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Microbial Biomass, Activity, and Community Structure of Water and Particulates Retrieved by Backflow from a Waterflood Injection Well

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Oil field injection water was allowed to back flow from two wells at the Packard drill site in Los Angeles, Calif., and was sampled at various times to obtain information about the biomass, potential activity, and community structure of the microbiota in the reservoir formation and in the injection water. Biomass was greatest in water samples that came from the zone near the injection site and dropped off sharply in subsequent samples, which were assumed to come from zones farther away from the well. Samples obtained from near the well also had visible exopolysaccharide blankets, as seen in scanning electron microscopic preparations. In one of the wells that was sampled, rates of glucose or acetate incorporation into microbial lipids correlated with biomass; but in the other well, activities correlated with the sampling time (volume of water that back flowed). Transmission electron micrographs showed a diverse, gram-negative bacterial population in a variety of physiological states. The analysis of the phospholipid ester-linked fatty acid profiles of the samples revealed consistently large proportions of 18:1ω7c fatty acids, indicating the presence of many anaerobes, facultative organisms, or both. Proportions of cyclopropyl fatty acids and ratios of *trans/cis* monoenoic compounds increased with the volume of water that back flowed (analogous with the distance into the formation), while the ratio of unsaturated/saturated compounds decreased, possibly indicating higher levels of stress or starvation in the microbial communities farthest from the injection well. Greater than 90% of the total biomass was trapped on glass fiber filters, indicating that the microbiota were largely attached to particles or were clumped. These sampling techniques and analytical methods may prove useful in monitoring for problems with microbes (e.g., plugging) in waterflood operations and in the preparation of water injection wells for enhanced oil recovery by the use of microbes.

The injection of pressurized water into oil reservoirs has been used since the early part of the twentieth century as a means of enhancing petroleum recovery. This method of secondary oil production has often been hindered by the effects of bacteria, which corrode piping and reduce the permeability of the reservoir matrix by effectively plugging the pore spaces with biomass, extracellular polysaccharide products, and precipitates such as iron oxides and iron sulfides (14). These bacteria, including sulfate reducers, iron bacteria, and slime-forming bacteria, are commonly found in the injection waters that are forced into the reservoirs during waterflooding (23). Bacteria which form extensive exopolysaccharide materials are also commonly isolated from corroding oilfield pipeline systems and crude oil (40, 44). As a remedial cleanup measure, injection wells are often stimulated by expensive physical and chemical treatments to remove or dissolve the fouling microbes and precipitates, thus enhancing the water injection rate of the reservoir (9).

The ability of various bacteria to penetrate and colonize petroleum reservoirs has led to the development of several microbial-enhanced oil recovery techniques. The use of in situ fermentation leading to the production of gas has resulted in periods of enhanced recovery from some formations (22, 32); and the potential of certain bacteria to enhance oil recovery by producing acids, biopolymers, or biosurfactants in situ is being evaluated. Perhaps the most promising use of microbial-enhanced oil recovery is in the reduction of reservoir heterogeneity through flow diversion and selective permeability reduction or plugging, which improves the sweep efficiencies of the injection water. If bacteria could be injected into the breakthrough zones caused by channeling or if the indigenous microbes in these zones could be stimulated to grow and produce exopolysaccharides, the permeability of these zones could be reduced enough to allow the injection water to penetrate into the other oil-bearing areas of the reservoir.

Before any of these techniques can be evaluated adequately and developed to their full potential, much more information is needed concerning the ecology of the microbial communities in water injection wells (32). The purpose of this study was to evaluate the microbiota of a waterflooded oil reservoir through the analysis of injection waters retrieved from the formation and to assess the applicability of various sampling and analytical methods to this system.

MATERIALS AND METHODS

Site description. Samples of injection water were obtained from the East Beverly Hills Field Packard drill site, operated by Chevron USA, Inc., in Los Angeles, Calif. Previously injected produced water was allowed to back flow out of the injection wellhead under reservoir pressure when the injection was discontinued temporarily for 1 to 7 h. Water samples were retrieved at intervals (Table 1) during this

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TABLE 1. Sampling dates, sample designations, elapsed time, backflow volumes, temperature, and pH for injection water samples retrieved from wells P-38 and P-46 at the Packard drill site of the East Beverly Hills Oil Field, Los Angeles, Calif.

Injection well	Sampling date (mo/day/yr)	Sample	Elapsed time (h)	Well vol	Backflow vol (m^3)	Temp (°C)	pH
P-38	3/26/85	A	0.05	0.02	0.16	47	
		B	0.35	1.0	6.68	45	
		C	0.60	1.8	12.24	42	
		D	1.03	3.3	22.10	46	
P-38	11/19/85	A	0.05	0.02	0.16	45	7.2
		B	0.83	1.21	8.11	45	7.2
		C	1.58	2.31	15.41	48	7.1
		D	2.17	3.16	21.08	47	7.0
		E	4.42	5.81	38.79	47	7.0
		F	7.00	7.90	52.78	47	7.1
P-46	11/21/85	A	0.05	0.02	0.16	47	7.0
		B	0.67	0.89	6.49	43	7.0
		C	1.50	2.00	14.60	47	7.0
		D	2.25	2.99	21.89	47	7.0
		E	4.08	5.43	39.73	47	7.0
		F	6.08	8.09	59.19	49	7.0

continuous backflow. The two injection wells sampled penetrate at various angles into a deep (Hauser) sandstone formation to a vertical depth of 6,300 to 7,300 ft (1,920 to 2,225 m). The formation is geologically complex, containing sharp asymmetrically folded anticlines. Well P-38 was originally drilled in 1968 as an oil production well; was converted to a freshwater injection well in 1970; was changed over to a filtered brine injection well in 1977; and was stimulated by acid, sodium hypochlorite, and physical treatments in 1980 (9). Injection well P-46 was drilled in 1981.

Table 1 summarizes the sampling dates, times, volumes, temperatures, and pH determinations. The first samples taken from each well (at 0.05 h of elapsed time) are assumed to most closely approximate the quality of the injection water before it entered the reservoir. Volumes of back flow water are commonly expressed as well volumes, or hole volumes, which is the total volume of the well casings. The volumes of wells P-38 and P-46 are 42 and 46 barrels (6.68 and 7.31 m^3), respectively. The rates of injection (cubic meters per day) and the surface tubing pressure (pounds per square inch) for each well just prior to the backflow were 133 $m^3\ day^{-1}$ at 2,850 lb/in^2 for well P-38 on 26 March 1985, 167 $m^3\ day^{-1}$ at 2,850 lb/in^2 for well P-38 on 19 November 1985, and 506 $m^3\ day^{-1}$ at 3,186 lb/in^2 for well P-46 on 21 November 1985. Injection rates for the wells in this field typically ranged from 80 to 500 $m^3\ day^{-1}$.

The injection water for all wells at this site comes from a large common pool. Chlorine dioxide is added to this pool twice per week. An analysis of this injection water on 12 November 1985 gave the following compounds, at concentrations of milligrams per liter: Cl^-, 14,700; Na^+, 8,866; HCO_3^-, 1,220; Ca^{2+}, 345; Mg^{2+}, 330; $B_4O_7^{2-}$, 133; NH_4^+, 115; SiO_2, 88; I^-, 62; Ba^{2+}, 25; total Fe, 5; SO_4^{2-}, 2; CO_3^{2-}, 0; (pH 6.96). All water samples taken in November were thoroughly mixed with a magnetic stirrer during the subsampling procedures.

Microbial substrate incorporation into lipids. Water samples (2.0 ml) were incubated in the presence of 0.2029 μCi of [U-^{14}C]glucose (296 mCi/mmol) or 0.0437 μCi of [U-^{14}C]acetate (56 mCi/mmol) in the dark for 1.0 h immediately after samples were obtained. For the assays performed in March, incubation was at room temperature (25°C), while for those performed in November incubation was at 45°C. Following the incubation period, the total lipids were extracted, dried, and counted as described previously (37).

