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# Variation in Microbial Biomass and Community Structure in Sediments of Eutrophic Bays as Determined by Phospholipid Ester-Linked Fatty Acids

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**The distribution of phospholipid ester-linked fatty acids (PLFA) in sediments of eutrophic bays (Hiroshima Bay and Aki Nada) was studied to quantify the microbial biomass, community structure, and nutritional status. A total of 63 fatty acids in the range of $C_{10}$ to $C_{24}$ were determined. They consist of saturated fatty acids, branched fatty acids, monounsaturated fatty acids, and polyunsaturated fatty acids, and variation was revealed in the relative proportions of these fatty acids in sediments. On the basis of the PLFA concentration in sediments, the calculated microbial biomass showed variation (mean ± standard deviation = $0.70 \times 10^8 \pm 0.53 \times 10^8$ cells per g [dry weight] of sediment) in the eutrophic bays. In sediments, a higher amount of biomass was observed in the coastal area of Hiroshima Bay than that observed in the rest of the bay and adjacent Aki Nada. The microbial community structure of the present study area, as characterized by the PLFA profiles, showed very low percentages of polyunsaturated fatty acids and long-chain fatty acids characteristic of microeukaryotes and terrestrial input, respectively, and high percentages of fatty acids characteristic of bacteria. The distribution of PLFA profiles also showed the relative contribution of both aerobic and anaerobic bacteria, especially sulfate-reducing bacteria, in the study area. The relative proportions of PLFA revealed distinctive differences among the stations of the study area, as is evidenced from six clusters obtained for the PLFA profiles. The results of Tukey's honestly significant difference test further confirmed that the sediments in the coastal area of Hiroshima Bay were significantly enriched by a number of fatty acids when compared with other areas investigated where relatively few fatty acids were present in significant quantities. No marked variation in environmental parameters in the surface- and bottom-water samples was observed, indicating the absence of any water movement in the study area. Furthermore, low redox potential and the levels of sulfide in the sediment revealed the reduced condition of the sediment. The existing environmental conditions and pollution of the study area were attributed to the observed microbial community structure in the sediments.**

The accurate determination of microbial biomass in sediments poses many problems. Changes in the structure and functional relationships in the microbial community of sediments have proven to be difficult to measure without distorting the in situ microbial population (4). Several techniques have been used to enumerate and identify bacterial types present in marine sediments, but all suffer from various limitations. Culture techniques using selective media also have their own limitations, and the results can distort and underestimate the in situ population. Epifluorescence microscopic analysis of the sediment can provide data on bacterial distribution, but it involves various disruptive techniques for the separation of microbes from sediments (9) and, also, the stripping process is supposed to be selective and is often not quantitative (43). Furthermore, microscopic analyses of the sediment provide little information on the types of organisms present.

To overcome these difficulties, methods involving biochemical components as biomarkers were proposed to identify and quantify the viable biomass in sediments. Microbial biomarkers which can be analyzed directly from environmental samples provide an alternative to enrichment methods for the in situ study of indigenous bacterial populations. Analyses of lipids, especially fatty acids, have been of great value in understanding bacterial phylogenic and taxonomic classifications (25). Perry et al. (32) proposed that bacteria make a major contribution to the solvent-extractable fatty acids found in the sediment. Fatty acids are particularly useful biomarkers since they are essential components of bacteria with the exception of archaebacteria. Analysis of fatty acids is used to indicate the presence and relative abundance of the microorganisms in a given environment (30).

Phospholipids can be used to identify and quantify the viable biomass in sediments (3, 33, 41). Phospholipids are not found in storage lipids and have relatively rapid turnover in sediments, so the analysis of phospholipids provides a better measure of the viable cellular biomass than other measures of biomass such as enzyme activities, muramic acid levels, and total ATP (41). Analysis of phospholipid ester-linked fatty acids (PLFA) is presently one of the more sensitive chemical methods to determine the microbial biomass and community structure (42). Analyses of PLFA have been extensively used to identify the bacteria and their community structure in sediments (2, 4, 26, 28, 45) and subsurface aquifer sediments (3). Validations of PLFA in the determination of microbial biomass and community structure have been extensively reviewed (42).

Eutrophication in the present study area makes the water

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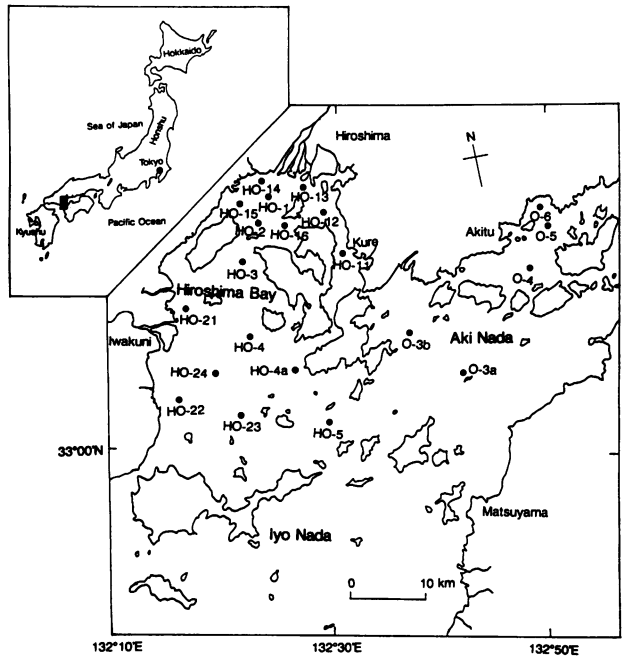


FIG. 1. Map of the study area showing the sampling stations.

polluted because of excessive growth of phytoplankton and not because of direct organic loadings from land. Microorganisms play a vital role in the mineralization of organic matter contributed by plankton. A diverse mixture of both aerobic and anaerobic bacteria is involved in the degradation of organic matter and cycling of nutrients in the aquatic environment. However, the information available on these microbial communities is rather limited. Hence, the present study was carried out to determine the distribution of bacterial biomass and community structure in the present study area, where the problems of eutrophication and organic pollution are acute. The distribution of microbial biomass and its community structure are also discussed in relation to the environmental parameters monitored in the study area.

## MATERIALS AND METHODS

**Study area and sample collection.** Hiroshima Bay is a semiclosed bay located in the western part of the Seto Inland Sea (Fig. 1); it has an area of about 900 $km^2$ and a mean depth of 24 m. This bay is approximately 30 km wide from east to west and 50 km long from north to south and reaches 41 m at its deepest point. This bay is one of the most polluted bays in the Inland Sea of Japan because of eutrophication. The presence of many islands near the mouth of the bay prevents water movement, and stagnation prevails in the bay. In summer, oxygen depletion in the bottom water and absence of a detectable oxidized layer in sediment were observed. The bay adjacent to Hiroshima Bay on the eastern side is called Aki Nada (Fig. 1), which is also reported to be polluted. The sediment of the present study area is mainly composed of silty clay, with a median grain size of less than 1.7 μm (21). Sixteen sampling stations were selected in Hiroshima Bay (HO-1 to HO-24), and four stations were selected in Aki Nada (O-3a to O-6) for the purposes of the present study. About 1 to 2 cm of the top layer of the sediment was sampled from the sediment collected by use of a Smith-McIntyre sampler (0.1 $m^2$) and frozen at −20°C. Surface- and bottom- (i.e., 2 m above the sediment) water samples were collected by use of a Van Dorn water sampler for dissolved oxygen analysis.

**Environmental parameters.** An STD monitor (Alec 1000; Alec Co., Kobe, Japan) was employed to monitor the salinity, temperature, and density (sigma 't') in the water column. The dissolved oxygen concentration was estimated as previously described (37), and the mud temperature was measured by using a thermometer. $E_h$ and pH of the sediment were determined with an ORP meter (RM 1; Toa Electronics Ltd., Tokyo, Japan) and a pH meter (pH S-33; Fujiwara Seisakusho, Tokyo, Japan), respectively. The sulfide concentration in sediments was estimated as previously described (22).

**Lipid extraction.** Freeze-dried sediment samples were solvent extracted by using the modified method of Bligh and Dyer as described by Baird and White (2). Phosphate buffer, chloroform, and methanol (2:3:6, vol/vol/vol) were used to extract lipid from sediment samples. The extracted lipids were fractionated into neutral lipids, glycolipids, and phospholipids by column chromatography on silica gel by using chloroform, acetone, and methanol, respectively. A mild alkaline methanolysis was carried out on the phospholipid fraction of the lipids to release and methylate the PLFA (43).

**Thin-layer chromatography.** The fatty acid methyl esters obtained from phospholipid fractions were purified by thin-layer chromatography. The precoated silica gel plates (20 cm by 20 cm by 250 μm; Merck, E. Merck, Darmstadt, Germany) were precleaned in hexane-diethyl ether (1:1, vol/vol). A standard $C_{19}$ fatty acid methyl ester was applied to the end lanes of the silica gel plate, and the sample was spotted in the middle. After development in the solvent system described above, the end lines were sprayed with Rhodamine 6G (0.01% [wt/vol] in water) and visualized while wet under UV light. The 2-cm-wide band corresponding to the standard fatty acid methyl ester was scraped from the sample line. The silica gel was collected in Pasteur pipettes plugged with glass wool preextracted with chloroform and methanol (1:1, vol/vol), and the PLFA were eluted from the silica gel with 10 ml of chloroform. The PLFA samples were dried under a stream of nitrogen and analyzed by gas chromatography (GC).

