth. Immediately before each test, the probe containing sterile sand was lowered approximately 450 feet into the observation well. It was removed immediately after injection ceased (Ehrlich and others, 1972).

After each test, the injection well was pumped until at least 1.5 times the volume of water injected had been removed. Samples for bacterial analyses were collected at appropriate intervals.

TABLE 3.—*Chemical and physical quality of typical reclaimed water*

[Modified from Vecchioli and others, 1975]

Constituent or characteristic (1)	Number of determinations (2)	Observed values <sup>1</sup>		Weighted average <sup>2</sup> (5)
		Maximum (3)	Minimum (4)	
Silica (SiO <sub>2</sub> )-----	35	15	12	13
Aluminium (Al), total-----	22	400	0	100
Iron (Fe), total-----	35	1,300	100	410
Iron (Fe), dissolved-----	35	780	70	230
Manganese (Mn), total-----	35	210	30	75
Manganese (Mn), dissolved-----	35	210	30	72
Calcium (Ca), dissolved-----	33	32	16	20
Magnesium (Mg), dissolved-----	34	10	5.2	6.4
Sodium (Na), dissolved-----	33	110	72	86
Potassium (K), dissolved-----	33	15	11	13
Bicarbonate (HCO <sub>3</sub> ), dissolved-----	33	108	30	59
Sulfate (SO <sub>4</sub> ), dissolved-----	35	210	120	160
Chlorine, total residual-----	207	3.5	0	2.1
Chloride (Cl), dissolved-----	34	150	65	99
Fluoride (F), dissolved-----	35	0.8	0.1	0.3
Organic nitrogen (N), dissolved-----	35	1.1	0	0.60
Nitrogen (N), total-----	35	27	21	24
Nitrite nitrogen (N), dissolved-----	35	0.02	0	0.01
Ammonia nitrogen (N), dissolved-----	34	26	20	23
Nitrate nitrogen, dissolved-----	35	0.1	0.0	0.1
Phosphorus, as P, total-----	35	0.48	0.01	0.08
Dissolved solids, residue 180°C-----	33	519	298	398
Dissolved solids, calculated from determined constituents-----	33	571	393	463
Total solids residue, volatile-----	33	52	15	33
Hardness, as CaCO <sub>3</sub> , (Ca, Mg)-----	33	121	63	79
Noncarbonate hardness-----	33	96	0	32
Specific conductance (μmho/cm at 25°C)-----	35	970	720	786
pH-----	210	6.6	4.8	6.1
Temperature, in °C-----	211	21	15	17.5
Turbidity, as SiO <sub>2</sub> -----	385	1.9	0	0.4
Dissolved oxygen (DO)-----	204	8.0	0	4.5
Chemical oxygen demand (COD) (0.025N K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub> )-----	35	21	2	9
Detergents (MBAS)-----	35	0.36	0.02	0.07

<sup>1</sup>Observed values are based on daily or weekly composite samples, consisting of four aliquots daily except for temperature, turbidity, chlorine residual, pH, and dissolved oxygen, which were determined at least twice daily onsite. Values for aluminum, iron, and manganese are in micrograms per liter; all other values except those for pH, specific conductance, and water temperature are in milligrams per liter.

<sup>2</sup>Weighted average is equal to (concentration 1 times injected volume 1 plus concentration 2 times injected volume 2, and so forth, to concentration n times injected volume n) divided by total injected volume.

### BACTERIAL ENUMERATION AND IDENTIFICATION

Total coliform determinations during tests RW1 through RW6 were made by the multiple tube technique (American Public Health Association and others, 1971, p. 662-678). In all other tests, total-coliform, fecal-coliform, and fecal-streptococcal counts were made by the membrane-filter method (American Public Health Association and others, 1971, p. 678-688; p. 690-691).

Total bacterial counts were made by the Standard Plate Count Method (American Public Health Association and others, 1971, p. 660-662) and by the following membrane filtration procedure: Total aerobic bacteria counts were made by filtering measured volumes of samples through membrane filters (mean pore size 0.45 μm). The filters were placed on plates of plate count agar or tryptone-glucose-yeast extract agar (American Public Health Association and others, 1971, p. 651) with the bacteria-laden side up and were incubated at 30°C for 48 hours. To enhance visibility of colonies on the membrane

filters, the filters with attached colonies were placed together on a pad saturated with dilute methylene blue dye solution before counting (Committee on Bacteriologic Technique, 1957, p. 13). Counting was done with a low-power binocular microscope.

Pure cultures from isolated colonies on membrane filters and standard plate count plates were obtained by picking with a sterile needle and streaking on plates of plate count agar. The process of picking and streaking was repeated at least three times after the initial isolation or until only one type of colony appeared on the streak plates. Material from one colony of each final isolation plate was transferred to Plate Count Agar Slants with monthly transfers.

Characterization of pure cultures was done according to methods described by the Committee on Bacteriologic Technique (1957) and Skerman (1967).

Bacterial densities of the sand probe after each test were determined in the following way: A portion of material from the center of the probe was suspended in sterile buffered dilution water, and the suspension was shaken vigorously to dislodge attached bacteria. Samples of this suspension and serial dilutions thereof were tested for total aerobic count as described above. Sand was collected on a preweighed filter and dried at 105°C. The weight of sand was obtained as the difference between the weight of dry sand and filter and the weight of the filter alone. Bacterial density was calculated from estimated pore volume based on sample weight and an assumed porosity of 30 percent and bulk density of 2 g/cm<sup>3</sup>.

TABLE 4.—*Chemical composition of water from observation wells at Bay Park artificial-recharge site*

[From Vecchioli and others, 1974. Chemical analyses by U.S. Geological Survey. All values in milligrams per liter unless otherwise noted]

Characteristic or constituent	Well number <sup>1</sup>		
	N7886	N7890	N8022
Silica (SiO <sub>2</sub> ), dissolved-----	7.2	7.5	7.4
Ferrous iron (Fe)-----	.30	.14	.14
Total iron (Fe)-----	.30	.14	.14
Total manganese (Mn)-----	.03	.03	.03
Calcium (Ca), dissolved-----	.37	1.39	.87
Magnesium (Mg), dissolved-----	.22	.21	.24
Sodium (Na), dissolved-----	3.90	3.82	3.68
Potassium (K), dissolved-----	.53	.62	.44
Bicarbonate (HCO <sub>3</sub> ), dissolved-----	4.5	7.5	6.0
Sulfate (SO <sub>4</sub> ), dissolved-----	3.55	3.95	3.75
Chloride (Cl), dissolved-----	3.90	3.90	3.85
Fluoride (F), dissolved-----	.13	.13	.13
Nitrate (NO <sub>3</sub> ), dissolved-----	.00	.00	.00
Phosphate (PO <sub>4</sub> ), dissolved-----	.02	.005	.015
Hydrogen sulfide (H <sub>2</sub> S), dissolved-----	.4	.0	.0
Dissolved solids-----	22	25	23
Specific conductance (μmho/cm at 25°C)-----	28	32	23
pH-----	5.22	5.72	5.45
Oxidation-reduction potential (volts)-----	-.03	-.10	-.06
Hydrogen sulfide (H <sub>2</sub> S)-----	.4	.0	.0
Temperature (°C)-----	15	15	15

<sup>1</sup>Locations of wells are shown in figure 2. N7886 is 20 ft from injection well; N7890 is 100 ft from injection well; N8022 is 200 ft from injection well.

Denitrifying bacteria counts were determined by the method described by Alexander (1965).

Presence of sulfate-reducing bacteria was determined by the method described by the American Petroleum Institute (1959).

