PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY A MATHEMATICAL, PHYSICAL & ENGINEERING SCIENCES

Bacterial Biomass and Activity in Deep Sediment Layers from the Peru Margin [and Discussion]

R. J. Parkes, B. A. Cragg, J. C. Fry, R. A. Herbert, J. W. T. Wimpenny, J. A. Allen and M. Whitfield

Phil. Trans. R. Soc. Lond. A 1990 **331**, 139-153
doi: 10.1098/rsta.1990.0061

Phil. Trans. R. Soc. Lond. A **331**, 139–153 (1990) [139]
Printed in Great Britain

Bacterial biomass and activity in deep sediment layers from the Peru margin

By R. J. Parkes[1]†, B. A. Cragg[1,2], J. C. Fry[2], R. A. Herbert[3] and J. W. T. Wimpenny[2]

[1] *Scottish Marine Biological Association, P.O. Box* 3, *Oban, Argyll PA*34 4*AD, U.K.*
[2] *University of Wales, Cardiff CF*1 3*XF, U.K.*
[3] *University of Dundee, Dundee DD*1 4*HN, U.K.*

The distribution of bacterial biomass and activity down to 80 m below the sea floor was investigated in sediments from the Peru margin, collected as part of the Ocean Drilling Programme, leg 112. Bacteria were present in all sediment depths sampled. Although direct bacterial counts decreased with depth there was no indication of a more rapid decline in the deeper layers and thus it is likely that bacteria should be present to much greater depths than those examined in this study. A significant number of the bacteria within the sediment were dividing and hence the bacteria were active and not just surviving. Bacteria were able to be cultured in laboratory media, from all depths, further confirming the viability of these organisms *in situ*. Bacterial sulphate reduction and methanogenesis were measured even in the deepest samples, although there were marked changes in rates with depth. There were also significant changes in the dominant populations of different types of viable bacteria and their associated activity with sediment depth. Although some of this succession in bacterial activity could be related to chemical changes in the pore water, there were some subtle changes in bacterial activity, possibly related to changes in the bioavailability of organic matter, which would not be anticipated from purely chemical data. Where numbers of viable bacteria and activity were decreasing a brine incursion, from below, dramatically stimulated further growth and activity, even in sediments approximately one million years old.

Introduction

Marine sediments play a major role in the global biogeochemical cycling of elements. Relative to sea water, organic matter is concentrated 10000–100000 fold in sediments and this material is used for energy by bacteria and as a consequence they drive the chemical cycles. Bacterial activity also determines the extent to which organic matter is degraded or preserved and thus has a profound effect on fossil and oil formation. The vast majority of research on sediment microbiology has concentrated on the surface few metres, where bacterial activity tends to be concentrated. These studies have demonstrated the close involvement of bacteria in diagenetic processes and mineral formation, some of which were initially thought to be purely chemical processes (e.g. pyrite formation, manganese and iron cycling). However, below these surface sediments bacterial activity is still present, albeit at a much reduced level, but because of the long timescales involved (millions of years) may continue to have a major effect on diagenesis (for example, in the modification and degradation of biological indicators of past depositional

† Present Address: Department of Geology, University of Bristol, Wills Memorial Building, Queen's Road, Bristol, BS8 1RJ, U.K.

environments, fossil and oil formation). Indirect geochemical evidence also suggests that microbial activity continues to considerable depth within sediments (e.g. chemical changes in pore water, gas production, modification of organic complexes such as kerogen, concretion formation, isotopic evidence (Krumbein 1983)). The thermal gradient of the Earth's crust is approximately 10–40 °C km^{-1} and bacteria grow at temperatures in excess of 100 °C at elevated pressure (Jannasch & Taylor 1984). So it is unlikely that bacteria would be inhibited by elevated temperatures until several kilometres below the Earth's surface.