**GC.** GC analyses were carried out with a Hewlett-Packard GC (HP 5890A) equipped with a 25-m cross-linked 5% phenylmethyl silicone fused capillary column (inside diameter, 0.20 mm) and a flame ionization detector. The sample was injected (by using an HP 7673A automatic sampler) in the splitless mode with a 30-s venting time at 250°C. The oven temperature was programmed to be at 50°C for 1 min, to increase at a rate of 20°C $min^{-1}$ for 3 min, to increase at a rate of 3°C $min^{-1}$ to 270°C, and then to be isothermal for 10 min. Helium was used as a carrier gas. Before GC-mass spectrometry (GC-MS) analysis, a comparison of retention times with known standards of fatty acid methyl esters (Supelco Inc.) was made for tentative peak identification of compounds separated by GC. Methyl nonadecanoate (19:0) was used as an internal standard.

**GC-MS.** GC-MS analyses (on a model GC HP 5890A with a model 5970 series mass selective detector) of PLFA samples were carried out by using the same type of column. Monoenoic double-bond position and geometry of PLFA were determined by GC-MS analysis of the adducts after reaction of the sample with dimethyl disulfide as described by Nichols et al. (27).

TABLE 1. Environmental characteristics of Hiroshima Bay and Aki Nada (September 1989)

| Station | Depth (m) | Temp (°C) | | Salinity (‰) | | Density (sigma 't') | | Dissolved oxygen (%) | | Sediment characteristics | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Surface water | Bottom water[a] | Surface water | Bottom water | Surface water | Bottom water | Surface water | Bottom water | Temp (°C) | pH | $E_h$ (mV) | Sulfide (mg of S/g) |
| HO-1 | 15 | 24.9 | 25.2 | 22.8 | 31.0 | 14.2 | 20.3 | 100.0 | 88.1 | 24.0 | 7.54 | 10 | 0.32 |
| HO-2 | 24 | 25.5 | 24.3 | 28.0 | 32.1 | 18.0 | 21.4 | 101.0 | 60.8 | 23.8 | 7.52 | −60 | 0.46 |
| HO-3 | 35 | 24.2 | 24.4 | 31.0 | 32.2 | 20.6 | 21.4 | 95.1 | 66.9 | 24.1 | 7.49 | −30 | 0.25 |
| HO-4 | 27 | 24.7 | 24.4 | 31.9 | 32.3 | 21.1 | 21.5 | 92.9 | 77.1 | 23.8 | 7.36 | 30 | 0.17 |
| HO-5 | 41 | 24.9 | 24.4 | 32.2 | 32.4 | 21.1 | 21.6 | 92.4 | 83.6 | 24.4 | 7.59 | 0 | —[b] |
| HO-11 | 27 | 26.6 | 23.8 | 30.0 | 32.3 | 19.0 | 21.8 | 116.7 | 29.8 | 23.4 | 7.56 | 0 | 0.22 |
| HO-12 | 27 | 26.6 | 24.1 | 26.2 | 32.4 | 16.4 | 21.6 | 132.3 | 40.6 | 24.6 | 7.52 | 50 | 0.13 |
| HO-13 | 22 | 26.6 | 24.1 | 27.3 | 32.4 | 17.1 | 21.6 | 123.9 | 43.2 | 23.6 | 7.46 | 0 | 0.30 |
| HO-14 | 14 | 26.5 | 24.6 | 26.1 | 31.4 | 16.3 | 20.8 | 136.4 | 46.5 | 23.8 | 7.39 | −40 | 1.59 |
| HO-15 | 16 | 25.8 | 24.4 | 29.0 | 32.2 | 18.6 | 21.4 | 114.9 | 55.4 | 24.0 | 7.54 | 60 | 0.21 |
| HO-16 | 22 | 25.6 | 24.2 | 30.1 | 32.4 | 19.5 | 21.7 | 90.6 | 50.4 | 24.4 | 7.47 | 30 | 0.04 |
| HO-21 | 29 | 25.8 | 24.2 | 30.3 | 32.4 | 19.6 | 21.6 | 109.4 | 68.6 | 23.4 | 7.42 | −130 | 0.39 |
| HO-22 | 29 | 23.1 | 24.4 | 31.1 | 32.0 | 13.4 | 21.3 | 95.3 | 82.5 | 23.9 | 7.64 | −20 | 0.22 |
| HO-23 | 29 | 25.2 | 24.6 | 31.5 | 32.0 | 20.7 | 21.2 | 96.6 | 82.9 | 24.5 | 7.38 | −80 | 0.16 |
| O-3a | 47 | 25.0 | 24.8 | 32.0 | 32.2 | 21.1 | 21.3 | 89.6 | 87.8 | 24.6 | 7.34 | 240 | 0.01 |
| O-3b | 28 | 25.1 | 25.0 | 32.0 | 32.0 | 21.1 | 21.1 | 90.2 | 87.0 | 24.0 | 7.28 | −20 | 0.08 |
| O-4 | 31 | 25.4 | 25.2 | 31.8 | 31.9 | 20.8 | 21.0 | 90.4 | 86.9 | 25.2 | 7.40 | −10 | 0.04 |
| O-5 | 25 | 25.8 | 25.4 | 31.5 | 31.8 | 20.5 | 20.8 | 89.2 | 87.8 | 25.5 | 7.53 | 250 | — |
| O-6 | 11 | 25.7 | 25.6 | 31.1 | 31.8 | 20.2 | 20.5 | 89.2 | 81.0 | 25.5 | 7.31 | 100 | 0.03 |

[a] Two meters above the bottom.
[b] —, not detectable.

**Nomenclature.** The fatty acid nomenclature used in the present study is as follows. The length of the carbon chain is indicated by a number, and the degree of unsaturation is indicated by a number separated by a colon from the chain length number. This degree of unsaturation number is followed by a number indicating the position of the double bond nearest the "d" (carboxyl) end of the molecule of the monounsaturated fatty acids. In cases where the exact positions of the double bonds were not determined, the "d" has been omitted. The prefixes "i" and "a" refer to iso and anteiso branching, and the suffixes "c" and "t" indicate *cis* and *trans* geometry. "br" indicates that the type of branching is not determined. Other methyl branching is indicated as the position of the additional methyl carbon from the carboxylic end. The presence of a cyclopropane ring in the fatty acid is indicated by "cy."

**Statistical analysis.** The similarity in PLFA composition of sediments in the study area was calculated by Bousfield's coefficient (7). It has been reported that Bousfield's coefficient is suitable to determine the difference in fatty acid composition since these values are smaller than that of the correlation coefficient (37a). On the basis of the results of the similarity analysis, the stations were grouped into two areas (coastal area and other areas) to find the difference in the PLFA composition between them. Tukey's honestly significant difference test was performed to determine the difference between the PLFA composition of these two areas by using a HITAC M-680H (VOS3) program with a mainframe computer available at the Hiroshima University Information Processing Saijo Center.

**Microbial biomass.** The following conversion factors were used to calculate the microbial biomass in sediments. It has been reported that the average bacterium, the size of *Escherichia coli* contains 100 μmol of PLFA per g (dry weight) (3, 43), and 1 g of bacteria is equivalent to $5.9 \times 10^{12}$ cells, which provides $1.7 \times 10^{-17}$ mol of PLFA per bacterial cell (26).

## RESULTS

On the basis of the vertical stability of the water column in summer, the present study area was divided into the coastal area of Hiroshima Bay, the southern region of Hiroshima Bay, and the adjacent bay. The environmental characteristics of the study area are shown in Table 1. The depth of the sampling stations varied from 11 to 47 m. A normal pattern of high saline and low thermal conditions was observed in the study area. The temperature and density (sigma 't') of the surface- and bottom-water samples did not show marked variations, whereas the salinity of the bottom-water samples was comparatively higher than that of the surface-water samples in Hiroshima Bay and no such difference in salinity was observed in Aki Nada. The percentage saturation of dissolved oxygen in surface water varied from 89.2 to 136.4%, whereas, in bottom water, it ranged from 29.8 to 88.1%, indicating that the oxygen saturation in the bottom water was low. The observed variations in density, temperature, and salinity between surface- and bottom-water samples of the study area were small, indicating the virtual absence of any water movement. The mud temperature ranged from 23.4 to 25.5°C, and the pH varied from 7.28 to 7.64. These values did not show much variation among the stations. $E_h$ values showed wide variation in the study area, ranging from −130 to +250. Higher values of hydrogen sulfide were determined in the sediments of stations in Hiroshima Bay than in those in Aki Nada, and the values ranged from 0.01 to 1.59 mg of S per g of sediment. The sediment samples were black, silty mud with an odor of hydrogen sulfide.