Anaerobic bacterial testing was done by two methods: (1) 0.1 mL of water sample was spread over the surface of a plate of predried Anaerobic Agar (BioQuest, Division of Becton Dickinson and Co.); (2) exposed membrane filters were laid on plates of Anaerobic Agar. Plates and membrane filters were inoculated under oxygen-free conditions in Gas Pak Jars (BioQuest, Division of Becton Dickinson and Co.) at room temperature.

### BACTERIAL CLOGGING OF WELL

Several investigations, including those of the California State Water Pollution Control Board (1954), Sniogocki (1963), Rebhun and Schwartz (1968), and Vecchioli and others (1975), have shown that suspended inorganic and organic particulate matter in the injectant collects and forms a mat at or near the well screen. Although the mat was never directly observed in these studies, it is expected to be similar to those formed on the surface of rapid sand filters.

Most of the deposit is assumed to be at the interface and to penetrate the interstices of the sand for only short distances. The effect of this mat in an injection well is the same as its effect on a filter, namely, to block pores and reduce hydraulic conductivity.

The California State Water Pollution Control Board (1954, p. 165) and Ehrlich and others (1972) found that the rate of clogging is directly proportional to the amount of solids injected in any period of time. The California State Water Pollution Control Board (1954) concluded that biological activity within the mat reduces the rate of clogging by breaking down the organic matter held in the mat. In contrast, Rebhun and Schwartz (1968) suggested that bacterial growth together with associated slimes enhance the rate of solids buildup and therefore increase the rate of well clogging.

Effects of microbial activity on the performance of the injection well at Bay Park were under investigation since the beginning of the program. Vecchioli and Ku (1972) suggested that bacterial growth may have contributed to well clogging observed during early tests. Treatment of the injection well after test RW4 with a biocidal agent resulted in a small improvement in well capacity, which suggests that biota had contributed to the head buildup observed in that test.

Elemental chlorine was selected as a bacterial growth inhibitor at the start of the injection program. To critically evaluate its effectiveness as a control agent, two comparative tests (RW7 and RW8) were run. In RW7, chlorinated reclaimed water was injected. After removal of the chlorinated injectant and restoration of the original specific capacity of the well, test RW8 was run with unchlorinated injectant. Results of the two tests are described in the following sections.

### INJECTION OF CHLORINATED RECLAIMED WATER

In test RW7, reclaimed water of virtually the same composition as that described in table 3 was injected. Residual chlorine level was maintained at about 2.5 mg/L (minimum 0 mg/L; maximum 4.0 mg/L). Specific capacity of the injection well decreased from about 24 (gal/min)/ft at the start of the test to 2.5 (gal/min)/ft at the end, 33 days later. Specific capacity as a function of time is depicted in figure 3; cumulative plots of turbidity values (suspended-solids concentration) in relation to head buildup are shown in figure 4. A rapid rise in head buildup followed a sharp increase in turbidity in the injectant from about the 14th to the 20th day. This correlation of decreased specific capacity with increased cumulative particulate matter was characteristic in all tests.

The first water recovered during redevelopment of the injection well during tests RW7 and RW8 was grayish tan, opaque, and very turbid. The volatility of the suspended matter was 25 percent, which indicates that it

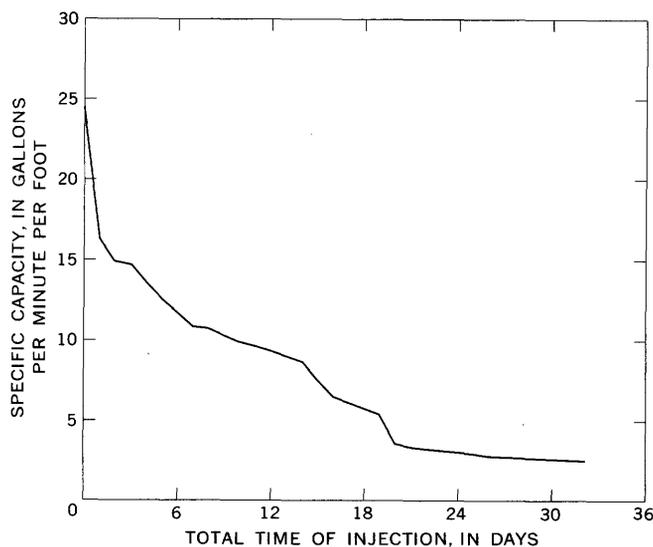


FIGURE 3.—Decrease of specific capacity of injection well with time during test RW7, with chlorinated water. (Modified from Ehrlich and others, 1972, fig. 1.)

<sup>1</sup> Use of trade names is for identification purposes only and does not imply endorsement by the U.S. Geological Survey.

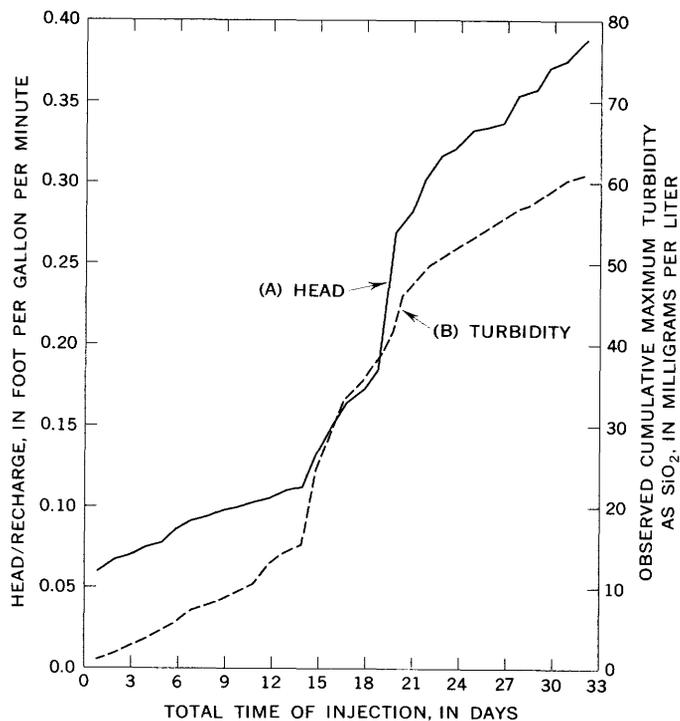


FIGURE 4.—Head in relation to turbidity during test RW7, with chlorinated water. A, head buildup divided by injection rate; B, cumulative maximum turbidity. (Modified from Ehrlich and others, 1972, fig. 2.)

was composed primarily of inorganic materials. The bacterial plate count of the first opaque, turbid water was near  $5 \times 10^3$  cells per 100 mL.

Clarity of repumped water gradually improved with pumping, and visible turbidity disappeared within several hours. After 30 minutes of pumping, the bacterial count had increased to  $5 \times 10^6$  cells per 100 mL (table 5). After 4 and 24 hours of pumping, bacterial counts remained at essentially that level.

The injection water contained significant concentrations of dissolved oxygen. (See table 3.) Water pumped from the formation immediately after cessation of injection had virtually the same dissolved-oxygen content as the injectant, but, as pumping continued, dissolved oxygen gradually decreased until it was no longer detectable. In a later test (Ragone and others, 1973), it was

TABLE 5.—Bacterial counts in water recovered from injection well after test RW7, with chlorinated water

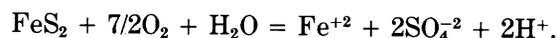
[Total bacteria and coliform counts given as cells per 100 mL; denitrifying bacteria given as most probable number per 100 mL]

Pumping time (min)	Total bacteria count	Denitrifying bacteria count	Coliform count
0-----	$5 \times 10^3$	--	0
30-----	$5 \times 10^6$	$5 \times 10^4$	67
240-----	$5 \times 10^6$	$5 \times 10^4$	70
1,440-----	$5 \times 10^6$	$5 \times 10^4$	90

determined that dissolved oxygen persists in the injectant to about 12 feet laterally into the formation. At greater distances, the water is essentially oxygen free.