Microbiological studies of deep sediment layers present considerable difficulties. These include the prevention of contamination from the more active surface layers, and the provision of both realistic enrichment conditions for bacterial isolation and realistic incubation conditions for activity measurements. There have been a few reports of the detection or cultivation of bacteria from sediments to depths of 200 m (Davies 1967; Oremland *et al.* 1982; Belyaev & Ivanov 1983; Bianchi 1986) but isolation does not demonstrate *in situ* activity and there are always concerns regarding contamination. Recently, positive indication of low levels of anaerobic bacterial activity have been found from between 4 and 167 m in subsurface sediments, this included both sulphate-reduction and methanogenesis (Whelan *et al.* 1985). There is a need to confirm this data and to use, in addition, a comprehensive suite of microbiological methods to obviate as much as possible contamination problems. In this paper we describe the results of such a study conducted on deep sediment layers from the Peru margin collected on the Ocean Drilling Programme, leg 112.

Methods

Sampling site

Sediment samples were collected on the Ocean Drilling Programme cruise, leg 112, site 681C on the Peru margin (10° 58.60′ S, 77° 57.46′ W). This is an area of upwelling, with a water depth of 150 m and is on the edge of an oxygen minimum. Sediments were collected with a high pressure corer down to 86.2 m below the sea floor (m BSF). The coring procedure disturbed approximately the top 1 m of sediment, hence the first samples were taken at 1.5 m to ensure that they were not disturbed. The downhole temperature at 63 m BSF was 15.6 °C. More details regarding this site can be found in the summary of drilling results Ocean Drilling Programme, leg 112 (Suess & von Huene 1989).

Experimental procedures

These are only briefly described as full details can be found in Cragg *et al.* (1990).

(*a*) *Shipboard handling*

Samples for bacterial enrichment and activity measurements were from whole round cores (10 cm long) cut from complete core sections with sterile equipment. These cores were capped while flushing with nitrogen, sealed with tape and paraffin wax and then enclosed in metal cans, which were flushed with nitrogen before storage at 5 °C.

Samples for direct microscopic analysis (1–2 cm^3) were taken from the core section immediately after the removal of the whole round cores and stored in 20 cm^3 of a 5% formalin solution in artificial seawater at 5 °C.

Sediments were collected on 8 November 1986, returned refrigerated to the U.K., and were opened for microbiological analysis from 7 January 1987.

(*b*) *Laboratory analysis*

Handling. All primary handling was conducted at 16 °C under a sterile jet of nitrogen and with sterile apparatus. The end caps were removed and the two exposed surfaces briefly flamed. The sample was split into 2×5 cm sections by extruding a portion into a 5 cm collar and cutting with a sterile slice. Small subcores were taken from both sections using 5 cm^3 plastic syringes from which the luer end had been removed. The syringes were sealed with butyl rubber 'suba seals' and stored temporarily in anaerobic jars under nitrogen at 16 °C.

Viable bacterial populations. The viable numbers of the following bacterial types were enumerated using a most probable number (MPN) technique (Colwell 1979): aerobic ammonifying bacteria, anaerobic heterotrophic bacteria, nitrate-reducing bacteria, sulphate-reducing bacteria (acetate and lactate utilizing), anaerobic hexadecane utilizing bacteria, methanogenic bacteria. Composition, preparation of media, and dilutions used are described in Cragg *et al.* (1990). Viable counts and confidence limits were calculated according to Hurley & Roscoe (1983). The total anaerobic viable count was the sum of all the anaerobic viable counts.

Bacterial activity. Bacterial activity was measured by injection of radioactive or stable isotopes into the sediment sub cores within the 5 cm^3 syringes, followed by incubation at 16 °C for 3, 10 and 20 days within anaerobic jars. Autoclaved sediments were used as controls and the incubation was stopped by freezing at −20 °C. Rates of activity were calculated from the mean rate of label turnover (after any DPM in the relevant control was subtracted) during the three incubation times multiplied by the pool size of the relevant compound in the sediment (Suess & von Huene 1986). ^{35}S-sulphate was used for sulphate-reduction, and sulphide in both acid volatile, pyrite and sulphur fractions determined (Parkes & Buckingham 1986). Sodium ^{14}C-bicarbonate was used for methanogenesis and ^{15}N-nitrate for rates of nitrate reduction. As these samples were stored prior to analysis and were not incubated under *in situ* pressure the measured rates may differ from the actual rates *in situ*, but the rates for the different activities relative to each other should be largely unaffected.

