Bacteria play a central role in the degradation and selective preservation of organic matter in marine sediments and are thus intimately involved in biogeochemical cycling of elements (Jørgensen, 1983). Their presence and activity in surface sediments has long been accepted (Nowitzky and Karl, 1986; Jørgensen et al., 1990). At the interface between the water column and the sediment surface, detrital microaggregates will accumulate in an oxic, or suboxic, environment, producing a favorable habitat for bacterial growth (Loche and Turley, 1988; Turley and Loche, 1990). Geochemical evidence (chemical changes in pore water, kerogen production, concretion formation, etc.) has suggested that bacterial populations remain active at considerable depths (Krumbein, 1983); some early evidence of the existence of such populations, based only on enrichment of viable numbers, was strongly correlated ($P < 0.002$) with total organic carbon. Near-surface concentrations of organic carbon rapidly decreased from approximately 3.6% to 2.0% at 13 mbsf, and thereafter remained at 1.5%–2.0% to the base of the core at 68.28 mbsf, indicating a high level of recalcitrance. The changes in the rate of bacterial population decrease with depth may be a response to increasingly recalcitrant organic carbon. High levels of methane (4100 $\mu$mol/L) were found at 9.0 mbsf, although the maximum concentration present in the sediment at this site may be in an unsampled horizon above this depth. At greater depth, methane concentrations were still high (>1000 $\mu$mol/L), and although this area has many seeps of oil and gas, the $C_{CH4}/C_{CO2}$ ratios indicate a biogenic rather than a thermogenic source. This work represents the first detailed microbiological analysis of deep sediment layers from the Santa Barbara Basin.

**INTRODUCTION**

In the marine environment, evidence for low levels of anaerobic bacterial methanogenesis and sulfate reduction has been reported in sediments to 167 mbsf in the Gulf of Mexico and the North Atlantic (Whelan et al., 1986; Tarafa et al., 1987). In a comprehensive study of high-organic-load sediments from the Peru Margin, Cragg et al. (1990) and Parkes et al. (1990) have described significant levels of bacterial sulfate reduction and methanogenic activity to 80 mbsf, and significant bacterial populations associated with low levels of activity have been reported to 500 mbsf in the Japan Sea (Cragg et al., 1992; Getliff et al., 1992; Parkes et al., 1994). Most recently, examination of sediments from sites of considerably lower oceanic productivity and, consequently, much lower sediment organic carbon concentrations has demonstrated the continued presence of significant numbers of bacteria, associated with low levels of bacterial activity, in sediments to 100 mbsf in the Lau Basin (Cragg, 1994) and to 310 mbsf in the Eastern Equatorial Pacific (Cragg and Kemp, in press).

The Santa Barbara Basin (SBB) sediments have long been recognized as providing a detailed index of recent environmental climatic history (Soutar and Crill, 1977). The surficial sedimentation rate is approximately 4 mm/yr (Schimmelmann et al., 1990), with sediment containing total organic carbon concentrations of 2–8 wt% laid down and preserved in the form of millimeter-scale laminae thought to be related to an annual cycle of oxygen replenishment and depletion in bottom waters (Reimers et al., 1990; Schimmelmann and Tegner, 1991). Bacterial degradation of organic matter settling out from the highly productive overlying surface waters reduces the oxygen content of the deepest waters, resulting in dysaerobic to anaerobic conditions with less than 0.1 mL/L dissolved oxygen (Kennedy and Brassell, 1992). This lack of oxygen inhibits the development of benthic animal populations and bioturbation of the surface sediments is prevented (Soutar and Crill, 1977; Savrda et al., 1984; Kennedy and Brassell, 1992). Disturbance is further reduced by the growth of bacterial mats covering some 20% of the sediment surface, coincident with the highest levels of total organic carbon (Grant, 1991), consisting predominantly of the filamentous sulfur-oxidizing Beggiatoa spp. with some iron-oxidizing bacteria (Soutar and Crill, 1986).
Bacterial Analysis

Contours are in meters. Redrawn from Kennett, Baldauf, et al. (1994).

Figure 1. Map of the Santa Barbara Basin showing the location of Site 893. Contours are in meters. Redrawn from Kennett, Baldauf, et al. (1994).