Two factors can account for the loss of oxygen from the injectant as it moved from the injection well into the aquifer.

1. Ragone and others (1973) and Ragone and Vecchioli (1975) suggested that oxygen in the injectant reacts with pyrite in the formation to produce ferrous iron, sulfate, and hydrogen ions according to the equation:



2. Microbial respiration can deplete dissolved oxygen. Apparently, only small amounts of soluble biotransformable compounds entered the aquifer with the injectant, but work by Moffett and Williams (1967) and Favaro, Petersen, Boyer, Carlson, and Bond (1971) has shown that substantial bacterial populations can arise in very dilute nutrient media. Therefore, it is possible that microbial activity was sufficient to account for a major part of the observed oxygen depletion. However, despite the potential for microbially induced oxygen depletion, the relative importance of this mechanism, as compared to other oxygen sinks, such as pyrite oxidation, cannot be assessed. Evidence presented later in this section suggests that microbial activity in the formation was restricted and that bacterial respiration would, therefore, be only a minor factor in oxygen uptake.

Several bacterial strains were isolated from the sand probe and from water pumped from the aquifer at selected times during initial well redevelopment. Twenty-five isolated colonies from the plate-count tests were identified as to genus. Sources and identity of isolates are given in table 6 (from Ehrlich and others, 1972).

The identified isolates were obligate aerobes and facultative anaerobes. Under the oxygen-poor conditions in the formation, only sparse growth of obligate aerobes such as *Alcaligenes*, *Micrococcus*, *Flavobacterium*, and *Acinetobacter* would be expected as was the case. A few genera of facultative anaerobes such as *Proteus*, *Aero-*

TABLE 6.—Genera of bacteria isolated from repumped water 2 days after cessation of test RW7, with chlorinated water

[From Ehrlich and others, 1972]

Genus	In sand-filled probe	Number of times isolated			
		In water samples			
		Pumping time, in minutes			
		0	30	240	1,440
<i>Pseudomonas</i> , type 1-----	1	0	0	0	0
<i>Pseudomonas</i> , type 2-----	1	0	0	0	0
<i>Pseudomonas</i> , type 3-----	0	0	4	2	0
<i>Micrococcus</i> -----	0	1	0	0	0
<i>Alcaligenes</i> -----	0	2	1	0	1
<i>Flavobacterium</i> -----	0	1	0	0	0
<i>Acinetobacter</i> -----	0	1	0	0	3
<i>Aeromonas</i> -----	0	0	1	1	0
<i>Serratia</i> -----	0	0	2	0	0
<i>Proteus</i> -----	0	0	0	2	1

monas, and *Serratia* were found; these can grow under anaerobic conditions if suitable fermentable substrates are available. Denitrifying strains of *Pseudomonas fluorescens* can grow under anaerobic conditions by using nitrate as a terminal electron acceptor. Although nitrate concentration was typically only about 0.4 mg/L, this concentration could sustain a small denitrifying bacterial population. Coliform (lactose-fermenting) bacteria were present in only small numbers in the repumped water.

Several *Pseudomonas* species, including *P. fluorescens*, *P. aeruginosa*, *P. putida*, *P. alcaligenes*, and *P. pseudoalcaligenes*, are able to sustain themselves under anaerobic conditions by decomposing arginine, an amino acid, through a process known as substrate level phosphorylation (Stanier, Douderoff, and Adelberg, 1970). The relative importance of arginine hydrolysis is unknown because no data on arginine concentrations are available.

#### INJECTION OF UNCHLORINATED RECLAIMED WATER

Results of test RW7, with chlorinated water, showed that the rate of clogging depended mainly on the concentration of suspended solids in the injectant. Suspended solids in the water first repumped from the injection well were mostly nonvolatile, which indicates that little organic matter had accumulated near the well.

In the next test (RW8), about 5 Mgal unchlorinated reclaimed water of nominally the same composition as that in test RW7 (table 3) was injected. After 10 days of injection, specific capacity of the injection well had decreased from 26 (gal/min)/ft of head buildup to 11 (gal/min)/ft. A curve showing rate of head buildup in this and three other tests is shown in figure 5. Turbidity of the injectant was consistently low in test RW8 (table 7) and averaged less than in other tests. Thus, the increased rate of head buildup observed in test RW8 could not be ascribed to physical clogging by inert particulate matter alone.

In all tests but RW8, head buildup in the observation well (well N7885) within the filter pack of the injection well was virtually the same as that in the injection well. In test RW8, however, after the second day of injection, the head in the injection well rose at a more rapid rate than in the observation well (fig. 6) and, near the end of the test, the head in the injection well was nearly 12 feet higher than in the observation well. This increase in head suggests that much of the clogging occurred in the filter pack immediately adjacent to, or on, the injection-well screen. A lesser amount of clogging probably also developed behind the filter pack at the aquifer face, where most of the clogging had been noted in other tests.

The water first recovered from the formation after the end of test RW8 was moderately turbid and showed little tendency for settlement of suspended material on

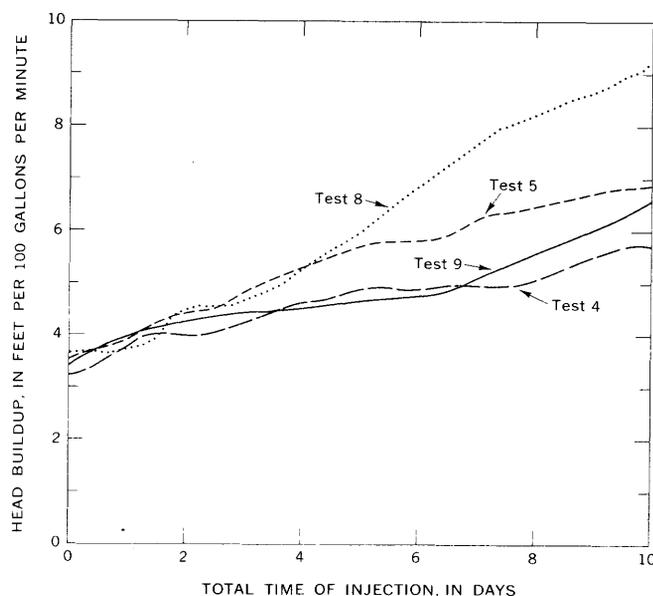


FIGURE 5.—Head as a function of time at the injection well during: (1) test RW8, with unchlorinated water, and (2) tests RW4, RW5, and RW9, with chlorinated water. (Modified from Ehrlich and others, 1973, fig. 2.)

standing. Approximately 75 percent of the suspended material was volatile at 550°C, which suggests that the material was largely oxidizable organic matter. In test RW7 (with chlorinated water), the volatility of comparable suspended matter was only about 25 percent. Because the injectant had received virtually the same treatment before both tests (except for chlorination), the threefold increase in volatile matter in the particulate material recovered after test RW8 cannot be attributed solely to differences in preinjection treatment.

Bacterial counts in the water first recovered from the filter pack after test RW8 were much higher than in comparable samples from test RW7 with chlorinated water ( $2.0 \times 10^7$  cells per 100 mL in RW8 as opposed to  $5 \times 10^5$  cells per 100 mL in RW7). This greater fraction of volatile matter in the residue after test RW8 suggests a larger bacterial biomass in the organic mat after test RW8.