Direct microscopic counts. Bacteria directly within the sediment were stained with acridine orange before counting with a epifluorescence microscope (Cragg *et al.* 1990; Fry 1988). Both total bacteria and numbers of dividing cells were counted. Dividing cells were counted as bacterial cells with a clear invagination as well as two identical cells joined together. Counts on sediment particles were multiplied by two to account for bacteria hidden beneath the particle. The frequency of dividing cells (FDC) was the percentage of dividing cells relative to the total count.

Results and discussion

The direct microscopic enumeration of bacteria within the sediment provides an accurate estimate of *in situ* distribution of bacterial populations, because, unlike the viable count procedure, the technique does not involve growth of the bacterial population and hence the potential for contamination from the more active surface layers is limited. Although the technique does not directly discriminate between living, dormant or dead cells, the number and frequency of bacterial cells dividing is an index of growth and hence viability (Hagstrom *et al.* 1979). Total bacterial counts obtained by this technique demonstrated the presence of significant bacterial populations in all the sediment samples, representing depths down to 80 m (figure 1). Bacterial numbers were highest at the surface (1.5 m, 1.05×10^9) and then gradually

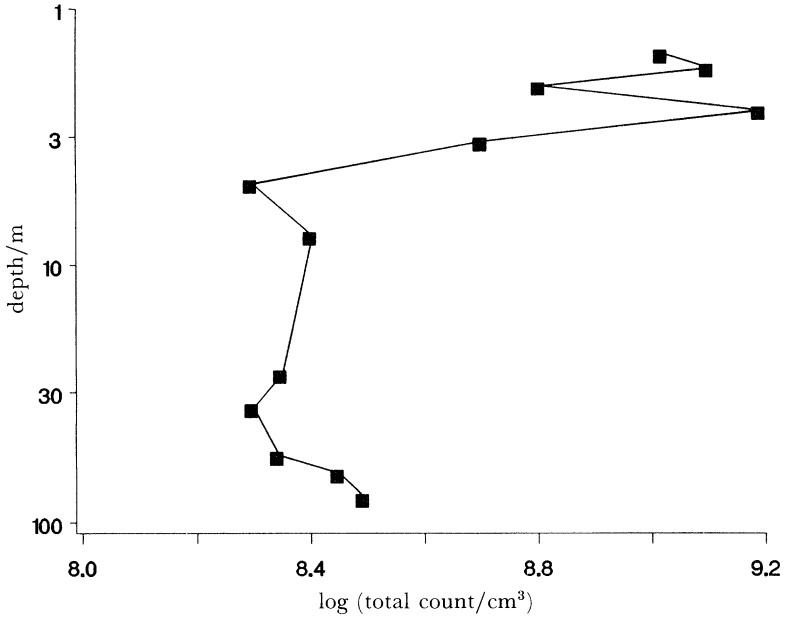

FIGURE 1. Total direct bacterial numbers with sediment depth.

decreased exponentially with depth (36.2 m, 1.99×10^8) by a factor of 5. The small increase in bacterial numbers after 50 m was not statistically significant. Dividing cells were present at all but one depth (36.2 m, figure 3) indicating that a portion of the bacterial population was actively growing and not just surviving, even in the deepest samples. The proportion of the total population that was dividing was relatively small (*ca.* 3%, figure 2) and decreased with depth down to 36 m followed by a small increase; a distribution similar to the total bacterial count. Anaerobic heterotrophic bacteria were cultured from all depths (figure 3). Numbers of these bacteria were highest at 1.5 m and then decreased rapidly down to 2 m (88000–55 bacteria cm^{-3}, a 1600 fold decrease). But at 55.2 and 64.7 m there was a significant ($P < 0.01$) increase in bacteria to 3553 bacteria cm^{-3}, followed by a decrease at 80.2 m. This distribution is similar but more exaggerated than either total bacterial counts (figure 1) or numbers of dividing cells, especially the increase at about 50 m (figure 3).