Lipid analysis. All extractions were performed at the field site immediately after samples were obtained. During the March survey, the backflow water was filtered through glass fiber filters (GFC; Whatman, Inc., Clifton, N.J.) and then through 0.45-μm-pore-size polycarbonate filters (Millipore Corp., Bedford, Mass.). During the November survey, the water was passed through 0.22-μm-pore-size polycarbonate filters (Millipore) after glass fiber filtration. The total lipids on the filters were extracted with chloroform-methanol (4) in glass bottles or Teflon centrifuge tubes (E. I. du Pont de Nemours & Co., Inc., Wilmington, Del.). The lipids were collected in the chloroform phase, the solvent was evaporated under a stream of nitrogen, and the samples were stored at −40°C until analysis. The phospholipids in the samples obtained in March were isolated by an acetone precipitation method (27), and silicic acid column chromatography (55) was used for the separation of the samples obtained in November. A mild alkaline methanolysis procedure was used to form the phospholipid ester-linked fatty acid derivatives (28), which were purified by thin-layer chromatography (27).

The phospholipid ester-linked fatty acids (PLFAs) were analyzed by high-resolution gas chromatography (flame ionization detector; 5880A; Hewlett-Packard Co., Palo Alto, Calif.) by using methylnonadecanoate (C^{19}) as an internal standard. A nonpolar, cross-linked methyl silicone capillary column (50 m by 0.2 mm [inner diameter]; Hewlett-Packard) was used with hydrogen as the carrier gas. After splitless injection, the temperature was programmed for a 5°C/min increase from 50 to 160°C, followed by a 2°C/min increase to 300°C. Peak identifications were made by retention time comparisons with known standards (3502 laboratory data system; Hewlett-Packard) and by gas chromatography-mass spectral analysis of representative samples from this study. The gas chromatographic-mass spectrometric system (5996A; Hewlett-Packard) had a direct capillary inlet and the same type of column described above. After splitless injection at 100°C, the temperature was increased to 300°C at 4°C/min. The carrier gas was helium, and the operating parameters of the mass spectrometer were as follows: electron multiplier, 1,300 to 1,400 V; transfer line, 300°C; source and analyzer, 250°C; autotune file normalized; optics tuned at *m/z* 502; electron impact energy, 70 eV. The mass spectral data were processed with a data system (RTE-6/vm; Hewlett-Packard).

The fatty acid nomenclature used is the number of carbon atoms in the chain: the number of double bonds, followed by the position of the double bond from the methyl (ω) end of the molecule. The prefixes *i* and *a* refer to *iso* and *anti-iso* branching, respectively; poly refers to a polyenoic compound; and cy refers to cyclopropane structures. The suffixes *c* and *t* refer to *cis* and *trans* bond orientations, respectively.

Transmission electron microscopy. Water samples were fixed for 2 h at 25°C in the field in 5% glutaraldehye in a 50:50 mixture of field water-cacodylate buffer (0.067 M; pH 7.2) immediately after recovery. The fixed samples were centrifuged, decanted, and washed twice in cacodylic acid buffer (0.067 M; pH 7.2). The material was later enrobed in 4% agar, postfixed with 2% OsO_4 for 2 h, washed, serially dehydrated, and embedded in Spurr resin (Electron Micros-

copy Sciences). Thin sections were stained with uranyl acetate and lead citrate (42).

Scanning electron microscopy. Samples collected on 0.45-μm-pore-size polycarbonate filters (Millipore) were fixed in 5% glutaraldehyde in cacodylate buffer (0.067 M; pH 7.2), washed in cacodylic acid buffer, serially dehydrated into ethanol and then into Freon 113 (du Pont), dried to the critical point, and coated with gold-palladium prior to examination (10).

RESULTS

Electron microscopy. Scanning electron microscopy of the particulate samples collected on 0.45-μm-pore-size filters revealed some qualitative differences in the water samples taken at various times (backflow volumes) from well P-38 in March. Individual bacteria were difficult to see because of the particulate matter, but many bacteria were visible in samples B, C, and D (Fig. 1). Samples B and C were distinguished from the others by the presence of large amounts of slime that coated the bacteria and particulates; this was presumably exopolysaccharide glycocalyx material. Few bacteria were distinguishable by scanning electron microscopy in the samples taken in November, perhaps because of the extensive buildup of particulates on the filters.

Transmission electron microscopy of the material centrifuged out of the water samples taken in November revealed a diverse assemblage of gram-negative bacteria (Fig. 2 and 3). The bacteria retrieved from well P-38, which had been sampled 7 months earlier, appeared to be numerous and diverse in all of the different samples, although diversity appeared to decline in the last two samples taken (Fig. 2C and D). Samples A and C (Fig. 2A and B, respectively) contained bacteria that appeared to be healthy, including some dividing cells. Other cells in these same samples appeared to be in various degrees of health or starvation, as indicated by the various degrees of centralization of the nuclear (electron-clear) region and cell membrane shrinkage (22). Some cells had irregular "blips" on their cell surfaces. In addition, a few remnants of dead cells were visible in these samples. In sample E (Fig. 2C), some large colonies of cells were found to be intact. The last sample, which was taken after 7.9 well volumes of backflow (sample F), contained many filaments of square-ended bacteria surrounded by electron-clear sheathlike structures (Fig. 2D), some of which were separated by spacers that were similar to those of some methanogenic archebacteria (3, 25).

The samples from injection well P-46 examined by transmission electron microscopy generally contained fewer bacteria than did those from well P-38, but again, several different types of gram-negative organisms were seen (Fig. 3). Most of the bacteria seen in the transmission electron micrographs from samples from this well appeared to be in a relatively healthy state, and all cells were seen to be surrounded by fibrous glycocalyx material.

Phospholipid fatty acids. Concentrated extraction solvent procedural blanks were processed along with the injection well lipids, to serve as contamination controls. No significant peaks with retention times corresponding to known PLFAs were found by gas chromatography. Small peaks corresponding to less than 0.5 pmol/ml of water sample were found in some of the blanks at retention times corresponding to those for 17:0, *i*18:0, 18:0, and 16:0 (less than 1.0 pmol/ml) fatty acids.

Most of the water samples were extracted and analyzed in triplicate. Of the samples taken in November, the variance in the fatty acid data was generally lower for the samples from well P-38 than for those from well P-46. For fatty acids present in concentrations of 5.0 pmol/ml or more, the coefficients of variation for well P-38 ranged from 0.5 to 35.1, with most being below 10.0, and for well P-46 they ranged from 1.6 to 36.8, with most being below 25.0. Variances were higher in the samples taken from well P-38 in March, possibly because of insufficient mixing during subsampling.

Table 2 shows the PLFA profiles of injection water samples taken at six different times during the backflow of well P-46. Very few of the PLFAs that were associated exclusively (5) with microeucaryotes or cyanophytes (polyenoic and long-chain fatty acids) were found in any of the samples from either well. Small amounts of polyenoic C_{16} and C_{18} PLFAs were present in some samples, totaling no more than 7.0% of the total PLFAs.

The total amounts of PLFAs per ml of water were generally highest in samples taken after approximately 2 well volumes of water had back flowed, subsequently dropping off by 2 orders of magnitude (Fig. 4). However, when well P-38 was sampled for the second time in November, the first sample taken had the highest biomass, with total PLFAs continuing to decrease in later samples and correlating with the volume of water that back flowed ($P < 0.01$; Fig. 4).

For some of the samples taken in November, the glass fiber filters were extracted separately from the 0.22-μm-pore-size filters. In all such cases, an overwhelming proportion of the total PLFAs recovered was associated with the glass fiber filter, with relatively little biomass passing through to the 0.22-μm-pore-size filter (Fig. 4). With the exception of the F samples (Table 1), the proportion of total PLFAs associated with the 0.22-μm-pore-size filters ranged from 0.5 to 5.6% for well P-38 and from 0.9 to 9.1% for well P-46. The F samples had a somewhat higher proportion of PLFAs in the smaller-size fraction (12.9 and 18.5% for wells P-38 and P-46, respectively). Some differences in the PLFA profiles between the two different size fractions were also found. In general, the glass fiber filter fractions had proportionally more *i*18:0 and 18:1ω7*c* fatty acids, while the fraction on the 0.22-μm-pore-size filter had more 18:0 and 18:1ω9*c* fatty acids.