TABLE 7.—Turbidity of reclaimed water during selected injection tests

Test	Turbidity	
	Range	Average
RW4	0.2-1.8	0.9
RW5	.1-1.6	.6
RW8	0 -0.3	.1
RW9	0 -1.5	.3

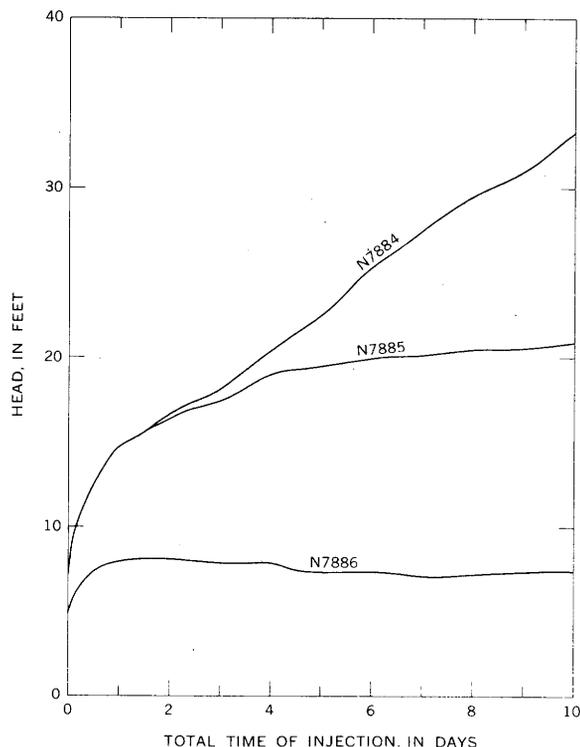


FIGURE 6.—Head buildup as a function of time at injection well, an observation well in filter pack, and an observation well 20 feet from the injection well during test RW8, with unchlorinated water. (Modified from Ehrlich and others, 1973, fig. 2.)

After test RW8, the sand-filled probe, which had been suspended in the observation well tapping the filter pack during injection, was coated with a slimy, gelatinous material that cemented some of the sand grains into larger aggregates. Such slime had not been observed in the probe after test RW7, in which chlorinated water had been used. Material from the center of the probe had a bacterial density of  $1.0 \times 10^8$  cells per 100 cm<sup>3</sup> of pore volume, about 500 times that of both the injectant and the water repumped after injection. The coliform bacteria count in the probe was about 10 times that of the injectant and the first repumped water, but fecal-coliform and fecal-streptococcal counts were about the same in the probe, the injectant, and the first repumped water.

Coliform bacteria, which formed approximately half the total bacterial count of the injectant during much of test RW8, constituted only 1 percent of the bacterial population in the probe. It was assumed that the ratio of coliform bacteria to total bacteria captured in the probe by filtration would be representative of the ratio in the injectant; therefore, the lower ratio noted in the probe must have resulted either from a more rapid die-off, or slower multiplication, of coliforms than of other bacterial

species. In any event, coliform bacteria during test RW8, in which the injectant was not chlorinated, were less significant.

From the preceding discussion, one can conclude that the impact of bacterial clogging is reduced in the presence of an effective bacteriostatic or bactericidal agent such as chlorine. When residual chlorine was maintained at a level of about 2.5 mg/L in the injected water, bacterial growth and attendant clogging did not occur either in the filter pack or at the formation face.

#### INJECTION OF DECHLORINATED RECLAIMED WATER

In test RW11, reclaimed water was dechlorinated with sodium thiosulfate before injection, so that the effects of chlorine on microbial growth could be studied further. The injection water was similar in chemical quality to that used in earlier tests (table 3). During test RW11, residual chlorine could not be detected in the injectant.

Total bacterial and total coliform counts in the injectant were monitored throughout test RW11 (table 8). Coliforms were detected only once, and total bacterial count never exceeded 37 cells per 100 mL. Specific capacity decreased from 24.9 (gal/min)/ft at the start of the test to 22.8 (gal/min)/ft after 10 days of injection. This relatively small (10-percent) loss of specific capacity contrasts sharply with the 58-percent loss observed in test RW8, in which unchlorinated water was injected.

Head buildup in test RW11 was moderate but less than expected. By analogy with test RW8, which had used unchlorinated water, accelerated clogging from accumulation of bacterial cells in the filter pack had been expected to appear during the first week. Chemical oxygen demand (COD) levels in the injectant were comparable in tests RW7 and RW11; therefore, about equal amounts of carbon and energy sources were available for cell building in the two tests.

Percentage volatiles content of solids recovered from the well and cell count of the sand probe were determined after test RW11. Solid material obtained during

TABLE 8.—Bacterial content of injectant during test RW11, with dechlorinated water

[Total bacteria and coliform counts given as cells per 100 mg/L]		
Days since beginning of test	Total bacteria count	Total coliform count
1	37	<1
2	14	<1
3	2	<1
4	1	<1
5	2	<1
6	2	<1
7	2	<1
8	1	<1
9	23	<1
10	4	1

redevelopment after test RW11 contained 86 percent volatile materials, which compares closely with the 68 percent found after test RW8 and is significantly greater than the 25 percent found after test RW7 (Ehrlich and others, 1972, p. 242). This high percentage shows that the clogging mat near the well was composed primarily of organic matter.

The bacterial density of the sand-filled probe after test RW11 was  $1.4 \times 10^4$  cells per 100 mL—significantly greater than the median bacterial counts of the injectant (table 8) and the probe after test RW7, but orders of magnitude less than the  $1.0 \times 10^8$  cells per 100 mL observed after test RW8. The high volatiles content of solids in water pumped after test RW11, and the nearly 10,000-fold increase of cells in the sand probe, suggest that bacterial growth had occurred both in the filter pack and the formation near the well during test RW11, although not nearly as rapidly as during test RW8.

A clogging mat did not form during test RW11. Its development may have been delayed by an initial lack of significant numbers of viable cells in the injectant because the rate of clogging-mat formation by bacteria and their associated slimes depends on the number and types of cells in the injectant as well as the quality and quantity of assimilable carbon sources. Inoculum size has long been known to be a major determinant of the rate of industrial fermentation processes; large inocula are generally administered to minimize the time required for the microbial population to build up to a high level.

The bacterial density of the injectant in test RW11 was consistently less than 100 cells per 100 mL, whereas in test RW8 it had been consistently greater than  $1 \times 10^5$  cells per 100 mL. The cell counts in the sand-filled probe after tests RW11 and RW8 ( $1.4 \times 10^4$  and  $1.0 \times 10^8$  colonies per 100 mL, respectively) indicate an increase of three to four orders of magnitude in each test. Thus, the absence of clogging during test RW11 may be attributed to a lower original count in the injectant. Had the test continued, a rapid increase in head buildup might have ensued.

#### EFFECT OF NUTRIENT LEVEL

Results of test RW7, with chlorinated water, suggest that the bacterial-growth-inhibiting effects of residual chlorine were exerted only over a short distance (less than 20 ft) within the formation. Once the chlorine had dissipated, microbial growth could proceed freely. Thus, it is possible that if a high level of soluble organic matter were introduced with the injectant, some of it would penetrate into the formation and support microbial growth.