**PLFA composition.** The percent distribution of PLFA in the study area is shown in Table 2 and includes saturated fatty acids ($C_{12}$ to $C_{24}$), branched fatty acids, monounsaturated fatty acids, and polyunsaturated fatty acids (Table 3). Sixty-three fatty acids were identified in the sediment samples analyzed. High percentages of 16:0 fatty acid were

determined in all of the samples. The fatty acids in the range of $C_{12}$ to $C_{19}$ are reported to be bacterial in origin. In the range of $C_{12}$ to $C_{19}$, branched and monounsaturated fatty acids were present in higher percentages than even-numbered saturated fatty acids (Table 3). Low percentages of odd-numbered saturated fatty acids were present in all of the samples. Monounsaturated fatty acids longer than 20 carbon atoms and polyunsaturated fatty acids were also determined to be present in small quantities. Longer-chain fatty acids with more than 20 carbon atoms were determined to be present in low percentages, indicating small input from a terrestrial source. The saturated fatty acids 16:0, 14:0, and 18:0 were present in high percentages. The iso, anteiso, and methyl branching fatty acids were determined to be present in sediment samples. The monounsaturated fatty acids with chain lengths shorter than 20 were in the range of $C_{14}$ to $C_{18}$, and the *cis* isomer of 16:1d9 was present in higher percentages than the *trans* isomer (Table 2). In general, the number of fatty acids present in the sediment of the study area showed variation, indicating the differences in the lipid-contributing communities.

To determine the similarity in the composition of PLFA in sediments in the study area, which is reported to be characteristic for different bacteria, a similarity analysis was performed. The results of cluster analysis of PLFA composition are shown in Fig. 2. Six clusters were obtained, and the PLFA compositions were similar at the 70% level. Clusters 1 and 2 are present in the coastal area of Hiroshima Bay, clusters 4 and 5 are present in southern Hiroshima Bay, and clusters 3 and 6 are present in Aki Nada (Fig. 3). The clusters obtained in the present study revealed the differences in the PLFA compositions and, in turn, indicated the differences in the microbial communities present in the study area.

To find the difference in the relative proportions of PLFA of stations grouped in cluster 2 with stations grouped in other clusters, Tukey's honestly significant difference test was performed and the results are shown in Table 4. The results illustrate the difference between the community structures of these two areas. CA refers to stations in the coastal area (cluster 2), and OA refers to stations in other areas (clusters other than cluster 2). Of the 40 fatty acids tested, 16 and 24 fatty acids were present in large amounts in OA and CA, respectively. Three fatty acids (a14:0, 14:1d7, and 14:2) of 16 fatty acids were significantly abundant in OA. In the CA, 10 fatty acids (i13:0, 16:1d9t, 16:1d11c, i17:1, cy17:0, 18:1d11t, 18:1, 10Me18:0, 20:1d11t, and 24:0) were present in significant quantities, and other fatty acids were present in large amounts (Table 4).

**Biomass.** The PLFA content in sediment samples showed marked variation, ranging from 0.26 (O-6) to 3.79 (HO-22) μg/g (dry weight) of sediment (Table 5). The variation in PLFA content of sediment samples was in conformity with the clusters obtained. The PLFA content in sediments of stations grouped in cluster 2 was higher than that measured in stations grouped in other clusters (Table 5). The PLFA content was generally lower in Aki Nada than in Hiroshima Bay. Microbial biomass calculated by using the conversion factor in the present investigation revealed that the bacterial biomass in sediments ranged between $0.1 \times 10^8$ cells per g (dry weight) at O-6 and $1.7 \times 10^8$ cells per g (dry weight) at HO-22 (Table 5). The calculated biomass seems to decrease with distance from the coast, and wide variation among the stations was also evident. The microbial biomass was higher in stations of cluster 2 than in the other stations grouped into other clusters. In Table 5, the ratios of branched fatty acids and monounsaturated fatty acids, the ratios of iso and anteiso fatty acids of 15:0 to 16:0, and the ratios of *trans* to *cis* of 16:1d9 are also illustrated.

## DISCUSSION

Of 63 PLFA identified in the sediments of Hiroshima Bay and Aki Nada, most of the fatty acids are considered to be of bacterial origin. Fatty acids in the range of $C_{12}$ to $C_{19}$ are reported to be characteristic of bacteria (25). A number of investigations have revealed that branched fatty acids, monounsaturated fatty acids, cyclopropyl fatty acids, and certain saturated fatty acids present in sediments are definitely contributed by the in situ bacterial populations (14, 32, 40, 45). High percentages of 16:0 fatty acids (15 to 22% of the total PLFA), which are ubiquitously present in most organisms, were determined in all sediment samples. Other saturated straight-chain fatty acids which are known to be present in both prokaryotes and eukaryotes were also detected in significant amounts. In all samples, odd-numbered fatty acids were detected in low percentages (Table 3).

The branched fatty acids are commonly encountered in bacterial lipids, and similar ranges of fatty acids were identified in the sediment and bacterial cultures. Branched fatty acids are reported as biomarkers in bacteria (39, 41), anaerobic bacteria (17, 23), and the sulfate-reducing bacteria (SRB) *Desulfovibrio* spp. (5, 6, 11). The 10Me16:0 fatty acid is a signature fatty acid for the SRB *Desulfobacter* spp. (10, 35) and is not detected in other SRB; approximately 27% of the total PLFA of the bacteria was reported to be this fatty acid (10). These acids are generally found in higher concentrations in bacteria than in other organisms, making them useful indicators of bacterial lipid contribution. The relative proportions of these branched fatty acids in sediments of the present study area reveal the contribution of anaerobic bacteria and SRB to sediments. The occurrence of SRB and the seasonal variation of both heterotrophic bacteria and SRB in sediments of Hiroshima Bay have been observed (29a). These results showed that higher numbers of SRB were distributed in coastal sediments than in other parts of the bay, and they were in the range of $10^1$ (southern Hiroshima Bay) to $10^4$ (coastal area of Hiroshima Bay). The above results of microbiological investigation also support the finding of the biomarker fatty acids of anaerobic bacteria in the present study, i.e., that anaerobic bacteria, especially SRB, were widely distributed in the sediments. In addition to the organic pollution, the environmental conditions of the study area confirm the existence of reduced conditions in the bays, which explains the presence of SRB.

The presence of monounsaturated fatty acids in sediments suggests the dominance of prokaryotic organisms in the sediment of the present study area (Table 2). Information on the presence of these fatty acids in bacteria has been available (31, 32), and the bacterial contribution of these fatty acids has also been reported for marine sediments (15, 31, 32). The unsaturated fatty acids in the range of $C_{12}$ to $C_{19}$ are indicative of gram-negative bacteria (24, 31). It has been reported that monoenoic fatty acids 16:1d9 and 18:1d9, which occur in bacterial cultures, can also be contributed by microalgae (12, 14). However, low percentages (1 to 8%) of polyunsaturated fatty acids (18:2, 20:4, 20:5), which are characteristic of microeukaryotes (4, 12, 41), suggest that the contribution of microeukaryotes is minor in the present study area. An estimation of the bacterial contribution to the sediment on the basis of the presence of unsaturated fatty acids and low percentages of polyunsaturated fatty acids supports the view that bacteria are the predominant contrib-