1977; Reimers et al., 1990). The presence of a sediment that is fully anaerobic to the surface is unusual (Soutar and Crill, 1977), and the lack of bioturbation will result in deposited organic carbon encountering only strictly anaerobic processes at the sediment surface before being incorporated into laminae. A considerable amount of work has been published on SBB sediments, although this has principally focused on sediment geochemistry and cores of less than 75 cm (Soutar and Crill, 1977; Lange et al., 1987; Reimers et al., 1990; Berthaud and Reimers, 1991; Grant, 1991; Schimmelmann and Tegner, 1991; Schmidt and Reimers, 1991; Kennedy and Brassel, 1992; Schimmelmann et al., 1992). Other investigators have obtained samples up to 9 mbsf using piston and gravity cores for the purpose of determining sedimentary structures and marker layers (Thornton, 1986; Schimmelmann et al., 1990), sulfur chemistry (Schimmelmann and Kastner, 1993), and bacterial methanogenesis and methane oxidation rates (Kosior and Warford, 1979; Doose and Kaplan, 1981).

Ocean Drilling Program (ODP) Site 893 (34° 17.07'N, 120° 02.20'W) is on the floor of the SBB (Fig. 1) at 538 m water depth (Kennett, Baldauf, et al., 1994). Sediments in this area are rich in organic carbon and methane (probably biogenic), with overlying water which is anoxic or suboxic (<0.1 mL/L O2). Sediment core samples were obtained from this site for a study which is, to our knowledge, the first investigation of bacterial populations and biomass in deep layers of SBB sediments.

MATERIALS AND METHODS

Shipboard Handling

Bacterial Analysis

Samples were removed from 25 core sections, between 0 and 68.28 mbsf, of Hole 893B. The surface 1-cm3 sample was taken from the loose slurry present at the top of the first core using a sterile (autoclaved) 5-mL syringe from which the luer end had been removed. The sample was ejected directly into a tared serum vial containing 9 mL of filter-sterilized (0.1 µm) 4% formaldehyde in artificial seawater. For all other samples, immediately after a core was cut into 1.5-m sections on the outside catwalk, a thin layer of sediment was removed from the section end using a sterile scalpel to expose an uncontaminated surface. A 1-cm3 sample was then taken with a sterile syringe as above and the samples were preserved as before.

Headspace Methane

Samples (5 cm3) from Hole 893B were removed from 9 core sections on the catwalk between 1.5 and 68 mbsf using a calibrated cork borer. The sediment was placed in a 21.5-cm3 glass serum vial and crimp-sealed. The vial was heated to 60°C in an oven for 30 min before a 5-cm3 gas sample was removed for analysis by gas chromatography (Kennett, Baldauf, et al., 1994).

Laboratory Handling

Direct Microscopic Observations

Acridine Orange staining and microscopic observations were based on the general recommendations of Fry (1988) with minor modifications. Preserved samples were vortex mixed, and between 5 to 10 µL were added to 10 mL of filter-sterilized (0.1 µm) 2% formaldehyde. Acridine Orange (50 µL) was added to give a final concentration of 5 mg/dm3. After 3 min, the solution was filtered through a 25-mm diameter Nucleopore black polycarbonate membrane (Appleton Woods, Birmingham, U.K.) of 0.2-µm pore size. The filter was rinsed with an additional 10 mL of filter-sterilized 2% formaldehyde and mounted in a minimum amount of paraffin oil under a coverslip. Triplicate membranes were prepared from each sample.

Mounted membrane filters were viewed under incident illumination with a Zeiss Axioskop microscope fitted with a 50-W mercury-vapor lamp, a wide-band interference set for blue excitation, a 100x (numerical aperture = 1.3) Plan Neofluor objective lens, and 10x eyepieces. Bacteriologically shaped green or red fluorescing objects were counted. Cells on or off particles were counted separately, and the numbers of those on particles were doubled in the final calculations to account for cells hidden from view by particles (Goulier, 1977). Dividing cells (those with a clear invagination) and divided cells (pairs of cells of identical morphology) were also counted. A minimum of 200 fields of view were counted on each membrane. Where replicate log10 counts differed by more than 0.5 log10 units a fourth membrane was prepared. The detection limit was calculated to be 1 × 105 cells/cm3 (Cragg, 1994).