To determine the effect of elevated nutrient levels in the injectant, the carbon filtration step was omitted from the tertiary-treatment process in test RW10. Water was

taken from the sand filters and chlorinated to a level of about 2.5 mg/L, and 5 Mgal was injected at a rate of 350 gal/min for 10 days. Chemical oxygen demand (COD) and methylene blue active substances (MBAS) concentrations were from two to eight times the concentrations usually observed in reclaimed water filtered through activated carbon. Nitrogen and phosphate concentrations were unaffected, and the bacterial count of the injectant was low (table 9).

Specific capacity of the well in test RW10 decreased from 26 (gal/min)/ft at the start of the test to 15.2 (gal/min)/ft after 10 days of injection. Pumping from the well was started shortly after the end of the injection period. The bacterial count of water was 1,400 cells per 100 mL after 14 minutes of pumping and 1,000 cells per 100 mL after 90 minutes, when pumping was discontinued. The bacterial count of the initially sterile sand probe suspended in the gravel pack was relatively low (300 cells per 100 mL), which indicates that little microbial growth had occurred in the vicinity of the well during the 10-day injection period.

Immediately after cessation of the 90-minute repumping cycle, the well was allowed to remain idle for 35 days. At the end of this time the well was again pumped, and the specific capacity of the well had increased from 15.2 (gal/min)/ft at the end of the injection test to 26 (gal/min)/ft, or roughly the same value as that at the start of injection. Bacterial counts in water pumped from the well after the 35-day rest period were in excess of  $10^5$  cells per 100 mL, as compared to 1,000 cells per 100 mL immediately after cessation of injection. The bacterial aftergrowth is consistent with observations of Rebhun and Schwartz (1968) and Vecchioli (1970), who found greatly increased coliform densities in water repumped from injection wells after long pauses between injection and redevelopment.

Restoration of specific capacity to pretest levels indicates that the clogging mat, which had accumulated during the injection phase and was not removed during the

TABLE 9.—*Bacterial quality of injectant during test RW10, with carbon filtration omitted*

[Total bacteria and coliform counts given as cells per 100 mL]

Days since beginning of test	Total bacteria count	Total coliform count
1-----	12	<1
2-----	9	3
3-----	7	2
4-----	6	4
5-----	7	2
6-----	28	2
7-----	15	<1
8-----	19	2
9-----	10	2
10-----	28	<1

initial 90-minute repumping cycle, had changed sufficiently to be removed during the 60-minute repumping cycle after the 35-day rest period.

#### LONG-TERM INJECTION TEST

Test RW13, the final test of the series, ran from October 24, 1972, to May 11, 1973. Recharge was intermittent, and the total volume of chlorinated reclaimed water injected during the test was 42 Mgal. Some of the water-quality aspects of test RW13 have been discussed by Ragono and others (1975) and Vecchioli and others (1975). About two-thirds of the way through the test, after injection of slightly more than 28 Mgal of reclaimed water, the sand-filled probe was removed from the observation well in the gravel pack. The bacterial count of the sand was  $1 \times 10^3$  cells per 100 mL of free pore volume—considerably more than that observed in the probe after any of the shorter tests with chlorinated water—which indicates that bacterial growth may occur in the proximity of chlorinated water.

During test RW13, the injection well was redeveloped by pumping whenever specific capacity decreased to 6 (gal/min)/ft. This was done seven times during the test. On three occasions, samples of repumped water were analyzed for bacterial count. Results are given in table 10.

The sample of December 11, 1972, taken 3 days after the cessation of injection, showed that bacterial populations increase rapidly during idle periods. The bacterial count was very high at first but decreased with pumping. Sulfate-reducing bacteria appeared after 60 minutes of pumping on February 14, 1973, but were present in the initial water repumped from the well on April 6, which shows that microbial growth occurred near the well even in the presence of residual chlorine. Apparently, this growth did not contribute significantly to well clogging. The appearance of sulfate-reducing bacteria near the

TABLE 10.—*Bacterial counts of water repumped from injection well during three redeveloped cycles, test RW13*

[Total bacteria counts given as cells per 100 mL; sulfate reducing bacteria given as most probable number per 100 mL. ND, not determined]

Date	Running time (min)	Total bacteria count	Sulfate-reducing bacteria count
12-11-72-----	10	100,000	ND
	30	3,400	ND
	60	260	ND
2-14-73-----	12	200	<50
	30	2	<60
	60	2	8
4- 6-73-----	12	64	43
	30	ND	9
	60	54	4
	<sup>1</sup> Surge	380	23

<sup>1</sup>Pump turned off then on at short intervals over a 10-minute period.

well bore toward the end of the test suggests that a nutrient buildup was occurring near the well, but at a sufficient distance to be in an anoxidative zone, since sulfate-reducing bacteria are obligate anaerobics.

#### BACTERIAL ALTERATIONS OF WATER QUALITY

Reclaimed water contains higher concentrations of most chemicals than native water from the Magothy aquifer. Chemical oxygen demand (COD) of reclaimed water from the tertiary-treatment plant was typically 9 mg/L, whereas COD of native water from the aquifer is below measurable levels. In addition, the concentrations of essential microbial nutrients such as  $\text{NH}_4^+$ ,  $\text{PO}_4^{-3}$ ,  $\text{Mg}^{+2}$ ,  $\text{K}^+$ , and  $\text{Fe}^{+2}$  in reclaimed water are sufficient to allow a considerable amount of microbial growth.

Abundant evidence has been presented to show that microbial growth supported by nutrients in the injectant occurs in the clogging mat, particularly in the absence of chlorine. During the process of microbial assimilation, part of an organic substrate will be used for energy and part will be incorporated into new cell mass. Simpler molecules are produced in the heterotrophic utilization of organic compounds, and sometimes these products of metabolism produce undesirable tastes and odors in the water.

Many microorganisms can cause transformations of organic compounds, although they cannot use these compounds as carbon and energy sources. This phenomenon, called cometabolism, can produce undesirable products from relatively innocuous substances.

During the evolution of any microbiological ecosystem, the rate of cell death will at some time equal or exceed the rate of new cell formation. After death, cells lyse and release their contents to the environment, where some of them may cause unpleasant tastes and odors.

Both particulate and soluble organic materials are present in reclaimed water. Although significant amounts of particulate materials are filtered from the injectant near the well screen at or near the well bore/aquifer interface, a small fraction of particulate matter is probably also carried into the formation, where it can provide nutrients for microbial growth.

The fate of dissolved organic substances is uncertain. Sorption processes could concentrate dissolved organic nutrients previously too dilute for microbial assimilation into local, highly concentrated areas on solids, where they might then be utilized by microbes. Kaolinite, illite, and lignite, which are present in the Magothy aquifer at Bay Park, are known to have sorptive capacity for organic molecules. However, no measurements of the sorptive capacity of materials in the Magothy aquifer have been made.

To investigate the effects of microbial activity on organic substances in the formation, a recharge test involving injection of reclaimed water having higher than average COD levels was run. In test RW10 (described above), 5 Mgal of reclaimed water having an average COD of 22 mg/L and a MBAS concentration of 0.55 mg/L was injected at a rate of 350 gal/min for 10 days. The injected water subsequently was repumped briefly on five occasions over a 2-month period. A second period of recharge followed, in which an additional 2 Mgal of reclaimed water with a COD of 9 mg/L and MBAS concentration of 0.07 mg/L was injected; this was followed by another repumping period to remove the injected water from the aquifer.

Ku and others (1975) presented graphs showing concentrations of nitrogen, phosphate, chloride, COD, and MBAS at the observation well 20 feet from the recharge well (well N7886) as the reclaimed water moved through the formation (fig. 7).