TABLE 2. Percent composition of PLFA in sediment samples

| Fatty acid | % of fatty acid at station | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | HO-1 | HO-2 | HO-3 | HO-4 | HO-4a | HO-5 | HO-11 | HO-12 | HO-13 | HO-14 | HO-15 | HO-16 | HO-21 | HO-22 | HO-23 | HO-24 | O-3a | O-3b | O-4 | O-6 |
| 10:0 | 0.06 | 0.84 | —[a] | — | — | — | 0.08 | — | — | — | — | — | — | — | — | 0.08 | — | — | — | — |
| i12:0 | 0.42 | 1.49 | 0.44 | 0.23 | 0.25 | 0.63 | 0.19 | — | 0.49 | 0.12 | 0.29 | 0.36 | 0.27 | 0.44 | — | 0.23 | 0.62 | 0.43 | — | 0.80 |
| a12:0 | 0.09 | — | 0.05 | — | — | — | 0.01 | 0.45 | 0.06 | — | — | — | 0.06 | 0.07 | — | 0.26 | 0.26 | — | — | — |
| 12:0 | 0.48 | 2.04 | 0.58 | 0.36 | 0.44 | 0.96 | 0.38 | 1.30 | 0.75 | 0.38 | 0.76 | 1.06 | 0.55 | 0.47 | 0.76 | 0.52 | 1.04 | 0.58 | — | 1.02 |
| i13:0 | 0.18 | — | 0.21 | — | — | — | 0.22 | — | 0.18 | — | 0.27 | 0.24 | 0.20 | 0.16 | — | 0.13 | 0.35 | 0.18 | — | — |
| a13:0 | 0.57 | 0.36 | 0.41 | 0.40 | 0.54 | 0.26 | 0.02 | 0.56 | 0.17 | 0.15 | 0.32 | 0.40 | 0.05 | 0.06 | 0.35 | 0.15 | 0.91 | 0.36 | 0.28 | 1.00 |
| 13:0 | 0.14 | — | 0.18 | — | — | — | 0.18 | — | 0.25 | 0.14 | 0.12 | — | 0.21 | 0.18 | — | 0.46 | — | 0.38 | — | — |
| br14:0 | 0.20 | 1.98 | 0.15 | 1.05 | 0.61 | 1.65 | 0.84 | 0.73 | 0.42 | 1.01 | 1.35 | 1.58 | 0.69 | 0.24 | 0.65 | 6.80 | — | 2.19 | 1.77 | — |
| i14:0 | 1.38 | 1.41 | 1.45 | 0.79 | 0.68 | 2.10 | 1.81 | 1.73 | 1.81 | 1.64 | 2.29 | 2.08 | 1.80 | 1.55 | 2.37 | 2.44 | 2.75 | 1.56 | 2.06 | 1.11 |
| a14:0 | 0.38 | 2.30 | 0.46 | 1.92 | — | 0.83 | 0.39 | 0.47 | 0.48 | 0.44 | 0.83 | 1.12 | 0.56 | — | 2.13 | 2.83 | 2.14 | 0.96 | 2.30 | — |
| 14:1d7 | 0.47 | 3.26 | 0.40 | 1.63 | — | 0.71 | 0.37 | 0.55 | 0.67 | 0.38 | 0.64 | 1.01 | 0.45 | 0.45 | 0.88 | 3.90 | 1.69 | 0.91 | 1.93 | 5.33 |
| 14:1d9 | 0.51 | 2.23 | 0.41 | 0.92 | 0.97 | 0.51 | 0.39 | — | 0.48 | 0.25 | 0.54 | 0.66 | 0.46 | 0.47 | — | 1.34 | 1.09 | 0.46 | 1.02 | — |
| 14:2 | 0.42 | 1.14 | 0.36 | 1.25 | 1.41 | 0.29 | 0.29 | — | 0.57 | 0.29 | 0.61 | 0.74 | 0.38 | 0.38 | 1.77 | 3.85 | 1.36 | 0.74 | 1.49 | — |
| 14:0 | 4.59 | 5.84 | 4.86 | 5.64 | 5.40 | 7.46 | 5.50 | 6.49 | 6.22 | 6.68 | 7.46 | 5.44 | 6.71 | 6.28 | 7.07 | 7.09 | 6.86 | 5.97 | 5.65 | 4.23 |
| br15:0 | — | — | 0.16 | — | — | — | 0.42 | — | 0.48 | 0.25 | 0.36 | — | 0.24 | 0.49 | — | 1.26 | — | 0.07 | — | — |
| i15:0 | 5.64 | 4.07 | 5.70 | 3.95 | 3.87 | 7.04 | 7.30 | 7.21 | 6.55 | 6.30 | 8.12 | 7.02 | 6.89 | 6.42 | 7.54 | 7.04 | 7.72 | 5.93 | 6.64 | 3.95 |
| a15:0 | 8.49 | 4.90 | 8.69 | 4.59 | 5.07 | 8.21 | 9.85 | 9.02 | 9.34 | 8.98 | 10.96 | 9.90 | 9.37 | 8.82 | 14.61 | 10.80 | 10.31 | 7.44 | 6.64 | 4.23 |
| 15:1d7 | 0.13 | — | — | — | — | — | 0.15 | — | — | — | — | — | 0.12 | — | — | 0.97 | — | — | — | — |
| 15:1d9 | 0.37 | 1.31 | 0.36 | 0.25 | 1.04 | — | 0.39 | — | 0.33 | 0.09 | 0.16 | 0.65 | 0.40 | 0.40 | — | 0.78 | 0.33 | 0.56 | 0.98 | — |
| 15:1 | 0.11 | — | 0.13 | — | — | — | 0.11 | 0.36 | 0.15 | 0.16 | — | 0.26 | 0.12 | 0.15 | — | 1.72 | 0.48 | — | — | — |
| 15:0 | 1.75 | 1.91 | 1.99 | 2.17 | 2.08 | 2.44 | 2.01 | 2.16 | 2.30 | 2.41 | 2.32 | 2.32 | 2.50 | 2.43 | 2.79 | 4.30 | 2.05 | 2.62 | 1.84 | 2.40 |
| br16:0 | — | — | 0.23 | — | 0.60 | — | 0.28 | — | 0.14 | — | — | — | 0.29 | 0.13 | — | 1.95 | 0.34 | — | — | — |
| 16:2 | 0.19 | — | 0.54 | — | — | — | 0.39 | — | — | 0.23 | — | 0.31 | 0.42 | — | — | 0.66 | — | — | — | — |
| i16:0 | 2.25 | 2.65 | 2.21 | 2.63 | 2.55 | 2.17 | 2.46 | 3.44 | 2.76 | 3.04 | 2.70 | 2.56 | 2.53 | 2.61 | 4.14 | 2.77 | 2.36 | 1.97 | 2.37 | 2.41 |
| 16:1d6 | 0.11 | — | 0.11 | 1.06 | 1.05 | — | 0.23 | — | 0.30 | 0.17 | — | — | 0.34 | 0.10 | — | 1.35 | — | 0.47 | 0.70 | 3.33 |
| 16:1d7 | 1.40 | 0.71 | 1.71 | 1.39 | 1.32 | 1.38 | 1.37 | 1.67 | 1.18 | 0.88 | 1.41 | 1.13 | 1.36 | 1.34 | — | — | 1.19 | 1.54 | 1.80 | 0.59 |
| 16:1d9c | 9.30 | 6.08 | 9.81 | 8.02 | 8.10 | 8.56 | 8.74 | 7.24 | 8.61 | 5.33 | 8.29 | 7.92 | 10.29 | 9.64 | 5.14 | 0.48 | 6.80 | 8.96 | 8.32 | 11.02 |
| 16:1d9t | 1.29 | 0.99 | 1.71 | 1.15 | 1.12 | 1.03 | 1.70 | 1.21 | 1.38 | 0.97 | 1.27 | 1.35 | 1.60 | 1.53 | 1.03 | — | 0.69 | 0.91 | — | — |
| 16:1d11c | 1.97 | 1.21 | 2.04 | 1.69 | 1.64 | 1.82 | 2.18 | 1.64 | 2.13 | 1.64 | 2.06 | 2.17 | 2.50 | 2.54 | 1.10 | 0.56 | 1.56 | 1.61 | 1.62 | 1.35 |
| 16:1d11t | 0.25 | — | 0.16 | — | — | — | 0.27 | — | 0.33 | — | — | — | 0.18 | 0.35 | — | — | 0.70 | 0.42 | 0.80 | — |
| 16:0 | 16.13 | 15.29 | 16.18 | 18.96 | 18.41 | 21.87 | 15.97 | 20.08 | 18.66 | 20.64 | 18.35 | 17.37 | 17.36 | 17.78 | 20.12 | 15.83 | 18.35 | 17.95 | 20.17 | 18.43 |
| i17:1 | 0.39 | — | 0.37 | 0.42 | 0.46 | — | 0.28 | — | 0.30 | 0.20 | 0.31 | 0.32 | 0.25 | 0.26 | — | 0.40 | 0.31 | 0.28 | — | — |
| 10Me16:0 | 5.32 | 7.83 | 4.23 | 5.68 | 5.70 | 1.82 | 3.75 | 2.25 | 3.61 | 3.05 | 3.82 | 2.75 | 3.74 | 3.72 | 4.32 | 4.65 | 3.04 | 4.59 | 2.25 | 4.73 |
| br17:1 | 0.48 | — | 0.49 | — | — | — | 0.47 | — | 0.46 | 0.60 | 0.43 | 0.45 | 0.41 | 0.46 | — | 0.52 | — | 0.54 | — | — |
| br17:1 | — | 3.30 | — | — | — | 0.67 | 0.27 | — | — | — | — | 0.32 | 0.39 | — | — | 0.41 | — | 0.86 | 0.65 | 0.94 |
| i17:0 | 1.30 | 0.84 | 1.11 | 1.20 | 1.21 | 1.22 | 1.36 | 1.40 | 1.31 | 1.39 | 1.28 | 1.38 | 1.16 | 1.23 | 1.29 | 0.88 | 1.31 | 1.47 | 1.35 | 0.84 |
| a17:0 | 2.33 | 1.82 | 2.05 | 2.07 | 2.11 | 1.50 | 2.23 | 2.51 | 2.40 | 2.18 | 2.15 | 2.53 | 2.16 | 2.24 | 3.27 | 1.79 | 2.22 | 2.58 | 1.71 | 1.64 |
| 17:1d8 | 0.75 | 0.57 | 0.92 | 0.99 | 1.01 | 0.76 | 0.75 | 0.73 | 0.82 | 0.68 | 0.57 | 0.80 | 0.79 | 0.85 | — | 0.52 | 0.89 | 1.47 | 0.76 | 1.07 |
| 17:1d11 | 0.68 | — | 1.11 | 0.80 | 0.83 | 0.91 | 1.12 | 1.04 | 1.01 | 1.13 | 0.89 | 1.34 | — | 1.06 | — | 0.31 | 0.56 | 1.99 | 0.13 | — |
| cy17:0 | 0.18 | — | 0.14 | — | — | — | 0.13 | — | 0.12 | 0.24 | — | — | 0.11 | 0.17 | — | — | — | — | — | — |
| 17:0 | 1.33 | 1.35 | 1.24 | 1.46 | 1.47 | 1.63 | 1.45 | 1.70 | 1.49 | 1.55 | 1.36 | 1.63 | 1.27 | 1.36 | 1.38 | 1.17 | 1.53 | 2.31 | 1.65 | 1.91 |
| i18:0 | — | — | — | — | — | — | — | — | — | — | — | 0.22 | — | — | — | — | — | — | — | — |
| a18:0 | — | — | 0.15 | — | — | — | 0.29 | — | — | 0.21 | — | 0.17 | 0.14 | 0.10 | — | 1.30 | — | — | — | — |
| 18:2 | 0.23 | 2.56 | 0.27 | 1.91 | — | 1.32 | 0.55 | — | 0.16 | 0.74 | 0.77 | 0.94 | 0.71 | 0.20 | — | — | — | 0.64 | 1.59 | 4.04 |
| 18:1d9c | 1.09 | 0.95 | 0.92 | 1.36 | 1.47 | 1.03 | 1.19 | 1.51 | 1.09 | 1.25 | 0.99 | 0.97 | 0.88 | 1.25 | 1.26 | 0.85 | 0.75 | 0.80 | 0.90 | 1.26 |
| 18:1d9t | 5.34 | 4.71 | 5.64 | 5.62 | 5.88 | 3.87 | 3.98 | 5.42 | 4.60 | 4.45 | 3.80 | 4.11 | 4.09 | 4.56 | 4.13 | 1.28 | 3.45 | 4.13 | 4.12 | 6.86 |