The exponential decrease in bacterial populations with depth for both the total bacterial and viable heterotrophic bacteria, is a profile commonly observed in marine sediments, although the bulk of any changes are thought to occur in the top 10 cm, and below 1 m changes in bacterial populations are considered to be extremely limited (Rheinheimer 1985). Our data is thus in marked contrast to this generally accepted view of bacterial distributions within deep sediment layers, particularly the presence of dividing cells even in the deepest sample (80 m) and the increase in numbers of viable heterotrophic bacteria, FDC and total bacterial count after 50 m (figures 1–3).

Comparison of our data on bacterial populations with previous work is difficult as most bacterial analysis tends to be confined to samples well within the top 1 m of sediment. To our knowledge this is the first report of direct bacterial counts, FDC, bacterial activity and estimates of the viable numbers of a range of bacterial types down to 80 m BSF. Our data confirms and extends the limited number of reports of the detection or cultivation of bacteria from deep sediment layers (see, for example, Davis 1967; Oremland *et al.* 1982; Belyaev & Ivanov 1983;

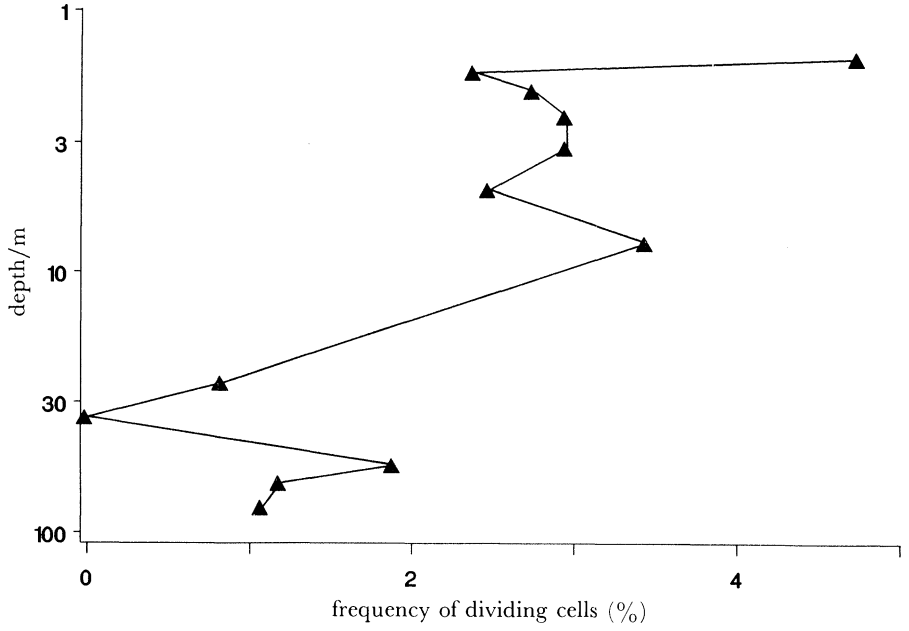

FIGURE 2. The frequency of dividing cells in the direct bacterial count with sediment depth.