Replacement of native water by reclaimed water at the observation well 20 feet from the injection well, as indicated by chloride concentrations, was complete after  $5 \times 10^5$  gal had been injected. After that, chloride concentration at the observation well remained essentially constant at a value close to that of the injected water. Total nitrogen concentration approached the nitrogen level of the injectant, but at a slightly slower rate than the chloride. MBAS and COD concentrations were about half their respective concentration in the injected water.

After the initial injection of 5 Mgal of reclaimed water, the injection well was left idle for 2 months except for brief pumping episodes. Analysis of water samples from the injection well 35 days after cessation of injection showed concentrations of dissolved iron, total organic carbon (TOC), and COD of 40 mg/L, 21 mg/L, and 50 mg/L, respectively. Concentrations of dissolved iron, TOC, and COD in the reclaimed water were 0.2 mg/L, 6 mg/L,

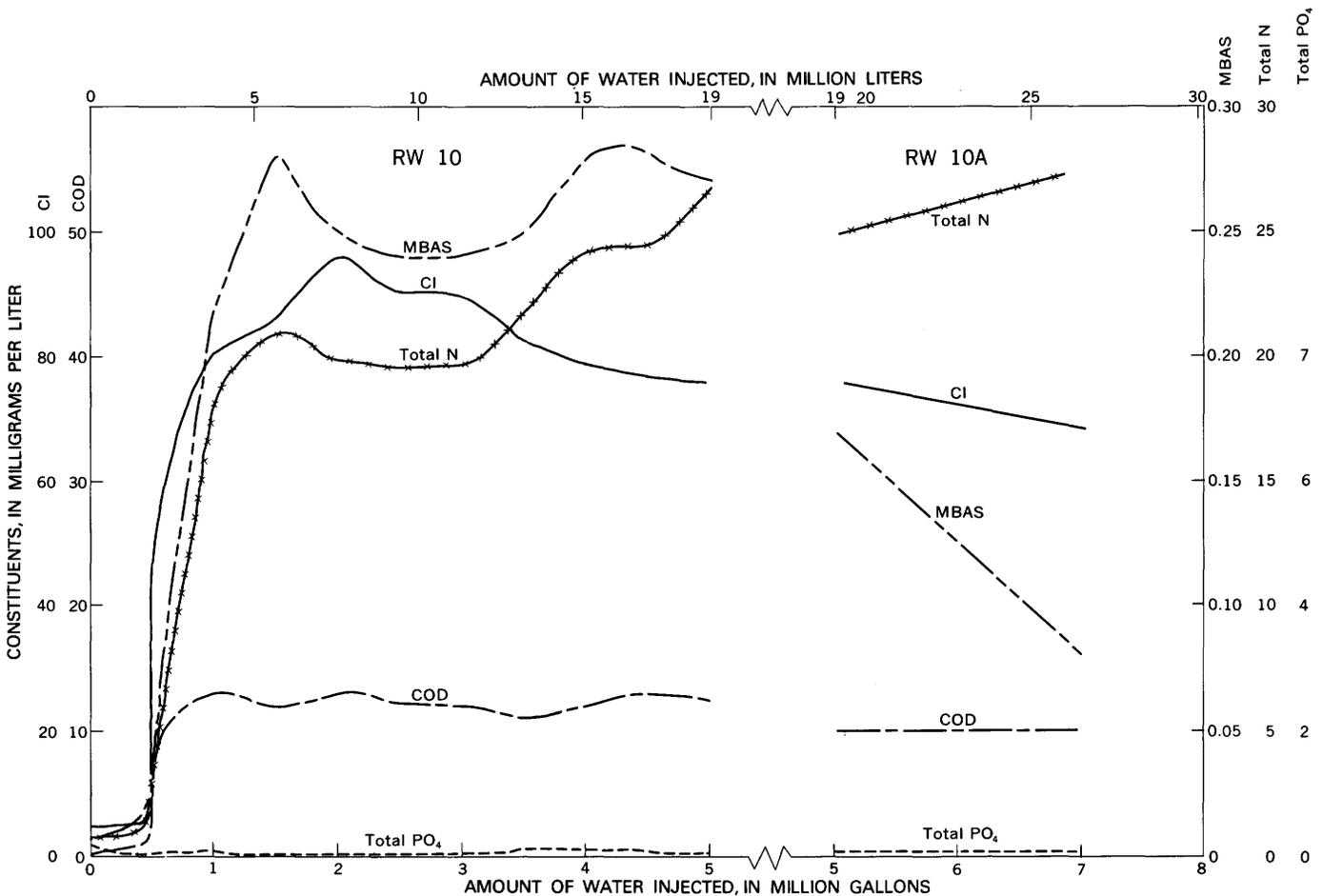


FIGURE 7.—Changes in concentration of total nitrogen, total phosphate, MBAS, COD, and chloride at observation well N7886, 20 ft from injection well during recharge. (Modified from Ku and others, 1975.)

TABLE 11.—Bacterial counts observed in injection well during 2-month resting phase, test RW10

[Total bacteria counts given as cells per 100 mL; denitrifying bacteria and sulfate reducers given as most probable number per 100 mL]

Date	Total bacteria count	Denitrifying bacteria count	Sulfate reducers count
11-11-71-----	1 x 10 <sup>8</sup>	140	--
11-23-71-----	9 x 10 <sup>8</sup>	--	--
12- 7-71-----	5 x 10 <sup>8</sup>	--	--
12-14-71-----	4.3 x 10 <sup>8</sup>	400	--
12-15-71 <sup>1</sup> -----	7.5 x 10 <sup>8</sup>	2.4 x 10 <sup>4</sup>	4.6 x 10 <sup>5</sup>
12-15-71 <sup>2</sup> -----	2.5 x 10 <sup>8</sup>	>2.4 x 10 <sup>4</sup>	4.6 x 10 <sup>5</sup>

<sup>1</sup>Taken 14 minutes after onset of pumping.<sup>2</sup>Taken 60 minutes after onset of pumping.

and 22 mg/L, respectively. Results of bacterial counts made during the resting period are given in table 11.

This approximate tripling of the TOC and doubling of COD indicates that the concentration of soluble organic compounds in the water near the well was increasing, probably at the expense of insoluble particulate organic matter in the filter mat.

The increase of Fe<sup>+2</sup> concentration in the area near the well was similar to the increase of Fe<sup>+2</sup> in soils shortly after waterlogging. Although the exact mechanism of this transformation is not known, it has been shown to be a consequence of bacterial metabolism (Alexander, 1961).

Many genera of bacteria are able to induce the reduction of Fe<sup>+3</sup> in compounds such as Fe(OH)<sub>3</sub>, FeOOH, FePO<sub>4</sub>, and Fe<sub>2</sub>O<sub>3</sub>. Included in table 11 are several genera reported to be active iron reducers. Tests of the iron-reducing ability of isolates from tests RW7 and RW8 were made by the method described by Ottow (1968). Several isolates of *Pseudomonas* type 2 showed small but definite iron-reducing activity. *Pseudomonas* type-2 organisms are also vigorous denitrifiers. This is in agreement with the observations made by Ottow (1968) that organisms having inducible nitrate-reductase activity frequently also showed iron-reducing activity.