Total Organic Carbon

Samples for organic carbon determination were removed during a shore-based sampling meeting approximately two months post-cruise. Core sections had been stored in sealed plastic tubes at 5°C (Kennett, Baldauf, et al., 1994) until this time. Samples were removed exclusively from Hole 893A, which was some 10 m laterally displaced from Hole 893B. Determination of total organic carbon was carried out on an HERAEUS CHN analyzer. Total carbon was measured from dried and ground bulk sediment samples. After washing in 10% hydrochloric acid and drying, organic carbon was measured on the carbonate-free samples (Stax and Stein, 1993). Organic carbon (TOC wt%) of the bulk sediment was calculated using the following equation:

TOC = 100 - (8.333 × TC) / (100/TOC') - 8.333

where TC is the total carbon of the bulk sample and TOC' is the organic carbon of the carbonate-free residue.

RESULTS AND DISCUSSION

Bacteria were present in all samples to 68.28 mbsf (Fig. 2). The near-surface total count of 1.27 × 109 cells/cm3 decreased rapidly to 5.0 × 108 cells/cm3 at approximately 12 mbsf (a 254-fold decrease),
and then more gradually to $2.51 \times 10^6$ cells/cm$^3$ at 68.28 mbsf, representing a 505-fold decrease in total. This overall rate of decrease is thought to be a minimum value as the near-surface sample was "soapy" and particularly difficult to sample. A layer of intact pelecypods at 0.25 mbsf was believed to reflect the "Macoma oxygenation event" (Schimmelmann et al., 1992) indicating that APC coring had washed away approximately the top 30 cm of sediment (Kennett, Baldauf, et al., 1994). The near-surface total count is therefore probably an underestimate. Surficial sediment porosities in the SBB are unusually high, ranging from 86% to 95%, and require specialized techniques for accurate retrieval (Soutar and Crill, 1977; Isaacs, 1985; Reimers et al., 1990). Total bacterial numbers obtained at nearby San Pedro Basin (Craven et al., 1986) using box cores that successfully captured the sediment-water interface were approximately 3 in the top 5 mm of sediment, some 5 times higher than reported here.

The minimum total count was $1.9 \times 10^8$ cells/cm$^3$ at 52.78 mbsf. A shift in the rate of population decrease with depth was apparent from the data, and from regression and simultaneous-equation analyses, this was calculated to be at $12.8 \pm 1.4$ mbsf (Fig. 2 inset). Above this depth bacterial populations decreased at approximately 50% per meter; however, below this depth, the reduction was only 1% per meter. The age of this horizon is estimated to be 8.5 ka at nearby Hole 893A (J. Kennett, pers. comm.). Similar changes in the rate of bacterial population decreases with sediment depth have been observed at other Pacific Ocean deep sediment sites (Cragg et al., 1990, 1992; Parke, 1990; Cragg, 1994; Cragg and Kemp, in press), although the reasons for such a change are not clear and the depth at which it occurred varied between sites.

Comparisons with total counts made at other ODP sites show that near-surface counts are higher than those at less productive, deep-water sites but lower than that at the highly productive, shallow-water site in the Peru Margin (Table 1). This suggests that overlying water depth and productivity are both important in determining sediment bacterial population size (Cragg and Kemp, in press). Correlation analysis produced a significant relationship between water depth and sediment-surface population size ($R = 0.911; N = 55; P < 0.05$). As water depth, productivity, and near-surface organic carbon are all interrelated, the above correlation is a reflection of a general relationship.

The dividing cell numbers correlated closely with the total count ($R = 0.981; N = 25; P < 0.002$), representing an average of 9.6% of the total count. This strong relationship has been observed at other sites (Cragg et al., 1990, 1992; Parke, 1990; Cragg, 1994; Cragg and Kemp, in press). Near-surface numbers were $2.15 \times 10^8$ cells/cm$^3$, which decreased rapidly to approximately 12 mbsf, and thereafter more slowly to $2.52 \times 10^7$ cells/cm$^3$ at 68.28 mbsf (Fig. 2). This represents a 850-fold decrease, indicating that the dividing cell population decreases more rapidly than the total count. Surprisingly, the minimum population of $1.7 \times 10^7$ cells/cm$^3$ was around the 12 mbsf change in the population depth profile (Fig. 2). Numbers of dividing cells at the near-surface (0.03 mbsf) of $2.15 \times 10^8$ cells/cm$^3$ were relatively high although within the range observed from other sites (Table 1). Dividing cell numbers, like the total counts, show a trend of decrease with increasing overlying water depth; however, the correlation is not quite statistically significant ($P = 0.1$).