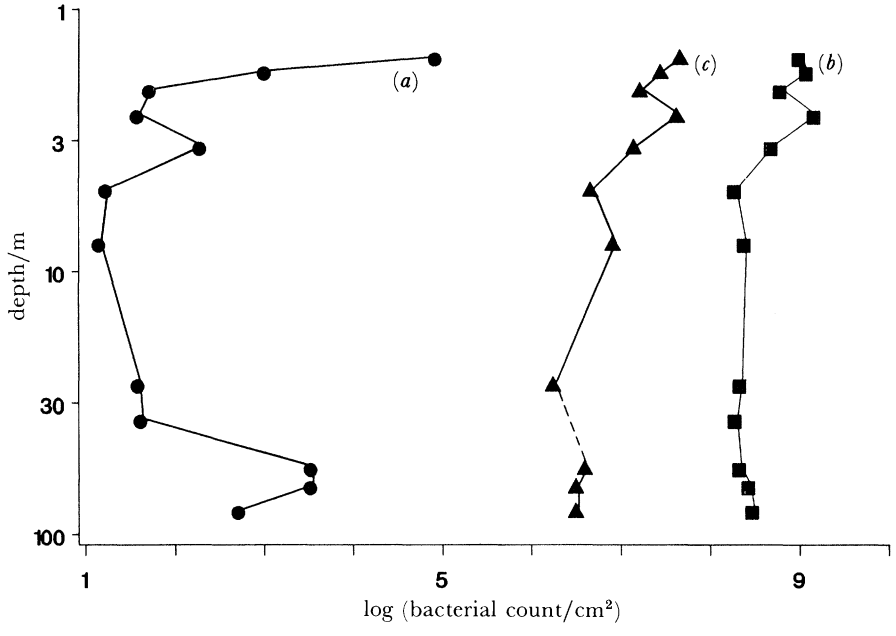

FIGURE 3. Numbers of (*a*) anaerobic viable heterotrophic bacteria, (*b*) total bacterial numbers and (*c*) numbers of dividing cells with sediment depth (dashed line indicates an omitted data point of zero).

Bianchi 1986). The direct bacterial count at 1.5 m is a very significant bacterial population representing 6% of the bacterial population in the surface 1 cm (1.78×10^{10} g^{-1} dry mass, unpublished data). The direct bacterial count even at 80 m represents approximately 1% of the surface bacterial population. Our estimate of the total bacterial population at the sediment surface is very similar to estimates of bacterial populations at the sediment surface in the deep sea. For example Rice *et al.* (1986) reported direct counts of 1.2×10^{10} g^{-1} (dry mass) from

sediments between 1000 and 4500 m in the Porcupine Sea Bight and Lochte & Turley (1988) found direct counts of between 2.2×10^9 and 1.6×10^{11} g^{-1} (dry mass) associated with phytodetritus on the sediment surface at 4500 m in the northeastern Atlantic. Therefore, it seems reasonable to assume that the distribution of bacteria with sediment depth at these sites would be similar to those found at our more shallow site. Despite these significant populations of bacteria, they only represent a small percentage of the total sediment carbon and this decreases significantly with depth (figure 4).

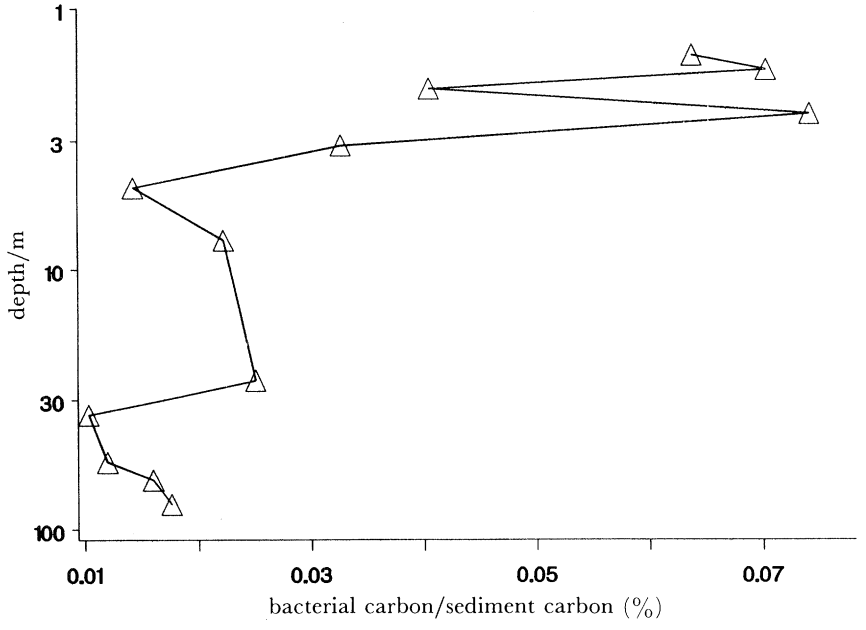

FIGURE 4. Bacterial carbon expressed as a percentage of the total sediment organic carbon, with sediment depth.