Some similar trends in the relative proportions of certain PLFAs with the backflow volumes were found for wells P-38 and P-46 in November (Table 3). The proportion of 15:0, 16:0, *a*15:0, 16:1ω7*c*, and 16:1ω7t in relation to the total amount of PLFAs was at a minimum in water from sample F, while conversely, the proportions of 17:0, 18:0, *i*17:0, *a*17:0, *i*18:0, cy19:0, and 18:1ω9*c* were at their highest in sample F (Table 3). In general, the proportions of 14-, 15-, and 16-carbon PLFAs correlated negatively with backflow volume (depth), while the 17- and 18-carbon PLFAs and cyclopropyl PLFAs correlated positively with backflow volume (Table 3). However, the polyenoic PLFAs and 18.1ω7*c* fatty acids did not follow these trends, and *i*16:0 fatty acids correlated positively with the volume that back flowed from well P-46. Similar trends in the proportions of 18:0, *a*15:0, *i*17:0, 16:1ω7c, and 18:1ω9C fatty acids with backflow volume were found in the fractions on the 0.22-μm-pore-size filter.

Microbial activity. The rates of incorporation of ^{14}C-labeled acetate into lipids by the microbiota in samples from wells P-38 and P-46 in November are shown in Fig. 5. The incorporation of ^{14}C-labeled glucose into lipids followed a similar pattern, and both generally decreased with increasing backflow volume. Acetate and glucose incorporation rates correlated with the volume of water that back flowed in well

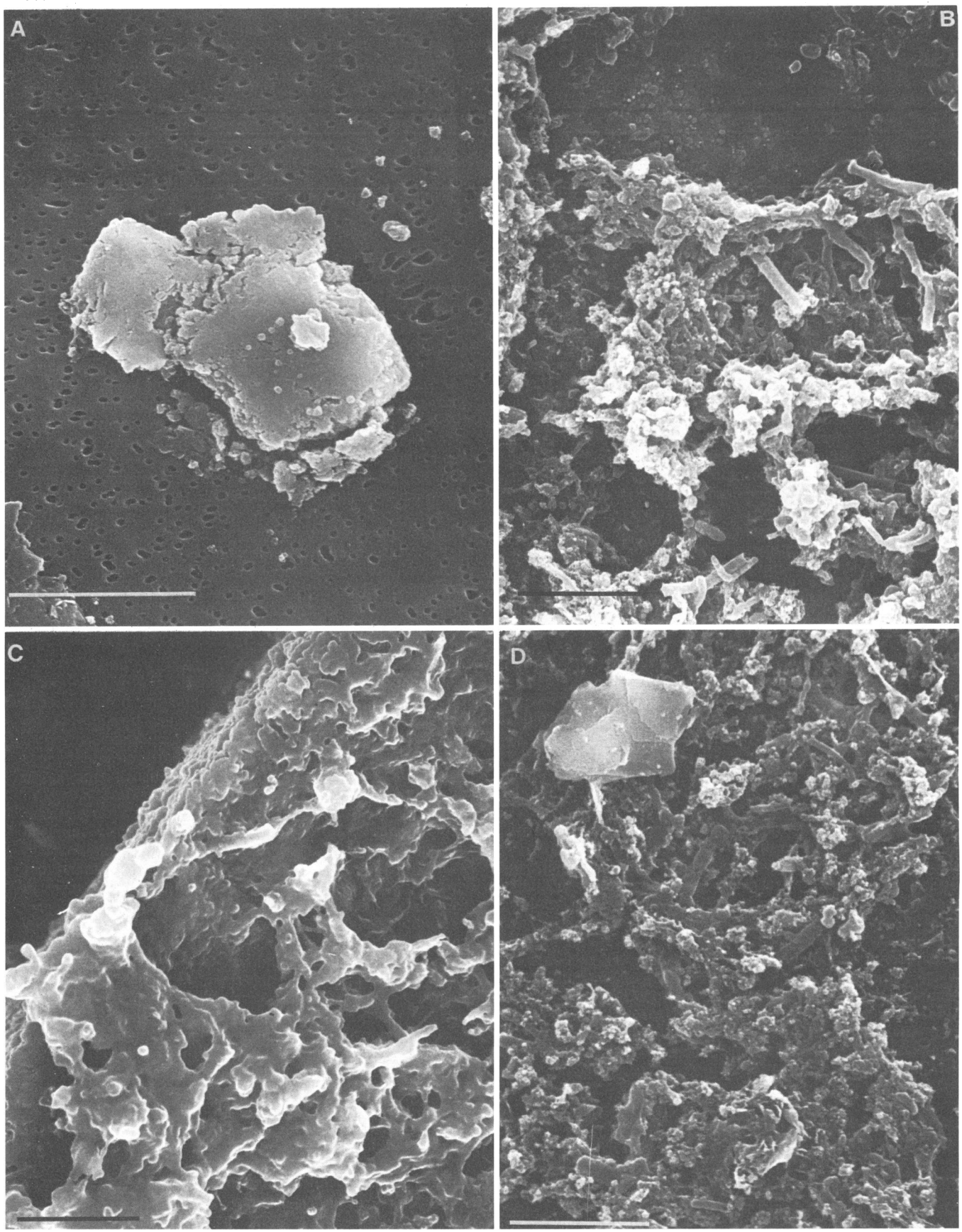

FIG. 1. Scanning electron micrographs of particles and bacteria trapped on 0.45-μm-pore-size membrane filters after 0.02 (A), 1.0 (B), 1.8 (C), or 3.3 (D) well volumes of injection water had back flowed out of well P-38 in March 1985. Note the large amounts of extracellular slime associated with the particles and organisms in panels B and C. Bars, 5.0 μm.