Sample depths for organic carbon determination are not entirely coincident with those for bacterial analyses, thus restricting interpretation. Average organic carbon levels over the top 1 m of sediment were 3.6%. This compares with 4.0%–5.1% between 0.30 and 0.89 mbsf (ten Haven et al., 1990; Patience et al., 1990) in Peru Margin sediments, which display greater numbers of dividing cells (Table 1). Conversely, between 1.5 and 2 mbsf, the average organic carbon concentration was 2.6%, compared to 1.6% over the same depth range in the Japan Sea (Ingle, Suyehiro, von Breymann, et al., 1990) which had much lower numbers of dividing cells at 0.03 mbsf (Table 1).

When calculated over the entire core length of 68.28 m, both total count and dividing cell counts correlate significantly with organic carbon concentrations ($R = 0.91$ and $R = 0.86$ respectively; $N = 25; P < 0.002$). Similar significance has been demonstrated at other sites (Parke et al., 1993; Cragg and Kemp, in press). The average or

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**Figure 2.** Depth distribution of total bacteria (open circles) and dividing/divided cells (open squares) to 68.28 mbsf in Hole 893B. Inset graph has the same axes and shows only total bacteria separated into two groups: upper group (solid circles) and lower group (triangles) with their respective regression lines. The two crosses indicate data points used in the construction of both regressions. The intersection was determined by simultaneous equation analysis.

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**Table 1.** Comparison of direct bacterial count data at 0.03 mbsf from Hole 893B with those from four other ODP sites.

<table>
<thead>
<tr>
<th>Source</th>
<th>Water depth (m)</th>
<th>Direct count (cells/cm$^3$)</th>
<th>Dividing count (cells/cm$^3$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eastern Equatorial Pacific</td>
<td>576.5</td>
<td>$1.27 \times 10^9$</td>
<td>$2.15 \times 10^9$</td>
</tr>
<tr>
<td>Lau Basin</td>
<td>576.1</td>
<td>$2.08 \times 10^8$</td>
<td>$4.85 \times 10^8$</td>
</tr>
<tr>
<td>Japan Sea</td>
<td>2692</td>
<td>$6.12 \times 10^7$</td>
<td>$9.75 \times 10^7$</td>
</tr>
<tr>
<td>Peru Margin</td>
<td>500</td>
<td>$7.82 \times 10^6$</td>
<td>$6.35 \times 10^6$</td>
</tr>
<tr>
<td>150</td>
<td>$3.30 \times 10^5$</td>
<td>$7.30 \times 10^5$</td>
<td></td>
</tr>
</tbody>
</table>

Notes: Depth data from Suess, Huenne, et al. (1988; ODP Leg 112 Site 681); Ingle, Suyehiro, von Breymann, et al. (1990; ODP Leg 128 Site 798); Parson, Hawkins, Allan, et al. (1992; ODP Leg 138 Site 851); and Mayer, Piksom, Jancek, et al. (1992; ODP Leg 138 Site 851). Bacterial data from Parkes et al. (1993; Charles Darwin cruise leg 38); Cragg et al. (1992; ODP Leg 128); Cragg (1994; ODP Leg 135); and Cragg and Kemp (in press; Leg 138).
Bacterial enumeration of SBB sediments has demonstrated high total and dividing cell counts consistent with the high organic carbon environment. Absolute bacterial numbers in near-surface sediment agree with an emerging relationship between surficial numbers of
bacteria and overlying water depth. Both total count and dividing cell count decrease rapidly to approximately 13 mbsf and then more gradually to 68.3 mbsf. The reason for the sudden change at 13 mbsf is unclear but may be due to an alteration in the biodegradability of organic carbon. Near-surface bacterial concentrations are less than those encountered in Peru Margin sediments but greater than those measured in Japan Sea sediments. Surface organic carbon concentrations of approximately 3.6% decrease rapidly to 2.0% at 13 mbsf and then remain at 1.5%–2.0% to the base of the hole at 68.3 mbsf, suggesting that a considerable proportion of the organic carbon actually incorporated into the sediment is recalcitrant. Methane concentrations are high at the surface and are probably biogenic in origin. At greater depths, methane concentrations, although lower, remain substantial and the C1/C2 ratio indicates a mainly biogenic source.

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