Although FDC can be used to calculate growth rates for pelagic bacteria (Hagstrom *et al.* 1979), at present, accurate growth rates cannot be calculated from FDC of sediment bacteria, as it has been shown that the procedure greatly overestimates bacterial growth rates in sediments (Newell & Fallon 1982; Fallon *et al.* 1983). This situation may indicate that sediment bacteria remain attached after division for long periods of time, making the interpretation of FDC difficult. It seems unlikely, however, that dividing cells have merely survived in this form from the surface sediment layers, as they decrease much faster with sediment depth than the numbers of total bacteria (4.5 times faster) and there is no obvious reason why the two cell forms should have different survival rates. However, as there is a significant ($p = 0.003$) correlation between sediment depth and FDC (figure 2) we can provide an indication of the depth at which bacterial division and hence growth might be expected to cease; in this case 275 m. It is likely that bacterial activity as distinct from growth would continue even deeper than this.

The percentage of the total bacterial population that could be grown anaerobically in the laboratory was generally low, but surprisingly the viability of the bacterial population at 55.2 m and below was similar to the bacterial viability in the upper sections (figure 5). The viability of natural bacterial populations are normally low, due to the absence of an effective growth medium for the majority of natural bacteria, and varies between 0.0001% and 10%

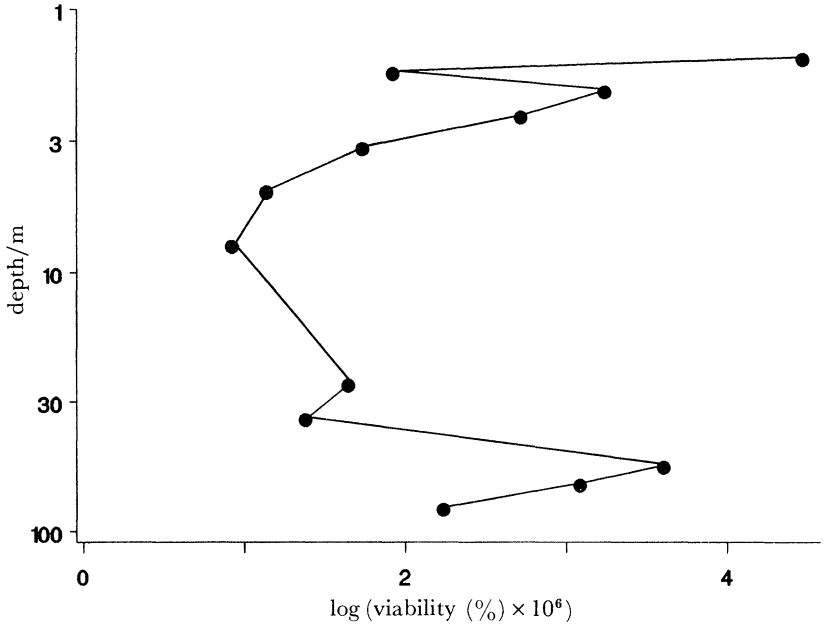

FIGURE 5. The viability of anaerobic bacteria (total viable anaerobic bacteria/total direct count × 100) with sediment depth.

of the direct count (van Es & Meyer-Reil 1982). The viability of bacteria at site 681 down to 2.5 m and also from 55.2 m is within the lower end of this range. However, the central zone of the core (4.8–36.2 m) had a particularly low viability (i.e. 0.0000087% at 7.7 m), this might indicate that the bacteria in this region were stressed *in situ* and/or unable to grow as satisfactorily in the various culture media as bacteria from other regions. FDC also decreases in this region (figure 2) but the decrease in the two measurements is not directly comparable, one possible explanation for this might be an increase in the bacterial death rate in the presence of a similar growth rate.