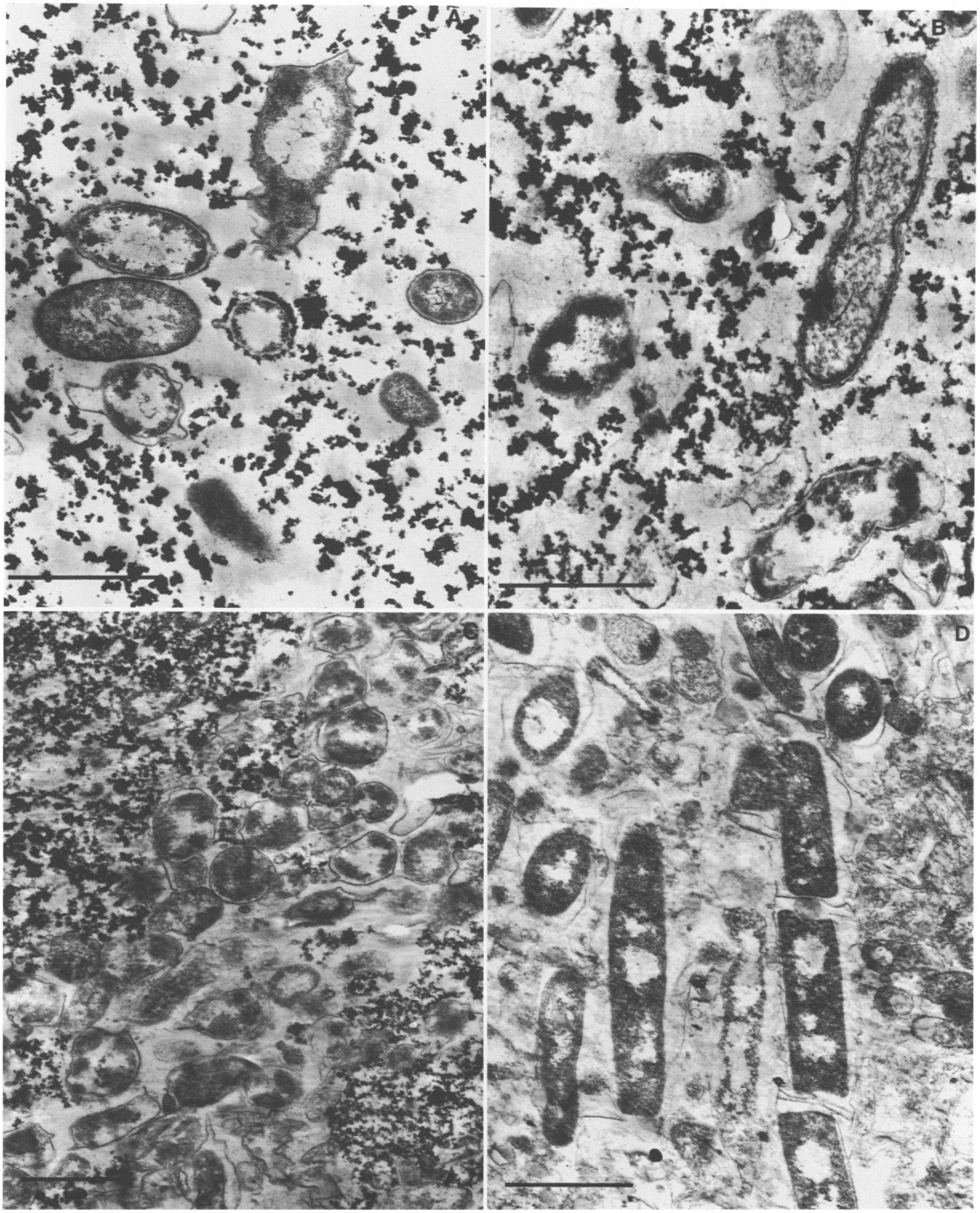

FIG. 2. Transmission electron micrographs of material centrifuged out of water samples taken after 0.02 (A), 2.31 (B), 5.81 (C), or 7.90 (D) well volumes had back flowed out of well P-38 in November 1985. All samples contained large numbers of glycocalyx-enclosed gram-negative bacteria, and sample F (Figure 2D) contained morphologically distinct square-ended archebacteria. Bars, 1.0 μm.

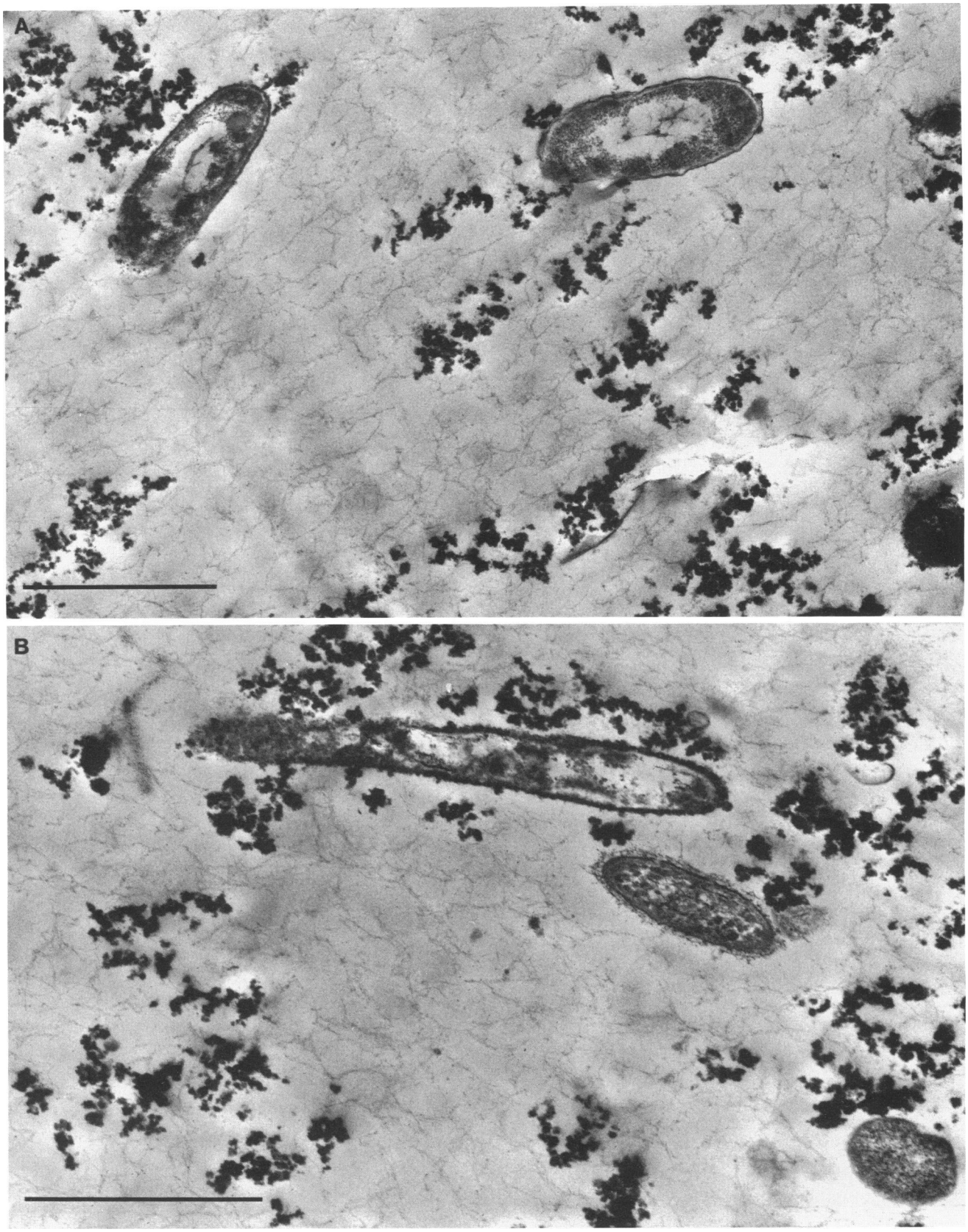

FIG. 3. Transmission electron micrographs of material centrifuged out of injection water after 2 well volumes of water had back flowed out of well P-46. All bacteria seen in this figure were gram-negative, and all were found within a fibrillar glycocalyx matrix in which electron-dense particulate material was also embedded. Bars, 1.0 μm.

P-46 ($P < 0.01$) but not that which backflowed in well P-38. In well P-38, however, incorporation rates correlated with total PLFA ($P < 0.01$).

DISCUSSION

Variation in the microbial communities with volume of backflow. The first samples taken from each injection well were of water from near the top of the well. These samples may be representative of the injection water as it enters the formation, although some accumulation of biofilm and particulates in dead spaces and on surfaces at the wellhead may lead to an overestimate of the biomass of the injection water as represented by these samples. As the backflow continues, water enters the well from a roughly spherical or ovular area that surrounds the perforated section of the well, which is the lower 1,000 and 800 ft (305 and 245 m) of pipe in wells P-38 and P-46, respectively. However, irregularities in the formation, channeling, and variable permeability and plugging of the sandstone and the perforations complicate the interpretation of the increasing volume of backflow (or elapsed time) as the distance from the injection site is increased.

(i) Biomass. The total PLFA component can be used as an estimate of biomass in bacterial communities (17, 52, 55), since phospholipids are essential to all cellular membranes, are not found in endogenous storage lipids, and are in relatively constant proportions in the various taxa (54). Since rates of phospholipid turnover are relatively rapid in both living and dead cells that are added to the environment (28, 54), they may serve as estimates of viable biomass. Such biochemical assays of viable biomass are relatively nonselective and quantitative compared with culturing techniques.