Samples of water from the observation well 20 feet away were analyzed for bacterial count during the initial

TABLE 12.—Bacterial counts in observation well 20 feet from injection well during initial injection phase, resting phase, and after final redevelopment phase of tests RW10 and RW10A

Date	Phase	Total bacterial count (cells per 100 mL)
11- 4-71-----	Initial injection-----	3,150
11- 6-71-----	Initial injection-----	2,600
11- 8-71-----	Initial injection-----	5,000
11-11-71-----	Initial injection-----	5,800
11-23-71-----	Resting-----	1,000
12- 1-71-----	Resting-----	13,100
12-14-71-----	Resting-----	3,700
2-23-72-----	Final redevelopment-----	5,400

injection phase, the resting phase, and the final injection phase of tests RW10-10A (table 12). Water from that well during the resting period showed that total N and COD concentrations had decreased slightly, whereas the MBAS concentration had dropped from 0.27 mg/L to 0.17 mg/L. The COD decrease was about 2 mg/L, which suggests that some degradation of organic matter took place; however, a significant increase in bacterial numbers was not noticed.

At the end of the 2-month resting stage, an additional 2 Mgal of reclaimed water was injected. The carbon filtration stage was employed during this phase, and COD and MBAS concentrations were 9 and 0.07 mg/L, as opposed to 22 and 0.55 mg/L during the first phase. COD concentration at the well 20 feet away did not change during the second phase, although MBAS concentration declined linearly from nearly 0.17 mg/L at the start of the second phase to about 0.07 mg/L after the injection of 2 Mgal.

A volume of water about 1.5 times the volume injected was withdrawn from the injection well after the second phase to restore the ground-water quality to pretest conditions. Ku and others (1975) calculated mass balances for the constituents as shown in table 13.

The near-total recovery of nitrogen suggests only limited assimilation by microbes during the test. If it is assumed that microbial assimilation and mineralization accounted for the entire COD loss and that the pertinent reactions involved carbon at the oxidation level of a carbohydrate, then the calculated amount of carbon assimilated in these processes was 172 lb. According to Stanier and others (1970), the maximum cell yield is at most 50 percent of the total of carbonaceous substrate converted by combined respiration and cell synthesis; hence, the microbial cell mass produced was no more than 86 lb. Since the carbon/nitrogen ratio in bacterial cells is normally about 8, it is seen that only about 11 lb of nitrogen could be accounted for in this way, although the total loss was 368 lb.

TABLE 13.—Mass-balance data on chloride, total nitrogen, methylene blue active substances, and chemical oxygen demand, tests RW10 and RW10A

[Modified from Ku and others, 1975]

Constituent	Mass, in kilograms		Percentage	
	In water injected	In water recovered	In water injected	In water recovered (assuming 100-percent recovery of chloride)
Chloride-----	2,100	1,780	84	100
Total nitrogen (N)-----	755	588	78	93
Methylene blue active substances (MBAS)-----	11.2	4.9	43	51
Chemical oxygen demand (COD)-----	494	207	42	50

Because MBAS and COD concentrations at the 20-foot-distant observation well changed little during the 2-month resting period, it is concluded that the major part of the microbial activity associated with the system remained in the near vicinity of the injection well. Apparently, significant concentrations of soluble and particulate organic materials capable of sustaining anaerobic microbes did not travel as far as 20 feet into the formation.

The final injection test (RW13) began on October 24, 1972, and ended on May 11, 1973. Results of test RW13 are given in Vecchioli and others (1975), and a summary log of the test was presented by Sulam (1973). In general, the results of test RW13 were similar to those of most earlier tests. Reclaimed water had effectively displaced the formation water at the observation well 20 feet from the injection well after  $5 \times 10^5$  gal had been injected. Some difficulties with COD measurement arose during the test, but the MBAS measurements indicated that both substances traveled at roughly the same rate as the reclaimed-water front. The steady-state concentration of MBAS at the observation wells 20 feet and 100 feet away was close to that in the injected water. Total nitrogen levels in water at both observation wells were close to those in the injected water.

Total bacteria count was measured in both observation wells during test RW13 (table 14).

Bacterial densities in the observation wells were highly variable during test RW13. Densities were generally higher at the well 20 feet away than at the well 100 feet away, and densities at the nearer well were generally somewhat lower during test RW13 than during test RW10, which may reflect the reduced COD levels in the injectant during test RW13. If large amounts of microbial nutrients had been transported into the formation,

TABLE 14.—Microbiological quality of water from observation wells 20 and 100 feet from injection well, test RW13

[Total bacteria counts given as cells per 100 mL; sulfate reducers given as most probable number per 100 mL. ND, not determined]

Well N7886			Well N7890		
Date	Total bacteria	Sulfate reducers	Date	Total bacteria	Sulfate reducers
11- 7-72----	320	--	--	--	--
11-12-72----	--	70	--	--	--
11-21-72----	1	<30	--	--	--
11-29-72----	1,400	ND	11-28-72	4	ND
12-15-72----	6,700	40	12- 5-72	400	30
12-20-72----	1	30	12-19-72	2	30
1- 3-73----	2,000	ND	--	--	--
1-10-73----	5	30	1- 9-73	73	--
1-17-73----	14	40	--	--	--
1-31-73----	30	186	1-30-73	14	80
2- 7-73----	--	30	2- 6-73	3	40
2-13-73----	690	ND	2-12-73	167	ND
3- 4-73----	49	ND	3-20-73	7,100	ND
3-29-73----	136	23	3-28-73	38	23
--	--	--	4- 3-73	206	ND
--	--	--	4-11-73	4	--
5- 2-73----	12	ND	5- 3-73	220	43
5- 9-73----	340	ND	--	--	--

high bacterial densities would be expected. However, the above test results suggest that little soluble biotransformable material remained in the reclaimed water.

Sulfate-reducing bacteria were detected in half the samples collected during test RW13. The presence of  $H_2S$  in native formation water suggests that sulfate-reducing bacteria might be indigenous to the aquifer. The effect of an increased potential nutrient supply upon the activity of these organisms is not clear from the results of these tests. It is possible, however, that an increased level of  $H_2S$  could result if a higher level of nutrients were available to those organisms. Also, the presence of sulfate-reducing bacteria indicates a relatively low Eh, which indicates that oxygen and chlorine were absent.

### PUBLIC HEALTH CONSIDERATIONS

Introduction of pathogenic organisms and their dissemination through the surrounding aquifer is one of the primary concerns when treated sewage is injected directly into ground-water bodies. The movement of bacteria through aquifers has been thoroughly studied. Romero (1970) summarized the characteristics of bacterial movement through porous media. A partial listing of these characteristics follows:

1. Bacteria travel with the flow of water; they do not move against the hydraulic gradient.
2. The rate of bacterial removal by filtering is a function of the nature of the aquifer materials. Aquifer materials best suited for removal of biological contaminants are silt and fine sand with high clay content.
3. The rate of bacterial removal by filtering can be characterized by a function called the filter efficiency of the aquifer (called filterability by California State Water Pollution Control Board, 1954, and Romero, 1970).
4. Typically, the maximum distance that bacteria travel in porous materials ranges from 50 to 100 feet.
5. Under favorable conditions (not necessarily applicable to the Magothy aquifer), bacteria may survive as long as 5 years.

In December 1970, during test RW8, unchlorinated reclaimed water containing substantial numbers of total coliforms, fecal coliforms, and fecal streptococci was injected for a 10-day period. Water from the observation well N7886, 20 feet from the injection well, was monitored for specific conductance to determine the arrival of injected water. Results showed that the injected water had almost displaced the native water at the nearest observation well after the second day of injection.