In addition to the MPN for anaerobic heterotrophic bacteria all the other MPN media successfully enriched bacteria allowing the distributions of aerobic ammonifying, nitrate-reducing (denitrifying and ammonium producing), sulphate-reducing (using lactate and acetate), anaerobic hexadecane oxidizing, and methanogenic (using H_2/CO_2) bacteria to be determined (figure 6 and table 1). There were marked changes in the distribution of the different bacterial groups with sediment depth, with heterotrophic and nitrate reducing bacteria dominating the top 2 m, followed by sulphate-reducing bacteria at 2.5 m (98% of the total anaerobic viable count), and then below this methanogenic bacteria became for the first time a significant component of the population (e.g. 24% at 4.8 m). Hydrocarbon oxidizers made a significant contribution to the population in the middle section of the sediment (3.3–36.2 m), at and below the peak in numbers of methanogens. In the deepest samples (36.2–80.2 m) the heterotrophs and nitrate utilizers were again dominant. This succession of different bacterial types with depth in the sediment is one that is well documented for surface sediments (Jorgensen 1983; Nedwell 1984) and reflects the preferential use of electron acceptors that provide the most energy for the degradation of common substrates in the absence of oxygen (Parkes & Senior 1988). It is, however, surprising to have significant changes in bacterial populations to such depths within the sediment as it is thought that

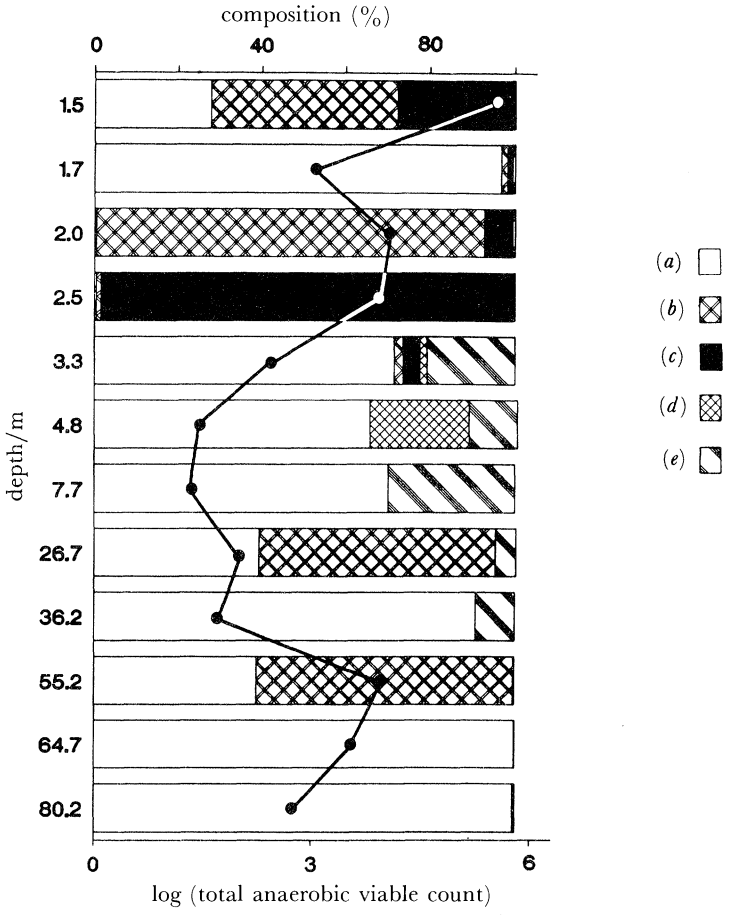

FIGURE 6. The distribution of different types of anaerobic viable bacteria and total anaerobic viable count with sediment depth. Heterotrophic bacteria (*a*), nitrate-reducing bacteria (*b*), sulphate-reducing bacteria (*c*), methanogenic bacteria (*d*), hexadecane oxidizing bacteria (*e*).