*Continued on following page*

TABLE 2—*Continued*

| Fatty acid | % of fatty acid at station | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | HO-1 | HO-2 | HO-3 | HO-4 | HO-4a | HO-5 | HO-11 | HO-12 | HO-13 | HO-14 | HO-15 | HO-16 | HO-21 | HO-22 | HO-23 | HO-24 | O-3a | O-3b | O-4 | O-6 |
| 18:1d11c | 9.83 | 6.15 | 9.53 | 9.64 | 10.19 | 6.92 | 7.75 | 9.00 | 7.59 | 6.29 | 6.56 | 7.18 | 7.47 | 7.51 | 5.92 | — | 5.22 | 7.03 | 7.17 | 6.05 |
| 18:1d11t | 0.62 | — | 0.58 | 0.74 | 0.75 | — | 0.68 | 0.78 | 0.56 | 0.67 | 0.47 | 0.56 | 0.53 | 0.51 | 0.72 | — | — | 0.35 | — | — |
| 18:1 | 0.21 | — | 0.20 | — | — | — | 0.22 | — | 0.17 | 0.33 | — | 0.19 | 0.18 | 0.22 | — | — | — | — | — | — |
| 18:0 | 2.75 | 2.84 | 3.02 | 3.07 | 3.01 | 3.81 | 2.95 | 3.27 | 2.87 | 3.96 | 2.44 | 2.80 | 2.35 | 2.75 | 3.08 | 2.46 | 2.99 | 2.33 | 3.65 | 4.06 |
| br19:1 | 0.88 | 2.46 | 0.80 | 0.57 | 0.64 | 0.71 | 0.40 | 0.82 | 0.44 | 0.82 | 0.39 | 0.45 | 0.48 | 0.63 | 0.73 | 0.24 | 0.53 | 0.69 | 0.89 | 1.00 |
| 10Me18:0 | 0.56 | — | 0.47 | 0.32 | 0.35 | — | 0.40 | 0.17 | 0.44 | 0.62 | 0.25 | 0.27 | 0.36 | 0.41 | — | 0.22 | 0.50 | 0.36 | — | — |
| cy19:0 | 0.54 | — | 0.46 | 0.49 | 0.49 | 0.46 | 0.40 | 0.55 | 0.41 | 2.61 | 0.41 | 0.46 | 0.37 | 0.40 | 0.70 | 0.35 | 0.69 | 0.53 | 1.28 | — |
| 20:5 | 1.46 | 0.64 | 1.09 | 0.89 | 1.05 | 0.85 | 1.02 | 0.41 | 0.48 | 0.13 | 0.36 | 0.34 | 0.81 | 0.85 | — | 0.17 | 0.37 | 0.34 | 0.62 | 1.31 |
| 20:4 | 1.92 | 0.61 | 1.23 | 1.77 | 2.12 | 1.74 | 1.65 | 0.54 | 0.61 | 0.16 | 0.33 | 0.33 | 0.98 | 1.29 | — | — | 0.49 | 0.48 | 0.60 | 3.10 |
| 20:1d11c | 0.56 | — | 0.49 | 0.32 | 1.00 | 0.36 | 0.72 | 0.32 | — | 0.32 | 0.29 | 0.51 | 0.34 | — | — | 0.22 | 0.53 | 0.42 | 1.36 | — |
| 20:1d11t | 0.39 | — | 0.45 | — | 0.54 | — | 0.39 | — | 0.38 | 0.23 | 0.26 | 0.29 | 0.30 | 0.43 | — | — | — | 0.30 | — | — |
| 20:1 | 0.17 | — | 0.24 | — | 0.55 | — | 0.32 | 0.65 | 0.13 | 0.22 | — | 0.32 | — | 0.19 | — | 0.29 | 1.48 | — | 0.96 | — |
| 20:0 | 0.53 | 0.66 | 0.42 | 0.61 | 0.71 | 0.50 | 0.36 | 0.62 | 0.52 | 1.25 | 0.41 | 0.46 | 0.40 | 0.76 | 0.73 | 0.54 | 0.43 | 0.35 | — | — |
| 22:1 | 0.17 | 0.71 | 0.21 | — | 0.53 | — | 0.20 | — | 0.12 | — | — | — | 0.12 | 0.16 | — | 0.34 | — | — | — | — |
| 22:1 | 0.33 | — | 0.12 | — | 0.46 | — | — | — | 0.14 | — | — | — | — | 0.10 | — | — | 0.72 | — | — | — |
| 22:0 | 0.20 | — | 0.19 | — | 0.35 | — | 0.13 | — | 0.28 | 1.36 | — | 0.28 | 0.15 | 0.46 | — | 0.30 | — | — | — | — |
| 24:0 | 0.16 | — | 0.14 | — | — | — | 0.10 | — | 0.27 | 0.81 | — | — | 0.13 | 0.41 | — | 0.23 | — | — | — | — |

[a] —, not detected.

utor of fatty acids to the sediment and the predominant source of monounsaturated fatty acids in these sediments. In mangrove-associated sediments, the presence of high percentages of these acids was attributed to microalgae and higher-order plants (14). The difference in the relative proportions of branched and monounsaturated fatty acids in sediments which are reported to be marker acids for gram-positive and gram-negative bacteria suggests the difference in the origins of these acids (Table 3).

Cyclopropyl fatty acids ($C_{17}$ and $C_{19}$) have also been proposed as bacterial signatures in sediments (2, 8, 32) and have been reported as major constituents in a large number of gram-negative bacteria (24) and *Desulfobacter* spp. (10), but are rare in eukaryotes. The results of Parkes and Taylor (31) suggest that cyclopropyl fatty acids are characteristic of aerobic sediment bacteria, and this was supported by the presence of high concentrations of cyclopropyl fatty acids in oxidized sediment surface layers and the decrease of these concentrations with increasing depth as anaerobic conditions prevailed. The observations of Parkes and Taylor (31) also suggested that cyclopropyl fatty acids are more indicative of aerobic bacteria than of anaerobic bacteria. The environmental characteristics of the study area revealed that the sediments are subject to less physical disturbance by currents, resulting in greater vertical stability, which favors the formation of microniches of anaerobic conditions. From the small amounts of these fatty acids in sediment of the present study area, it could be inferred that there is a shift in the microbial community structure from aerobic to anaerobic bacteria.

One of the characteristic features of the sediment samples analyzed in the present study, in comparison to estuarine and abyssal marine sediments (1, 44, 45), was the relatively low percentage of polyunsaturated fatty acids longer than 19 carbon atoms in the PLFA profiles. A plausible cause of the relatively low quantities of these biomarker acids in the present study area could be the existing environmental conditions and the extent of organic pollution in the study area. An $E_h$ value of +100 mV or higher indicates that oxidizing conditions prevail (16). Variation in the $E_h$ values from +100 to −130 mV except at station O-3a revealed that the sediments were in a reduced condition. At this redox level, the presence of sulfide in sediments supports the mineralization of organic matter by sulfate reduction. Dissolved oxygen is present in the bottom water (2 m above the sediment) but at low concentrations, and, at the sediment-water interface, the dissolved oxygen concentration must be lower than that in the bottom water. As observed by Findlay et al. (13), the reduced availability of oxygen in sediments may cause the loss of microeukaryotic biomass, the decrease in aerobic bacteria, and the increase in anaerobic bacteria and SRB. Survival of microeukaryotes might be restricted in polluted bays like Hiroshima Bay, which is one of the heavily polluted bays in the Seto Inland Sea. The results of the present study are in agreement with the results of Smith et al. (36), who reported a relative lack of polyenoic fatty acids longer than 19 carbon atoms in organically contaminated sediments. Low percentages of these acids in coastal sediments were attributed to the organic pollution and the prevailing anoxic conditions (32a). Low percentages of long-chain fatty acids in sediments, which are known to be contributed to the sediment by terrigenous input, indicate that the terrestrial contribution in the present study area is minor. In the study area, the land-based organic matter is considerably lower than that contributed by primary production (34).