Both injection wells examined during this study had their highest biomass (total PLFAs) levels in the water samples taken after 2 well volumes (12.2 to 14.6 m^3) of water were allowed to back flow, when sampled the first time (March 1985 for well P-38; November 1985 for well P-46). The levels of biomass fell exponentially as the back flowing continued. These results were corroborated by scanning electron micrographs of samples from well P-38 (Fig. 1), in which increased

TABLE 2. Profiles of the PLFA extracted from injection water samples back flowed from well P-46 on 21 November 1985

Fatty acid	pmol of PLFA/ml of backflow water (mean ± SD) for the following samples[a]:					
	A	B	C	D	E	F
Saturated						
14:0	0.8 ± 0.5	0.9 ± 0.3	2.1 ± 0.7	1.0 ± 0.7	0.3 ± 0.4	
15:0	2.8 ± 1.0	2.7 ± 0.6	6.8 ± 1.8	3.6 ± 1.1	1.6 ± 0.4	0.1 ± 0.1
16:0	57.6 ± 15.6	51.8 ± 16.1	198.7 ± 28.4	114.4 ± 22.6	53.8 ± 4.0	2.8 ± 0.7
17:0	11.1 ± 2.0	9.0 ± 3.2	46.2 ± 4.0	26.1 ± 5.0	14.6 ± 0.4	1.6 ± 0.2
18:0	12.7 ± 2.2	9.2 ± 2.8	70.0 ± 6.8	38.4 ± 7.1	24.7 ± 0.8	4.3 ± 0.4
20:0	0.2 ± 0.2	2.0 ± 1.9	3.5 ± 0.5	1.8 ± 0.3	1.4 ± 0.1	0.3 ± <0.1
Branched						
*i*14:0	0.4 ± 0.5	0.7 ± 0.3	2.9 ± 1.4	1.1 ± 0.8	0.4 ± 0.6	
*i*15:0	1.0 ± 0.4	1.1 ± 0.3	3.5 ± 0.9	1.9 ± 0.6	1.0 ± 0.5	0.1 ± 0.1
*a*15:0	4.5 ± 2.0	4.3 ± 1.1	23.9 ± 5.8	10.5 ± 3.4	5.7 ± 2.1	0.2 ± 0.2
*i*16:0	1.7 ± 0.5	1.4 ± 0.4	7.2 ± 1.0	3.8 ± 0.9	2.2 ± 0.3	
*i*17:0	0.4 ± 0.3	0.2 ± 0.2	2.5 ± 0.3	1.3 ± 0.2	0.2 ± <0.1	0.1 ± <0.1
*a*17:0	2.8 ± 0.5	2.3 ± 0.8	11.0 ± 1.1	6.0 ± 1.1	3.8 ± 0.2	0.4 ± <0.1
*i*18:0	3.2 ± 0.4	2.2 ± 0.8	28.1 ± 2.9	14.4 ± 2.6	10.2 ± 0.3	1.5 ± 0.2
Cyclopropyl						
cy17:0	2.6 ± 0.7	2.2 ± 0.7	7.4 ± 1.6	4.8 ± 0.8	2.4 ± 0.1	0.4 ± <0.1
cy19:0	3.3 ± 0.5	2.6 ± 1.0	11.2 ± 1.9	7.4 ± 1.2	3.9 ± 0.1	0.7 ± <0.1
Unsaturated						
Poly 16	0.3 ± 0.3	TR	5.2 ± 1.1	2.3 ± 0.5	1.6 ± 0.2	
16:1ω9*c*	0.1 ± 0.1	0.1 ± 0.1	2.6 ± 0.5	1.4 ± 0.4	0.5 ± 0.4	0.3 ± <0.1
16:1ω7*c*	13.3 ± 3.7	12.4 ± 4.4	27.0 ± 4.7	15.2 ± 4.3	6.7 ± 1.3	0.2 ± 0.1
16:1ω7*t*	1.4 ± 0.4	1.3 ± 0.5	4.4 ± 1.0	2.6 ± 0.8	1.1 ± 0.2	TR
Poly 18	1.2 ± 0.1	0.4 ± 0.4	3.6 ± 0.4	1.7 ± 0.6	1.1 ± <0.1	0.1 ± 0.1
18:1ω9*c*	4.7 ± 0.4	4.2 ± 1.1	12.4 ± 1.2	6.6 ± 1.6	3.5 ± 0.1	0.8 ± 0.1
18:1ω7*c*	50.7 ± 8.2	43.7 ± 15.6	141.2 ± 21.1	85.6 ± 19.8	42.0 ± 0.7	5.2 ± 0.5
18:1ω7*t*	0.9 ± 0.6	0.9 ± 0.5	5.6 ± 1.0	3.4 ± 0.8	1.8 ± <0.1	0.3 ± 0.1
Total PLFA	179 ± 40	156 ± 51	629 ± 82	356 ± 76	185 ± 10	19.4 ± 2.1
Total branched	14.0 ± 4.3	12.2 ± 3.4	79.1 ± 12.0	39.1 ± 9.5	24.2 ± 3.4	2.5 ± 0.3
Total cyclopropyl	5.8 ± 1.2	4.8 ± 1.6	18.7 ± 3.5	12.2 ± 2.0	6.3 ± 0.2	1.1 ± 0.5
Unsaturated/saturated	0.72 ± 0.04	0.68 ± 0.01	0.48 ± 0.02	0.50 ± 0.02	0.46 ± 0.01	0.54 ± 0.01
trans/cis	0.035 ± 0.013	0.037 ± 0.006	0.059 ± 0.007	0.058 ± 0.006	0.060 ± 0.004	0.060 ± 0.020
C_{16} *trans/cis*	0.108 ± 0.005	0.101 ± 0.001	0.163 ± 0.015	0.168 ± 0.007	0.168 ± 0.005	0.059 ± 0.102

[a] The sample codes A to F are described in Table 1. For all samples $n = 3$, except for sample B, where $n = 2$.

biomass and extracellular polysaccharidelike material in the samples from the backflow of 2 well volumes are shown. The levels of fatty acids found in these samples corresponded to 1.2×10^7 and 6.3×10^6 bacterial cells that were the size of logarithmic-phase *Escherichia coli* per ml of water for wells P-38 and P-46, respectively.

If one considers that the first well volume of water retrieved during the backflow was mainly water that was in the well pipe itself, then it is reasonable to conclude that the peak of biomass found in the samples from the backflow of 2 well volumes came primarily from the large amounts of bacteria that were commonly assumed to plug the zone immediately surrounding the injection site, which partially reduced permeability. Results of experiments in the laboratory with packed sand has shown that microbial plugging after flooding with water is greatest at the front end of the injection face (20), where the bacteria first encounter large surface areas for attachment or entrapment and where they are exposed to a continuing input of nutrients. The plugging of scintered glass bead cores was also found to be predominantly caused by the formation of a thick, exopolysaccharide-laden biofilm of aerobic bacteria at the injection face, although facultative anaerobes are able to penetrate the rest of the core to some extent (45).

These results are very similar to those found by Belyaev et al. (1) for water that was back flowed from a freshwater injection well in the Bondyurg (USSR) oil field, where the highest numbers of total bacteria (assessed on 0.22-μm-pore-size membrane filters) were found after 12.5 m^3 (1.7 well volumes) of water was back flowed (1.3×10^7 cells per ml); biomass was subsequently found to drop off rapidly in the samples after 25.0 to 166 m^3 (3.4 to 25 well volumes; 5.3×10^6 to 2.6×10^6 cells per ml, respectively) of water was backflowed. In a well from the Romashkino (USSR) oil field, however, the maximum biomass was found in the backflow samples of 5.0 m^3 (9.5×10^6 cells per ml), subsequently decreasing in the samples of 10.0 to 30 m^3 (3.0×10^6 to 1.4×10^6 cells per ml) (1). These zones of high biomass were found to coincide with a drop in redox potential to less than +80 mV E_h. Higher rates of methanogenesis were found in these zones; they subsequently dropped off with total biomass as backflowing continued. Berdichevskaya (2) also sampled water from freshwater injection wells in the Yarino-Kemenolozh and the Shemeti (USSR) oil fields and found 1.0×10^7 to 2.0×10^7 cells per ml by a direct counting method.

When well P-38 was sampled for the second time in November 1985, the first sample taken (0.16 well volumes) had the highest levels of biomass, which then dropped off sharply as the backflow continued. This may indicate that backflow obtained the previous March disturbed the particulates and the microbiota sufficiently to cause a redistribution of the biomass.

White et al. (55) used phospholipid analyses to quantify microbial biomass in a clay groundwater aquifer (depth, 410 m deep), a shallow aquifer, and surface estuarine sediments. These sediments yielded 1.07, 5.33, and 77.6 nmol of total PLFA/g (dry weight), respectively. These results can be compared with those we obtained (Table 2).