Neither fecal-coliform nor fecal-streptococcal bacteria were found in any samples collected from the observation

TABLE 15.—*Bacterial colony counts of water sampled during and after test RW8, with unchlorinated water*

[From Vecchioli and others, 1972. All results in number of cells per 100 mL; (e), estimated count based on nonideal colony count]

Date	Volume of injected water (gal)	Observation wells		Injection well <sup>1</sup>
		20 feet from injection well	100 feet from injection well	
Total coliform				
12- 1-70-----	9,700	19	--	--
12- 2-70-----	27,000	<1	--	--
12- 3-70-----	10,000	<1	--	--
12- 4-70-----	47,000	<1	--	--
12- 5-70-----	60,000	1(e)	--	--
12- 6-70-----	61,000	1(e)	--	--
12- 7-70-----	60,000	1(e)	<1	--
12- 8-70-----	54,000	<1	<1	--
12- 9-70-----	55,000	<1	1	--
12-10-70-----	--	--	--	83,000
1- 5-71-----	--	4(e)	<1	3,200
Fecal coliform				
12- 1-70-----	200	<1	--	--
12- 2-70-----	1,200	<1	--	--
12- 3-70-----	--	--	--	--
12- 4-70-----	2,000	<1	--	--
12- 5-70-----	1,500	<1	--	--
12- 6-70-----	750(e)	<1	--	--
12- 7-70-----	1,100	<1	<1	--
12- 8-70-----	2,200	<1	<1	--
12- 9-70-----	960	<1	<1	--
12-10-70-----	--	--	--	2,800
1- 5-71-----	--	<1	<1	10(e)
Fecal streptococcus				
12- 1-70-----	1(e)	<1	--	--
12- 2-70-----	80	<1	--	--
12- 3-70-----	11(e)	<1	--	--
12- 4-70-----	39	<1	--	--
12- 5-70-----	31	<1	--	--
12- 6-70-----	30	<1	--	--
12- 7-70-----	10(e)	<1	<1	--
12- 8-70-----	15(e)	<1	<1	--
12- 9-70-----	22	<1	<1	--
12-10-70-----	--	--	--	57
1- 5-71-----	--	<1	<1	32

<sup>1</sup>Water pumped from injection well after end of injection on Dec. 10, 1970.  
<sup>2</sup>All counts reported as <1 represent no colonies counted in a 100-ml sample.

wells during test RW8 (table 15). Low numbers of total coliform bacteria were found in the observation well on the 2d, 6th, 7th, and 8th days of the test and also in a sample obtained from that well 26 days after the end of the test.

Rate of travel of bacterial cells through an aquifer is controlled by complex interactions of physical, chemical, and biological factors. Nevertheless, in an investigation of the travel of pollutants, the California State Water Pollution Control Board (1954) found that, at maximum penetration of coliforms into the aquifer, the logarithm of the coliform density decreased linearly with distance from the injection well. The Board called this property of impeding bacterial travel the filterability of the aquifer, herein called the coefficient of filter efficiency.

The filter efficiency of the aquifer is defined by the following equation:

$$\text{Log } N_2 = \text{Log } N_1 - F (r_2 - r_1)$$

where:

- $N_1$  = most probable number of organisms at any sampling point in the aquifer ( $r_1$ );
- $N_2$  = most probable number at any other point ( $r_2$ );
- $r_1, r_2$  = distance between sampling points and point of injection with  $r_2 > r_1$ ; and
- $F$  = coefficient of filter efficiency of the aquifer.

When the equation is applied to the Bay Park observations with values of 50,000 cells per 100 mL for the water injected ( $r_1 = 0$ ), and 1 cell per 100 mL for water from the observation well 20 feet from the injection well ( $r_2 = 20$ ), the calculated coefficient of filter efficiency of the Magothy aquifer is 0.23/foot. The coefficient is conservatively low because the coliform density used in the calculation (1 per 100 mL) was the highest count observed during the test. The high first-day count was not included in the interpretation.

Fractional reduction in bacterial density per foot of travel may be determined from the following formula (California State Water Pollution Control Board, 1954, p. 106):

$$-F = \log(1 - R)$$

where  $R$  = fractional reduction of bacteria per foot of travel. With an  $F$  value of 0.23, the computed  $R$  value is 0.41. This is interpreted as a 41-percent reduction in bacterial count per foot of travel.

In the California study, the coefficient of filter efficiency varied somewhat with direction of water movement from the injection well, but the average  $R$  values ranged from 19 to 24 percent (California State Water Pollution Control Board, 1954, p. 107). The aquifer under test in the California study, a stratum of sand and pea gravel, had a hydraulic conductivity of about 250 ft/d. The injection zone in the Magothy aquifer at Bay Park, a slightly silty fine to medium sand with thin beds of coarse sand, had an average hydraulic conductivity of about 130 ft/d (G. D. Bennett, written commun., 1970). On the basis of grain size and hydraulic conductivity, the greater fractional reduction values found for the Magothy aquifer seem reasonable.

The California State Water Pollution Control Board Study of 1954 noted that coliform counts in water from observation wells reached maximum values and then decreased as injection continued, possibly because of the filtering effect of a mat that formed in the vicinity of the injection well during injection. Some bacteria in the injectant were presumably captured by the filter mat,

which consisted of solids accumulated at the interface between the injection well and the aquifer (California State Water Pollution Control Board, 1954, p. 153). Formation of a filter mat and (or) microbial slime deposits was noted also in the Bay Park study. Bacterial capture by the filter mat complicates evaluation of computed aquifer filter efficiency. However, this effect can be minimized by using bacterial-density data from earlier in the injection period, before the filter mat had developed appreciably. The computed coefficient of filter efficiency for the early period would approximate the filtration component related to the effective porosity of the aquifer.

In this study, a significant number of indicator bacteria survived for at least 26 days in the vicinity of the injection well. Virtually undiluted injected water reached the observation well 20 feet from the injection well within 2 days. Only a nominal coliform count was observed in this water. If the bacterial cells had been traveling at a rate comparable with that of the injectant, significant coliform counts should have been observed on the second day. Large diminution of counts as a result of die-off would not be expected in only 2 days because the bacteria could survive for as long as 100 days (Romero, 1970, p. 42). Therefore, filtration phenomena rather than organism die-off were probably the primary reason for the large reduction in numbers. Similar conclusions were reached by the California State Water Pollution Control Board (1954, p. 113).

The results of the Bay Park study are consistent with the conclusion reached in other studies (Romero, 1970) that coliform bacteria travel only short distances in fine-grained material. Comparatively little work has been done with bacterial species other than coliform. Results of such studies suggest, however, that other species behave in the same way as the coliforms, and there is no reason to suppose that they would be more mobile than the coliforms.

### SUMMARY

Tertiary-treated sewage (reclaimed water) was injected into a fine to medium sand aquifer through a well. As long as a chlorine residual of 2.5 mg/L or greater was maintained in the injectant, interference from biological growth was minimal. The effect of residual chlorine did not extend more than 12 feet into the aquifer from the injection well, as indicated by the isolation of significant numbers of bacteria in water recovered from the injection well after relatively short pumping periods.

The composition of the bacterial population in the formation is not the same as that in the injectant. The proportion of coliform bacteria in the aquifer population was consistently much lower than in the injectant.

During injection, essentially aerobic assimilation of organic matter filtered from the injectant occurs in the vicinity of the well bore/aquifer interface. Continued microbial activity after cessation of injection leads to establishment of anaerobic conditions and the appearance of obligate anaerobic bacteria. Biotransformation under anaerobiosis can decompose the mat and thereby diminish the effects of clogging built up during injection.

Soluble organic material in the reclaimed water may be adsorbed onto clay and lignite in the formation. Additional soluble organic matter may be released by microbial action onto insoluble particulate organic matter in the clogging mat. The soluble matter could support an indigenous microbial population in the formation.

Travel of bacterial cells in the Magothy aquifer at Bay Park during injection was found not to exceed distances of 20 feet laterally from the injection point.

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