TABLE 3. Distribution of different groups of PLFA in sediments

| Station | % Fatty acid present | | | | | | |
|---|---|---|---|---|---|---|---|
| | Even-numbered saturated fatty acids (<19)[a] | Odd-numbered saturated fatty acids (<20) | Branched fatty acids | Monounsaturated fatty acids (<19) | Polyunsaturated fatty acids | Saturated fatty acids (>20) | Monounsaturated fatty acids (>19) |
| Cluster 1: HO-2 | 26.85 | 3.26 | 32.11 | 31.46 | 4.94 | 0.66 | 0.71 |
| Cluster 2 | | | | | | | |
| HO-1 | 24.00 | 3.23 | 31.11 | 34.93 | 4.22 | 0.90 | 1.62 |
| HO-3 | 24.69 | 3.42 | 29.94 | 36.21 | 3.29 | 0.75 | 1.50 |
| HO-11 | 24.89 | 3.64 | 33.04 | 32.32 | 3.90 | 0.59 | 1.63 |
| HO-12 | 31.14 | 3.86 | 31.31 | 31.15 | 0.95 | 0.62 | 0.97 |
| HO-13 | 28.50 | 4.04 | 31.94 | 31.85 | 1.83 | 1.06 | 0.77 |
| HO-14 | 31.66 | 4.10 | 33.23 | 25.27 | 1.55 | 3.42 | 0.77 |
| HO-15 | 29.00 | 3.80 | 36.09 | 28.08 | 2.06 | 0.41 | 0.55 |
| HO-16 | 26.67 | 3.95 | 33.81 | 31.06 | 2.66 | 0.73 | 1.12 |
| HO-21 | 26.97 | 3.99 | 31.75 | 32.54 | 3.30 | 0.69 | 0.76 |
| HO-22 | 27.27 | 3.97 | 30.15 | 33.38 | 2.72 | 1.63 | 0.87 |
| Cluster 3 | | | | | | | |
| HO-5 | 34.10 | 4.07 | 28.61 | 28.15 | 4.21 | 0.50 | 0.36 |
| O-3a | 29.25 | 3.58 | 36.37 | 25.40 | 2.23 | 0.43 | 2.74 |
| O-3b | 26.82 | 5.31 | 31.59 | 33.02 | 2.20 | 0.35 | 0.71 |
| O-4 | 29.47 | 3.50 | 29.54 | 30.88 | 4.30 | 0.00 | 2.33 |
| Cluster 4 | | | | | | | |
| HO-4 | 28.03 | 3.63 | 26.33 | 35.27 | 5.81 | 0.61 | 0.32 |
| HO-4a | 27.26 | 3.54 | 25.13 | 35.36 | 4.57 | 1.06 | 3.07 |
| Cluster 5 | | | | | | | |
| HO-23 | 31.04 | 4.16 | 42.11 | 20.18 | 1.77 | 0.73 | 0.00 |
| HO-24 | 25.98 | 5.93 | 46.49 | 15.00 | 4.68 | 1.08 | 0.85 |
| Cluster 6: O-6 | 27.73 | 4.31 | 21.71 | 37.79 | 8.46 | 0.00 | 0.00 |

[a] Values in parentheses indicate number of carbon atoms.

On the basis of the presence of biomarker fatty acids in sediments, Findlay et al. (13) classified the microorganisms into four distinct functional groups, namely, microeukaryotes (polyunsaturated fatty acids), aerobic prokaryotes (monounsaturated fatty acids), gram-positive and other anaerobic bacteria (saturated and branched fatty acids in the range

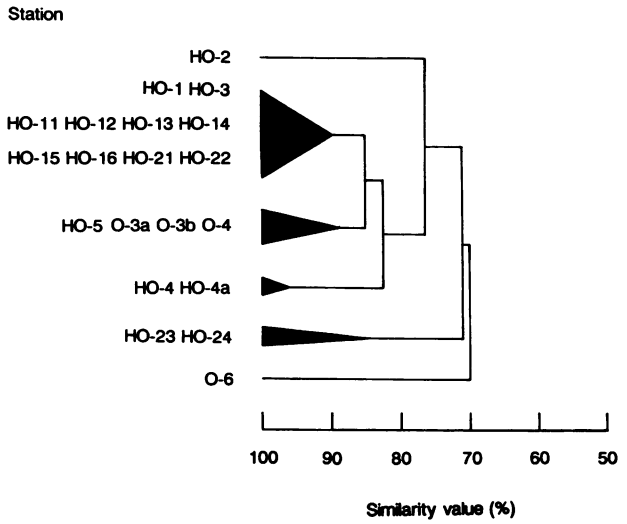


FIG. 2. Simplified dendrogram based on average linkage method.

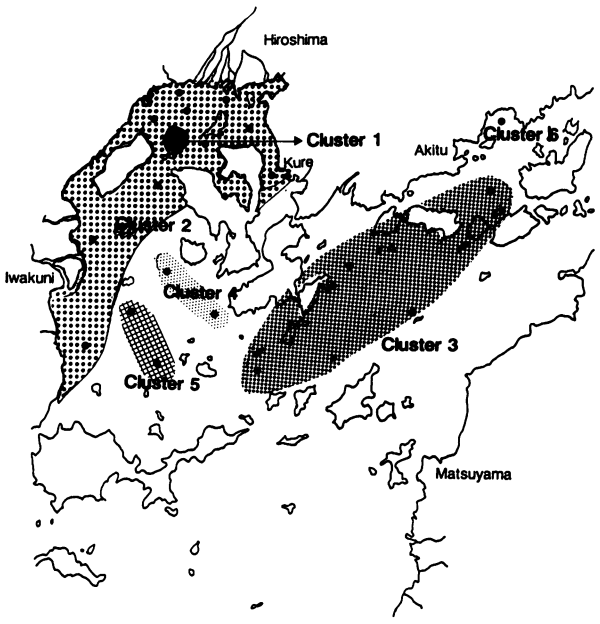


FIG. 3. Distribution of clusters in the study area.

TABLE 4. Tukey's significant difference maps generated from Tukey's honestly significant difference test (HITAC) of the study area[a]

| Fatty acid | Low | High | Fatty acid | Low | High |
|---|---|---|---|---|---|
| 12:0 | CA | OA | i17:0 | OA | CA |
| i13:0 | OA | CA | a17:0 | OA | CA |
| 13:0 | OA | CA | 17:1d8 | CA | OA |
| i14:0 | OA | CA | 17:1d11 | OA | CA |
| a14:0 | CA | OA | cy17:0 | OA | CA |
| 14:1d7 | CA | OA | 17:0 | CA | OA |
| 14:2 | CA | OA | 18:2 | CA | OA |
| 14:0 | CA | OA | 18:1d9c | OA | CA |
| i15:0 | OA | CA | 18:1d9t | OA | CA |
| a15:0 | OA | CA | 18:1d11c | OA | CA |
| 15:0 | CA | OA | 18:1d11t | OA | CA |
| i16:0 | OA | CA | 18:1 | OA | CA |
| 16:1d6 | CA | OA | 18:0 | CA | OA |
| 16:1d9c | OA | CA | 10Me18:0 | OA | CA |
| 16:1d9t | OA | CA | cy19:0 | OA | CA |
| 16:1d11c | OA | CA | 20:1d11c | CA | OA |
| 16:1d11t | CA | OA | 20:1d11t | OA | CA |
| 16:0 | CA | OA | 20:4 | CA | OA |
| 10Me16:0 | CA | OA | 20:5 | OA | CA |
| i17:1 | OA | CA | 24:0 | OA | CA |

[a] Values increase from left to right and are significantly different if not joined by a line.

TABLE 5. PLFA content and biomass in sediments of the study area

| Station | Ratio of monounsaturated fatty acids (<19)[a] to branched fatty acids | Total PLFA content (μg/g) | Biomass ($10^8$ cells/g) | Ratio of i+a15:0 to 16:0 | Ratio of *trans* to *cis* 16:1d9 |
|---|---|---|---|---|---|
| Cluster 1: HO-2 | 0.98 | 0.34 | 0.2 | 0.59 | 0.16 |
| Cluster 2 | | | | | |
| HO-1 | 1.12 | 3.33 | 1.5 | 0.88 | 0.14 |
| HO-3 | 1.21 | 3.04 | 1.4 | 0.89 | 0.17 |
| HO-11 | 0.98 | 3.26 | 1.5 | 1.07 | 0.19 |
| HO-12 | 1.00 | 1.21 | 0.6 | 0.81 | 0.17 |
| HO-13 | 1.00 | 3.25 | 1.5 | 0.85 | 0.16 |
| HO-14 | 0.76 | 1.70 | 0.8 | 0.74 | 0.18 |
| HO-15 | 0.78 | 1.61 | 0.7 | 1.04 | 0.15 |
| HO-16 | 0.92 | 1.17 | 0.5 | 0.97 | 0.17 |
| HO-21 | 1.03 | 2.33 | 1.1 | 0.94 | 0.16 |
| HO-22 | 1.11 | 3.79 | 1.7 | 0.86 | 0.16 |
| Cluster 3 | | | | | |
| HO-5 | 0.98 | 0.86 | 0.4 | 0.70 | 0.19 |
| O-3a | 0.70 | 0.52 | 0.2 | 0.98 | 0.10 |
| O-3b | 1.05 | 1.64 | 0.7 | 0.74 | 0.10 |
| O-4 | 1.05 | 0.49 | 0.2 | 0.66 | 0.00[b] |
| Cluster 4 | | | | | |
| HO-4 | 1.34 | 0.59 | 0.3 | 0.45 | 0.14 |
| HO-4a | 1.41 | 0.72 | 0.3 | 0.49 | 0.14 |
| Cluster 5 | | | | | |
| HO-23 | 0.48 | 0.45 | 0.2 | 1.10 | 0.20 |
| HO-24 | 0.32 | 0.58 | 0.2 | 1.13 | 0.00 |
| Cluster 6: O-6 | 1.74 | 0.26 | 0.1 | 0.44 | 0.00 |

[a] Value in parentheses indicates number of carbon atoms.
[b] *trans* fatty acid was not detected.

of $C_{14}$ to $C_{16}$), and SRB and other anaerobic bacteria (saturated and branched fatty acids in the range of $C_{16}$ to $C_{19}$). The presence of these fatty acids in the present study area (Table 3) also results in the inference that all four of these functional groups of microorganisms are present in sediments in different proportions.