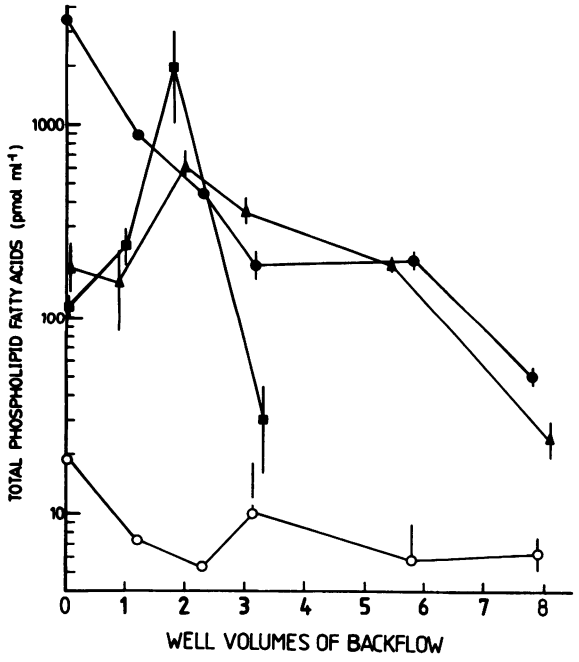

FIG. 4. PLFA estimates of total biomass in injection well water that was back flowed out of well P-38 in March (■), well P-38 in November (●), or well P-46 in November (▲). The much lower levels of biomass that passed through glass fiber filters and that were caught on 0.22-μm-pore-size filters are shown for samples obtained from P-38 in November (○). Values are means ± 1 standard deviation ($n = 3$).

TABLE 3. Comparison of trends in proportions of each PLFA found in water samples taken from wells P-38 and P-46 in November 1986[a]

PLFA	Well P-38			Well P-46		
	Min	Max	Pearson's *r*	Min	Max	Pearson's *r*
Saturates						
14:0			−0.47F	E,F	A,B	−0.71***
15:0	E,F		−0.82***	F	A,B	−0.84***
16:0	F		−0.61***	F		−0.83***
17:0		F	0.09	A,B	F	0.84***
18:0		F	0.17	A,B	F	0.94***
Branched						
*i*14:0			−0.74***			−0.42
*i*15:0			−0.32			−0.49*
*a*15:0			−0.66***	F		−0.28
*i*16:0			0.30			0.58***
*i*17:0		F	0.56**	A,B	F	0.81***
*a*17:0		F	0.79***	A,B	E,F	0.84***
*i*18:0		F	0.82***	A,B	F	0.92***
Cyclopropyl						
cy17:0			0.49*		F	0.62***
cy19:0		F	0.65***	B	F	0.85***
Unsaturates						
Poly 16:0			0.53*			−0.03
16:1ω9c			−0.08			−0.72***
16:1ω7c	F	B	−0.81***	F	A,B	−0.68***
16:1ω7t	F	B	−0.62***	F	A,B	−0.85***
Poly 18			0.25			0.07
18:1ω9c	B	F	0.88***		F	0.48*
18:1ω7c			−0.28		A,B	−0.22
18:1ω7t	A,B,C	D,E,F	0.53*	A,B	F	0.82***

[a] Sample codes A through F are explained in Table 1. Coefficients (Pearson's *r*) for the correlations between the mole% of PLFA and backflow volume (cubic meter) and their significance levels (*, $P < 0.05$; **, $P < 0.02$; ***, $P < 0.01$) are given. The minimum (Min) and maximum (Max) columns indicate those samples or groups of samples with PLFAs that were significantly different from the others. The multiple comparisons of the means were tested by Tukey's and Scheffe's method for contrasts ($\alpha = 0.05$).

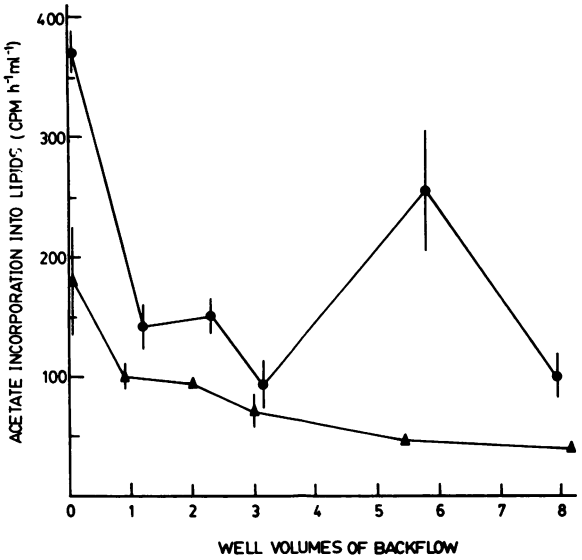

FIG. 5. Rates of incorporation of ^{14}C-labeled acetate into total lipids by the microbiota in injection waters back flowed out of well P-38 (●) or well P-46 (▲) in November. Values shown are means ± 1 standard deviation (n = 4).

(ii) Community structure. White (52, 53) reviewed the use of PLFAs for the examination of microbial community structures in marine sediments, detritus, and biofilms, which cause the fouling of metal in seawater. Analysis of subsurface clays and sands from a variety of locations indicates that a relatively specific subsurface microbial community exists that is different from that in surface soils and that responds to organic contamination by marked changes in the PLFA (48).

Several interesting trends in the fatty acid profiles of the samples from injection wells obtained during this study can be noted. The proportion of the fatty acid 18:1ω7*c* was high in all of the samples, most often ranging from 20 to 30% of the total PLFAs in a sample. The levels of 18:1ω7*c* were more variable in well P-38 in March, ranging from 13 to 69% of the total PLFA, and were generally higher than those in the samples obtained in November. No significant trends in 18:1ω7*c* were found with backflow volume (distance from the injection site; Table 3). High levels of 18:1ω7*c* suggest enrichment in anaerobes, since this fatty acid is synthesized by the anaerobic desaturase pathway in bacteria. White (52) found higher proportions of 18:1ω7*c* in deep groundwater sediments than were found in shallow aquifer sediments.

Other trends in fatty acids with increasing backflow volume found during this study include increasing proportions of cyclopropanes (Table 3), increasing ratios of *trans*- to *cis*-monoenoic fatty acids, and decreasing ratios of unsaturated to saturated fatty acids (Table 2). These trends may be due to a number of factors, some of which may interact. Cyclopropyl fatty acids have been found to accumulate in some species of bacteria when exposed to stress or adverse conditions (29) or in stationary-phase cultures (5, 26, 31, 34). Guckert et al. (19) found an increase in the proportions of saturated fatty acids, *trans*-monoenoic fatty acids (primarily C_{16}), and cyclopropyl fatty acids during nutrient deprivation of *Vibrio cholerae*, along with an overall decrease in the amounts of total phospholipids per cell. Similar changes in the ratios of *trans*- and *cis*-monoenoic fatty acids have been observed during the starvation of other species (33, 50). An increase in cyclopropyl fatty acids accompanied by a decrease in their *cis*-monoenoic precursors have also been found as a result of lowered pH (7), lowered oxygen tension, increase in Mg^+ ions (30), and increased temperatures (18, 30, 36). It has been suggested that it may be possible to use the *trans*/*cis* ratio as a stress or starvation index for microbial communities, with ratios greater than 0.1 being indicative of such conditions (19). The total *trans*/*cis* ratios found during this study were generally below 0.1, but for the C_{16} components the ratios were commonly above 0.1 (Table 2).

Increases in the relative amounts of saturated versus unsaturated fatty acids have been found during adaptations by mesophiles to higher temperatures (13, 15, 47) or community shifts toward thermophilic conditions (6). In this study, the unsaturated/saturated fatty acid ratio decreased with increasing distance from the injection site (or backflow volume), although the water temperature of these samples increased only a few degrees (Table 1), indicating that these changes may be due instead to stress or limited nutrients. However, the ratios of *iso*- to *anti-iso*-branched-chain fatty acids in these samples also increased with distance from the injection site. This ratio has been found to increase when facultatively thermophilic bacilli are exposed to increasing temperatures (8, 43). High hydrostatic pressures may also induce changes in the fatty acids of bacteria, but they lead to an increase in the unsaturated/saturated fatty acid ratio (15).