The calculated biomass was found to be higher in coastal sediments of Hiroshima Bay than the sediments of the southern region of Hiroshima Bay and the adjacent Aki Nada (Fig. 4), where the bacterial biomass was less than $0.5 \times 10^8$ cells per g (dry weight) of sediment. A similar observation on fatty acid composition was reported for coastal sediments (32a). Microbiological studies in the Seto Inland Sea also indicated that the densities of heterotrophic bacteria decreased as the distance from the coast increased (38). Station 2, located in the CA, contained a low amount of biomass, which may be due to the tidal current flow in the narrow passage between the islands. It has been reported that calculated values of bacterial biomass based on PLFA may underestimate the actual number of cells if the cells present are smaller than *E. coli* cells (2). However, the results of microcomputer-assisted image analysis of the bacterial cells in the sediments of Ohmi Bay, which is located on the western side of Hiroshima Bay, showed that more than 95% of the bacteria are in the size (equivalent spherical diameter) range of 0.4 to 1.0 μm (20), and the results of the present study are also in agreement with epifluorescence direct counts of the bacteria in Ohmi Bay (20).

The microbial community structure of the study area showed wide variation, as characterized by the relative proportions of fatty acids, and this pattern is evidenced by the results of cluster analysis. Cluster 1 contains high amounts of branched fatty acids and lesser amounts of monounsaturated and straight-chain fatty acids (Table 3). High amounts of both branched and monounsaturated fatty acids were present in clusters 2 and 3, whereas high quantities of monounsaturated fatty acids and low percentages of branched fatty acids were present in clusters 4 and 6. In cluster 5, branched fatty acids were present in high percentages, and saturated and monounsaturated fatty acids were present in somewhat lower percentages. The presence of low percentages of polyunsaturated fatty acids provides additional information about the contributing communities. In addition to this, the difference among the clusters showed the shift in the contributing microbial communities. Furthermore, the PLFA content and the calculated biomass were also higher in cluster 2 than in other clusters.

From the above discussion, it is understood that branched and monounsaturated fatty acids are characteristic of anaerobic and aerobic bacteria, respectively, and hence the ratios will provide the relative dominance of these bacterial groups. A ratio less than 1 will indicate the dominance of anaerobic bacteria, and a ratio above 1 will indicate the predominance of aerobic bacteria. These ratios in the study area varied from 0.32 to 1.74 (Table 5). Stations in cluster 4 (HO-4 and HO-4a) and cluster 6 (O-6) had higher percentages of monounsaturated fatty acids than of branched fatty acids (Table 3), and the ratios were also higher than 1.34. The lowest ratios observed at stations HO-23 and HO-24 indicated the dominance of anaerobic bacteria. Similarly, the sum of iso and anteiso fatty acids of 15:0 to 16:0 will give an indication of the proportions of the bacteria in sediments (26). These ratios ranged from 0.44 to 1.13, indicating an approximately 2.5-fold increase in the abundance of bacte-

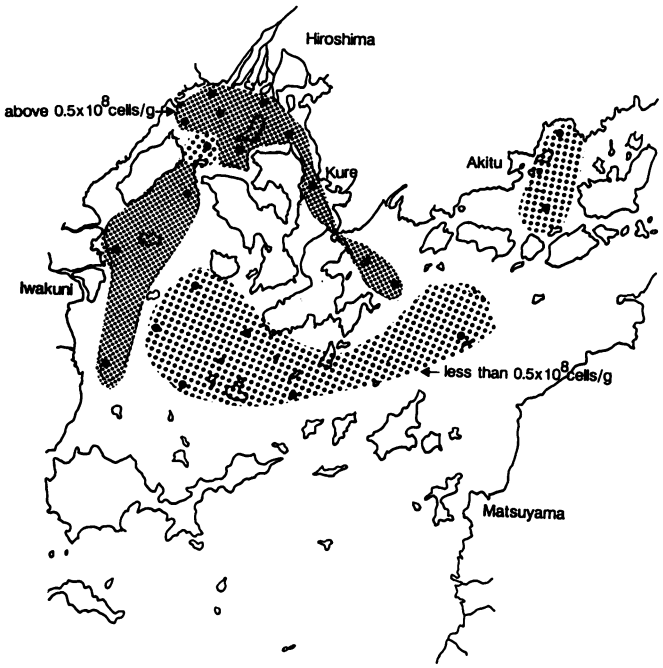


FIG. 4. Distribution of microbial biomass in the study area.

rial signatures in the study area. The PLFA profiles provide not only information on the bacterial biomass and community structure but also information on the metabolic status of the microorganisms. The presence of *trans* fatty acids has been associated with the physiological status of the microorganisms (17, 18, 19, 29), and *cis* isomers of monounsaturated fatty acids are commonly present in microbes (19). The ratios of *trans* to *cis* fatty acids in most bacterial and sediment samples were found to be less than 0.1 (14, 17, 29, 32), but the ratio was greater than 1 during starvation (26). In the present study area, the *trans/cis* ratio of fatty acid 16:1d9 in sediments ranged from 0.1 to 0.2, suggesting that microbes are likely to be exposed to some physiological stress.

The results of Tukey's honestly significant difference test provide additional information on the difference between the PLFA profiles of the sediments of the CA and those of the OA. In the OA, the saturated fatty acids are present in large percentages, whereas in the CA, branched and monounsaturated fatty acids are present in large percentages (Table 5). Although the long-chain fatty acids are present in low percentages, that fatty acid 24:0 is present in significant percentages in the coastal sediments indicates the terrestrial input. The presence of a significantly large number of fatty acids in the CA suggests that the microbial community is dominated by prokaryotes and is contributed to by anaerobic bacteria, which is different from the situation in the OA.

PLFA profiles of the sediment in the range of $C_{12}$ to $C_{19}$ and significantly low percentages of fatty acid markers (polyunsaturated fatty acids) for eukaryotic organisms and long-chain fatty acids suggest that the PLFA determined in the sediments are predominantly bacterial in origin. The PLFA profiles of the present study area are notably different from the reported results of PLFA in sediments of other studies. Significant amounts of polyunsaturated fatty acids were reported in deep-sea and estuarine sediments (1, 2), marine sediments (32), and intertidal sediments (40). PLFA profiles in sediments of an Antarctic lake system showed that polyunsaturated fatty acids were present at a significant level (26). As described earlier, the observed PLFA profile with high amounts of bacterial biomarkers and considerably low amounts of polyenoic fatty acids longer than 19 carbon atoms could be attributed to the existing pollution and environmental conditions, and these patterns also suggest that the PLFA analysis could be a useful tool in assessing the pollution. Similarly, the reported absence of polyunsaturated fatty acids in the contaminated sediments (36) also supports the observations of the present study.

The above data indicate that analysis of PLFA in sediments is a useful additional means for the comparison of microbiota in bay sediments for routine analysis in terms of both biomass and community structure. From the results, the presence of aerobic and anaerobic bacteria and of SRB in the study area was understandable. One of the characteristic features of the results of the present study has been the relatively very low percentages of both polyunsaturated fatty acids and long-chain fatty acids in sediments, indicating that the source of these fatty acids is extremely limited, possibly because of the existing pollution and the reduced oxygen conditions in the bays.

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## REFERENCES

1. **Baird, B. H., D. E. Nivens, J. H. Parker, and D. C. White.** 1985. The biomass and community structure and spatial distribution of the sedimentary microbiota from a high-energy area of the deep sea. Deep-Sea Res. **32**:1089–1099.
2. **Baird, B. H., and D. C. White.** 1985. Biomass and community structure of the abyssal microbiota determined from the ester-linked phospholipids recovered from Venezuela Basin and Puerto Rico Trench sediments. Mar. Geol. **68**:217–231.
3. **Balkwill, D. L., F. R. Leach, J. T. Wilson, J. F. McNabb, and D. C. White.** 1988. Equivalence of microbial biomass measures based on membrane lipid and cell wall components, adenosine triphosphate, and direct counts in subsurface aquifer sediments. Microb. Ecol. **16**:73–84.
4. **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.
5. **Boon, J. J., J. W. De Leeuw, G. J. van der Hoek, and J. H. Vosjan.** 1977. Significance and taxonomic value of iso and anteiso monoenoic fatty acids and branched β-hydroxy acids in *Desulfovibrio desulfuricans*. J. Bacteriol. **129**:1183–1191.
6. **Boon, J. J., W. Liefkens, W. I. C. Rijpstra, M. Baas, and J. W. DeLeevw.** 1978. Fatty acids of *Desulfovibrio desulfuricans* as marker molecules in sedimentary environments, p. 355–372. *In* W. E. Krumbein (ed.), Environmental and geomicrobiology biogeochemistry. Ann Arbor Science Publishers, Ann Arbor, Mich.
7. **Bousfield, I. J., G. L. Smith, T. R. Dando, and G. Hobbs.** 1983. Numerical analysis of total fatty acid profiles in the identification of coryneform, nocardioform and some other bacteria. J. Gen. Microbiol. **129**:375–394.
8. **Cranwell, P. A.** 1973. Branched chain and cyclopropenoid acids in a recent sediment. Chem. Geol. **11**:307–312.
9. **Dale, N. G.** 1974. Bacteria in intertidal sediments: factors related to their distribution. Limnol. Oceanogr. **19**:509–518.
10. **Dowling, N. J. E., F. Widdel, and D. C. White.** 1986. Phospholipid ester-linked fatty acid biomarkers of acetate oxidizing sulfate reducers and other sulfide forming bacteria. J. Gen. Microbiol. **132**:1815–1825.
11. **Edlund, A., P. D. Nichols, R. Roffey, and D. C. White.** 1985.

Extractable and lipopolysaccharide fatty acid and hydroxy acid profiles from *Desulfovibrio* species. J. Lipid Res. **26**:982–988.
12. **Findlay, R. H., J. W. Fell, N. K. Coleman, and J. R. Vestal.** 1986. Biochemical indicators of the role of fungi and thraustochy trids in mangrove detrital systems, p. 91–104. *In* S. T. Moss (ed.), Biology of marine fungi. Cambridge University Press, Cambridge.
13. **Findlay, R. H., M. B. Trexler, J. B. Guckert, and D. C. White.** 1990. Laboratory study of disturbance in marine sediments: response of a microbial community. Mar. Ecol. Prog. Ser. **62**:121–133.
14. **Gillan, F. T., and R. W. Hogg.** 1984. A method for the estimation of bacterial biomass and community structure in mangrove associated sediments. J. Microbiol. Methods **2**:275–293.
15. **Gillan, F. T., R. B. Johns, T. V. Verhey, and P. D. Nichols.** 1983. Monounsaturated fatty acids as specific bacterial markers in marine sediments, p. 198–206. *In* M. Bojory (ed.), Advances in organic geochemistry. John Wiley & Sons, Inc., New York.
16. **Graetz, D. A., D. R. Keeney, and R. B. Aspiras.** 1973. Eh status of lake sediment-water systems in relation to nitrogen transformations. Limnol. Oceanogr. **18**:908–917.
17. **Guckert, J. B., C. P. Antworth, P. D. Nichols, and D. C. White.** 1985. Phospholipid ester-linked fatty acid profiles as reproducible assays for changes in procaryotic community structure of estuarine sediments. FEMS Microbiol. Ecol. **31**:147–158.
18. **Guckert, J. B., M. A. Hood, and D. C. White.** 1986. Phospholipid ester-linked fatty acid profile changes during nutrient deprivation of *Vibrio cholerae*: increase in the *trans/cis* ratio and proportions of cyclopropyl fatty acids. Appl. Environ. Microbiol. **52**:794–801.
19. **Guckert, J. B., D. B. Ringelberg, and D. C. White.** 1987. Biosynthesis of *trans* fatty acids from acetate in the bacterium *Pseudomonas atlantica*. Can. J. Microbiol. **33**:748–754.
20. **Imai, I.** 1987. Size distribution, number and biomass of bacteria in intertidal sediments and seawater of Ohmi Bay, Japan. Bull. Jpn. Soc. Microb. Ecol. **2**:1–11.
21. **Inouchi, Y.** 1982. Distribution of bottom sediments in the Seto Inland Sea—the influence of tidal currents on the distribution of bottom sediments. J. Geol. Soc. Jpn. **88**:665–681.
22. **Japan Fisheries Resource Conservation Association.** 1980. Manual for water pollution analyses, p. 242–257. Koseisha Koseikaku, Tokyo. (In Japanese.)
23. **Kaneda, T.** 1977. Fatty acids of the genus *Bacillus*: an example of branched chain preference. Bacteriol. Rev. **41**:391–418.
24. **Kates, M.** 1964. Bacterial lipids. Adv. Lipid Res. **3**:17–90.
25. **Lechevalier, M. P.** 1977. Lipids in bacterial taxonomy—a taxonomist's view. Crit. Rev. Microbiol. **5**:109–210.
26. **Mancuso, C. A., P. D. Franzmann, H. R. Burton, and P. D. Nichols.** 1990. Microbial community structure and biomass estimates of a methanogenic Antarctic Lake ecosystem as determined by phospholipid analyses. Microb. Ecol. **19**:73–95.
27. **Nichols, P. D., J. B. Guckert, and D. C. White.** 1986. Determination of monounsaturated fatty acid double-bond position and geometry for microbial monocultures and complex consortia by capillary GC-MS of their dimethyl disulfide adducts. J. Microbiol. Methods **5**:49–55.
28. **Nichols, P. D., C. A. Mancuso, and D. C. White.** 1987. Measurement of methanotroph and methanogen signature phospholipids for use in assessment of biomass and community structure in model ecosystems. Org. Geochem. **11**:4512–461.
29. **Nichols, P. D., A. C. Palmisano, G. A. Smith, and D. C. White.** 1986. Lipids of the antarctic sea ice diatom *Nitzschia cylindrus*. Phytochemistry **25**:1649–1653.
29a. **Okada, T.** 1991. Study on the seasonal variations of sediment characteristics and sulphate reducing bacteria in eutrophic coastal bays. M.S. thesis. Hiroshima University, Hiroshima, Japan. (In Japanese.)
30. **Parkes, R. J.** 1987. Analysis of microbial communities within sediments using biomarkers, p. 147–177. *In* M. Fletcher, T. R. G. Gray, and J. G. Jones (ed.), Society of General Microbiology Symposium—Ecology of microbial communities. Cambridge University Press, Cambridge.
31. **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.
32. **Perry, G. J., J. K. Volkman, and R. B. Johns.** 1979. Fatty acids of bacterial origin in contemporary marine sediments. Geochim. Cosmochim. Acta **43**:1715–1725.
32a. **Rajendran, N., O. Matsuda, and Y. Urushigawa.** 1991. Microbial biomarker fatty acid composition in coastal sediments. J. Fac. Appl. Biol. Sci. Hiroshima Univ. **30**:31–42.
33. **Ringelberg, D. B., J. D. Davis, G. A. Smith, S. M. Pfiffner, P. D. Nichols, J. S. Nickels, J. M. Henson, J. T. Wilson, M. Yates, D. H. Kampbell, H. W. Read, T. T. Stocksdale, and D. C. White.** 1988. Validation of signature phospholipid fatty acid biomarkers for alkane-utilizing bacteria in soils and subsurface aquifer materials. FEMS Microbiol. Ecol. **62**:39–50.
34. **Seiki, T., E. Date, and H. Izawa.** 1991. Eutrophication in Hiroshima Bay. Mar. Pollut. Bull. **23**:95–99.
35. **Smith, G. A., P. D. Nichols, and D. C. White.** 1986. Fatty acids composition and microbial activity of benthic sediments from McMurdo sound, Antarctica. FEMS Microbiol. Ecol. **38**:219–231.
36. **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.** 1985. Quantitative characterization of microbial biomass and community structure in subsurface material: a prokaryotic consortium responsive to organic contamination. Can. J. Microbiol. **32**:104–111.
37. **Strickland, J. D. H., and T. R. Parsons.** 1972. A practical handbook of seawater analysis. Bulletin no. 167. 2nd ed. Fisheries Research Board of Canada, Ottawa.
37a. **Suwa, Y., Y. Urushigawa, Y. Matsui, and F. Yamaguchi.** 1990. Changes in fatty acid composition of activated sludges acclimated with starch and butylate. Bull. Nat. Res. Inst. Pollut. Res. **25**:19–26. (In Japanese.)
38. **Venkateswaran, K., H. Nakano, T. Okabe, K. Takayama, O. Matsuda, and H. Hashimoto.** 1989. Occurrence and distribution of *Vibrio* spp., *Listonella* spp., and *Clostridium botulinum* in the Seto Inland Sea of Japan. Appl. Environ. Microbiol. **55**:559–567.
39. **Volkman, J. K., and R. B. Johns.** 1977. The geochemical significance of positional isomers of unsaturated acids from an intertidal zone sediment. Nature (London) **267**:693–694.
40. **Volkman, J. K., R. B. Johns, F. T. Gillan, G. J. Perry, and H. J. Bavor, Jr.** 1980. Microbial lipids of an intertidal sediment. I. Fatty acids and hydrocarbons. Geochim. Cosmochim. Acta **44**:1133–1143.
41. **White, D. C.** 1983. Analysis of microorganisms in terms of quantity and activity in natural environments. Microbes in their natural environments. Soc. Gen. Microbiol. Symp. **34**:37–66.
42. **White, D. C.** 1988. Validation of quantitative analysis for microbial biomass, community structure, and metabolic activity. Arch. Hydrobiol. Beih. Ergebn. Limnol. **31**:1–18.
43. **White, D. C., W. M. Davis, J. S. Nickels, J. D. King, and R. J. Bobbie.** 1979. Determination of the sedimentary microbial biomass by extractable lipid phosphate. Oecologia **40**:51–62.
44. **White, D. C., J. S. Nickels, J. H. Parker, R. H. Findlay, M. J. Gehron, G. A. Smith, and R. F. Martz.** 1985. Biochemical measures of the biomass, community structure and metabolic activity of the ground water microbiota, p. 307–329. *In* C. H. Ward, W. Giger, and P. L. McCarty (ed.), Ground water quality. Wiley Interscience, New York.
45. **White, D. C., G. A. Smith, and G. R. Stanton.** 1984. Biomass, community structure and metabolic activity of the microbiota in Antarctic benthic marine sediments and sponge spicule mats. Antarct. J. U.S. **29**:125–126.