Munbaeva and Kolpakov (39) analyzed water samples from various depths in the Mangyshlak (USSR) oil-bearing reservoir by standard culture techniques. They found saprophytes to be dominant in the oxidizing zones (depth, 0 to 263 m), sulfate reducers to be prevalent in the transitional redox zones (depth, 263 to 560 m), and methanogens to be dominant in the reducing zones (depth, 560 to 2116 m). The water temperatures increased with depth in these zones, from 20 to 38 to 97°C, respectively, and the salinity increased from 3 to 11 to 150 g/liter, respectively. Sulfate reducers were also found in the oil reservoir waters that were sampled by Bedrichevskaya (2); hydrocarbon oxidizers and methanogens were also found (2). In the wells in California from which samples were obtained during this study, however, none of the fatty acid markers specific to sulfate reducers (10 Me16:0 for *Desulfobacter* sp. [49], *i*17:1ω7 [41, 49, 51] and *a*17:1ω7 [49] for *Desulfovibrio desulfuricans* were found in any of the samples. This does not preclude the presence of a mixed sulfate-reducing population, however (41).

In general, the fatty acid analyses support the transmission electron microscopy observations of a predominantly gram-negative (high levels of unsaturated and cyclopropyl fatty acids) (46), diverse, and possibly stressed bacterial community with decreasing biomass in samples from beyond 2 well volumes of backflow.

(iii) Microbial activity. Both times that samples from injection well P-38 were obtained, glucose incorporation rates (and acetate incorporation rates in November) into lipids correlated with biomass. On the contrary, in well P-46 incorporation rates correlated with the volume of water that was back flowed, but not with biomass. Although the biomass levels found in the water farthest from the injection site were very low, the level of activity per cell may have been greater.

Size fractionation. When the injection water samples were fractionated by glass fiber filtration, less than 5% of the total phospholipid fatty acids recovered passed through the glass fiber filter to be caught on the 0.22-μm-pore-size filter. Exceptions to this rule were the F samples, which were taken from water that was farthest from the injection site.

These samples had very low biomass levels, but 13 to 18% of the fatty acids showed up on the 0.22-μm-pore-size filters. It appears that a majority of the organisms recovered by this backflow sampling technique were either attached to particles or clumped together, as opposed to being free-living, singly dispersed cells. Some microcolonies were seen in the transmission electron microscopy samples, and the slime found on some scanning electron microscopy samples indicated that these bacteria may have been originally attached and clumped.

Microbial attachment to surfaces and the formation of biofilms are found in all natural and industrial environments, even when smooth surfaces are exposed to relatively high sheer forces (35). This appears to be the preferred mode of growth for bacteria in nature (11, 12, 16). Aggregates and chains of cells have been found to be more efficient in plugging reservoir materials than singly dispersed cells (24). In addition, although permeability reduction during injection of packed sand correlates with the number of bacteria injected (20), much of the actual plugging is due to the production of metabolic products such as exopolysaccharides (38, 45). It has been suggested that injection waters should contain less than 10 slime-producing bacteria per ml, to avoid potential permeability reduction problems (21). The results of this study indicate that a large population of bacteria exists in the areas of the reservoir near the injection site, possibly leading to reductions in permeability in these areas, but that far fewer bacteria reside beyond this zone.

In conclusion, the microbiota found in the injection waters retrieved from this oil reservoir were found to be numerous, diverse in bacterial morphology and physiological state, and predominantly gram negative. They also contained a significant proportion of anaerobic or facultative organisms. The fact that most of the bacteria retrieved from the reservoir were attached to particles or were clumped indicates that this backflow method of sampling recovers many organisms that are representative of the microbial communities present in the reservoir. These methods of analysis may be useful in monitoring water injection wells and in preparing these injection wells for microbial-enhanced oil recovery operations.

It should be kept in mind, however, that this method of sampling by the backflowing of water in injection wells probably underestimates the biomass in the reservoir. In addition, there may be a bias in that there is a favorable probability that samples containing unattached or loosely attached bacteria, single cells and smaller-sized bacteria, and the non-slime-forming bacteria of the community are obtained. These factors, combined with the characteristics of the reservoir formation mentioned above, may also affect the distribution of bacteria found in the water samples taken at particular times during the backflow, since some of the organisms present may detach and migrate through the reservoir at different rates.

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LITERATURE CITED

1. **Belyaev, S. S., K. S. Laurinavichus, A. Y. Obraztsova, S. N. Gorlatov, and M. V. Ivanov.** 1982. Microbiological processes in the critical zone of injection wells in oilfields. Microbiologiya **51**:997–1001.
2. **Berdichevskaya, M. V.** 1982. Effect of prolonged flooding of an oil pool on development of a biocenosis and activity of stratal microflora. Mikrobiologiya **51**:146–151.
3. **Beveridge, T. J., G. B. Patel, B. J. Harris, and G. O. Sprott.** 1986. The ultrastructure of *Methanothrix concillii*, a mesophilic acetoclastic methanogen. Can. J. Microbiol. **32**:703–710.
4. **Bligh, E. G., and W. J. Dyer.** 1959. A rapid method of lipid extraction and purification. Can. J. Biochem. Physiol. **35**:911–917.
5. **Bobbie, R. J., and D. C. White.** 1980. Characterization of benthic microbial community structure by high-resolution gas chromatography of fatty acid methyl esters. Appl. Environ. Microbiol. **39**:1212–1222.
6. **Brock, T. D.** 1967. Life at high temperatures. Science **158**:1012–1019.
7. **Buist, P. H., and J. M. Findlay.** 1985. The biosynthesis of cyclopropane fatty acids. III. pH dependence of methyl hydrogen exchange: gas chromatographic-mass spectral studies. Can. J. Chem. **63**:971–974.
8. **Chan, M., Y. P. Virmani, R. H. Himes, and J. M. Akagi.** 1973. Spin-labeling studies on the membrane of a facultative thermophilic *Bacillus*. J. Bacteriol. **113**:322–328.
9. **Clementz, D. M., D. E. Patterson, R. J. Aseltine, and R. E. Young.** 1982. Stimulation of water injection wells in the Los Angeles basin by using sodium hypochlorite and mineral acids. J. Petrol. Technol. **34**:2087–2096.
10. **Cohen, A. L., D. P. Marlow, and G. E. Garner.** 1968. A rapid critical point method using fluorocarbons ("Freons") as intermediate and transitional fluids. J. Microsc. (Paris) **7**:331–342.
11. **Costerton, J. W., G. G. Geesey, and K.-J. Cheng.** 1978. How bacteria stick. Sci. Am. **238**:86–95.
12. **Costerton, J. W., R. T. Irvin, and K.-J. Cheng.** 1981. The bacterial glycocalyx in nature and disease. Annu. Rev. Microbiol. **35**:299–324.
13. **Cronan, J. E., and E. P. Gelmann.** 1975. Physical properties of membrane lipids: biological relevance and regulation. Bacteriol. Rev. **39**:232–256.
14. **Davis, J. B.** 1967. Petroleum microbiology. Elsevier, New York.
15. **DeLong, E. F., and A. A. Yayanos.** 1985. Adaptation of the membrane lipids of a deep-sea bacterium to changes in hydrostatic pressure. Science **228**:1101–1103.
16. **Geesey, G. G., R. Mutch, J. W. Costerton, and R. B. Green.** 1978. Sessile bacteria: an important component of the microbial population in small mountain streams. Limnol. Oceanogr. **23**: 1214–1223.
17. **Gehron, M. J., and D. C. White.** 1983. Sensitive measurements of phospholipid in environmental samples. J. Microbiol. Methods **1**:23–32.
18. **Gill, C. O., and J. R. Suisted.** 1978. The effects of temperature and growth rate on the proportion of unsaturated fatty acids in bacterial lipids. J. Gen. Microbiol. **104**:31–36.
19. **Guckert, J. B., M. A. Hood, and D. C. White.** 1986. Phospholipid ester-linked fatty acid profile changes during nutrient deprivation of *Vibrio cholerae*: increases in the *trans/cis* ratio and proportions of cyclopropyl fatty acids. Appl. Environ. Microbiol. **52**:794–801.
20. **Gupta, R. P., and D. Swartzendruber.** 1962. Flow-associated reduction in hydrolic conductivity of quartz sand. Soil Sci. Soc. Proc. **26**:6–10.
21. **Hensel, W. M., Jr., R. L. Sullivan, and R. H. Stallings.** 1981. Understanding and solving injection well problems. Petrol. Eng. Int. **May**:155–170.
22. **Hood, M. A., J. B. Guckert, D. C. White, and F. Deck.** 1986. Effect of nutrient deprivation on lipid, carbohydrate, DNA, RNA, and protein levels in *Vibrio cholerae*. Appl. Environ. Microbiol. **52**:788–793.
23. **Ivanov, M. V., and S. S. Belyaev.** 1983. Microbial activity in waterflooded oilfields and its possible regulation. *In* E. C.

Donaldson and J. B. Clark (ed.), Proceedings of the 1982 International Conference on Microbial Enhancement of Oil Recovery. U.S. Department of Energy, Bartlesville, Okla.

24. **Kalish, P. J., J. A. Stewart, W. F. Rogert, and E. O. Bennett.** 1964. The effect of bacteria on sandstone permeability. J. Petrol. Technol. **16**:805–814.
25. **Kandler, O., and H. Konig.** 1985. Cell envelopes of archaebacteria. *In* C. R. Woese and R. S. Wolfe (ed.), The bacteria, vol. VIII: archaebacteria. Academic Press, Inc., New York.
26. **Kates, M.** 1964. Bacterial lipids. Adv. Lipid Res. **2**:17–90.
27. **Kates, M.** 1972. Techniques of lipidology. North-Holland Publishing Co., Amsterdam.
28. **King, J. D., D. C. White, and C. W. Taylor.** 1977. Use of lipid composition and metabolism to examine structure and activity of estuarine detrital microflora. Appl. Environ. Microbiol. **33**: 1177–1183.
29. **Knivett, V. A., and J. Cullen.** 1965. Some factors affecting cyclopropane acid formation in *Escherichia coli*. Biochem. J. **96**:771–776.
30. **Law, J. H.** 1971. Biosynthesis of cyclopropane rings. Accounts Chem. Res. **4**:199–203.
31. **Law, J. H., H. Zalkin, and T. Kaneshiro.** 1963. Transmethylation reactions in bacterial lipids. Biochim. Biophys. Acta **70**: 143–151.
32. **Lazar, I.** 1983. Some characteristics of the bacterial inoculum used for oil release from reservoirs, p. 73–82. *In* J. E. Zajic, D. G. Cooper, T. R. Jack, and N. Kosaric (ed.), Microbial enhanced oil recovery. PennWell Publishing Co., Tulsa, Okla.
33. **Malmcrona-Friberg, K., A. Tunlid, P. Marden, S. Kjelleberg, and G. Odham.** 1986. Chemical changes in cell envelope and poly-β-hydroxybutyrate during short-term starvation of a marine bacterial isolate. Arch. Microbiol. **144**:340–345.
34. **Marr, A. G., and J. L. Ingraham.** 1962. Effect of temperature on the composition of the fatty acids in *Escherichia coli*. J. Bacteriol. **84**:1260–1267.
35. **McCoy, W. F., J. D. Bryers, J. Robbins, and J. W. Costerton.** 1981. Observations of fouling biofilm formation. Can. J. Microbiol. **27**:910–917.
36. **McGarrity, J. T., and J. B. Armstrong.** 1981. The effect of temperature and other growth conditions on the fatty acid composition of *Escherichia coli*. Can. J. Microbiol. **27**:835–840.
37. **McKinley, V. L., and J. R. Vestal.** 1984. Biokinetic analyses of adaptation and succession: microbial activity in composting municipal sewage sludge. Appl. Environ. Microbiol. **47**:933–941.
38. **Mitchell, R., and Z. Nevo.** 1964. Effect of bacterial polysaccharide accumulation on infiltration of water through sand. Appl. Microbiol. **12**:219–223.
39. **Munbaeva, O. T., and V. B. Kolpakov.** 1975. Microflora of underground waters of oil- and gas-bearing Mangyshlak complexes. Mikrobiologiya **44**:321–324.
40. **Obwekwe, C. O., D. W. S. Westlake, F. D. Cook, and J. W. Costerton.** 1981. Surface changes in mild steel coupons from the action of corrosion-causing bacteria. Appl. Environ. Microbiol. **41**:766–774.
41. **Parkes, R. J., and J. Taylor.** 1983. The relationship between fatty acid distributions and bacterial respiratory types in contemporary marine sediments. Estuarine Coastal Shelf Sci. **16**: 173–189.
42. **Reynolds, E. S.** 1963. The use of lead citrate at high pH as an electron opaque stain in electron microscopy. J. Cell Biol. **17**: 208–212.
43. **Rilfors, L., A. Wieslander, and S. Stahl.** 1978. Lipid and protein composition of membranes of *Bacillus megaterium* variants in the temperature range 5 to 70°C. J. Bacteriol. **135**:1043–1052.
44. **Ruseska, I., J. Robbins, and J. W. Costerton.** 1982. Biocide testing against corrosion-causing oilfield bacteria helps control plugging. Oil Gas J. **March**:253–264.
45. **Shaw, J. C., B. Bramhill, N. C. Wardlaw, and J. W. Costerton.** 1985. Bacterial fouling in a model core system. Appl. Environ. Microbiol. **49**:693–701.
46. **Shaw, N.** 1974. Lipid composition as a guide to the classification of bacteria. Adv. Appl. Microbiol. **17**:63–108.
47. **Sinensky, M.** 1971. Temperature control of phospholipid biosynthesis in *Escherichia coli*. J. Bacteriol. **106**:449–455.
48. **Smith, G. A., J. S. Nickels, B. D. Kerger, J. D. Davis, S. P. Collins, J. T. Wilson, J. F. McNabb, and D. C. White.** 1986. Quantitative characterization of microbial biomass and community structure in subsurface material: a prokaryotic consortium responsive to organic contamination. Can. J. Microbiol. **32**:104–111.
49. **Taylor, J., and R. J. Parkes.** 1983. The cellular fatty acids of the sulfate-reducing bacteria, *Desulfobacter* sp., *Desulfobulbus* sp., and *Desulfovibrio desulfuricans*. J. Gen. Microbiol. **129**:1–7.
50. **Thomas, T. D., and R. D. Batt.** 1969. Degradation of cell constituents by starved *Streptococcus lactis* in relation to survival. J. Gen. Microbiol. **58**:347–362.
51. **Ueki, A., and T. Suto.** 1979. Cellular fatty acid composition of sulfate-reducing bacteria. J. Gen. Microbiol. **25**:185–196.
52. **White, D. C.** 1983. Analysis of micro-organisms in terms of quantity and activity in natural environments. *In* J. H. Slater, R. Whittenbury, and J. W. T. Wimpenny (ed.), Microbes in their natural environments. Thirty-fourth Symposium of the Society for General Microbiology. Cambridge University Press, Cambridge.
53. **White, D. C.** 1986. Environmental effects testing with quantitative microbial analysis: chemical signatures correlated with *in situ* biofilm analysis by FT/IR. Toxicity Assessment **1**:315–338.
54. **White, D. C., W. M. Davis, J. S. Nickels, J. D. King, and R. J. Bobbie.** 1979. Determinations of the sedimentary microbial biomass by extractable lipid phosphate. Oecologia **40**:52–62.
55. **White, D. C., G. A. Smith, M. J. Gehron, J. H. Parker, R. H. Findlay, R. F. Martz, and H. L. Fredrickson.** 1983. The groundwater aquifer microbiota: biomass, community structure, and nutritional status. Dev. Ind. Microbiol. **24**:201–211.