TABLE 1. THE DISTRIBUTION OF AEROBIC AMMONIFYING BACTERIA WITH SEDIMENT DEPTH

depth/(m BSF)	viable count/cm^3	depth/ (m BSF)	viable count/cm^3
1.5	404600	7.7	66040
1.7	60080	26.7	341000
2.0	845300	36.2	133600
2.5	939300	55.2	55200
3.3	160400	64.7	9728
4.8	281100	80.2	21560

bacterial populations are relatively constant below the top 1 m (Rheinheimer 1985). The depth distributions of the heterotrophic and nitrate reducing bacteria were similar, which is not surprising as nitrate reduction is a common characteristic of facultative anaerobic bacteria and hence the two groups may represent complementary bacterial types, especially as nitrate was probably absent from the *in situ* sediment. Aerobic ammonifying bacteria represented by far the largest viable bacterial population (table 1), but most of these were spore formers and were probably present as spores in the sediment. As the sediment was probably anoxic below the top few millimeters (Revsbech *et al.* 1980) the survival of these spores down to 80 m plus in the sediment in a viable form is remarkable. It is unlikely that the other groups of bacteria

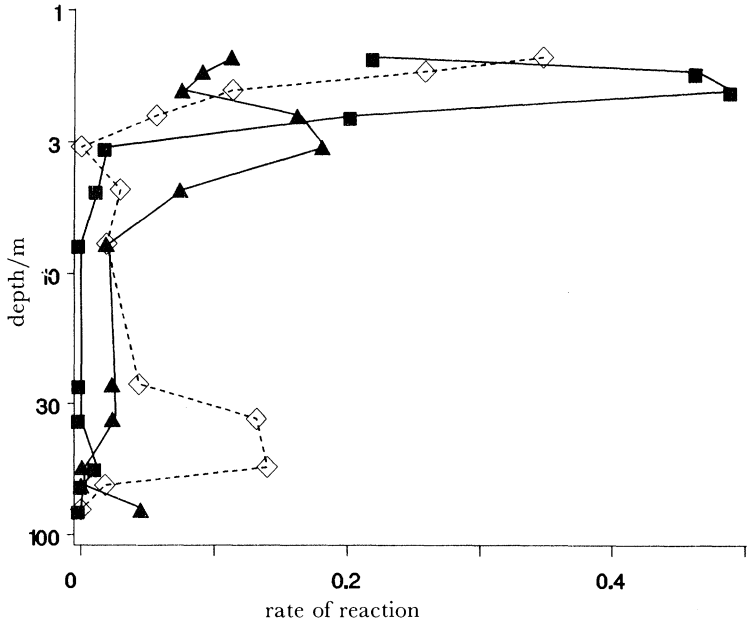

FIGURE 7. Depth distributions of rates of sulphate-reduction (■), and methanogenesis (▲) (nmol cm^{-3} d^{-1}), and potential rates of nitrate-reduction (◇) ((1/turnover time) × 100 d^{-1}).

enumerated represented spore formers as: (i) there are no known spore forming methanogenic bacteria; (ii) spore forming sulphate-reducing bacteria are relatively few in number and not normally present in marine environments; (iii) microscopic observation of anaerobic heterotrophs did not reveal any obvious spore formers.

The succession in bacterial types is also shown in the succession of bacterial activities with sediment depth, nitrate-reduction, sulphate-reduction and then methanogenesis (figure 7). The depth profiles of the viable counts and their corresponding activities were very similar and for some the relation was statistically significant (e.g. methanogenic viable counts and rates of methanogenesis, $r = 0.625$, $p < 0.05$). It is very unlikely that nitrate was actually present in the sediment, although pore water analysis did give some very low unreliable positive values, and hence these results are expressed as rate of label turnover and only represent a potential for nitrate reduction. In addition, as nitrate reduction is a common attribute amongst facultative anaerobic bacteria it seems most appropriate to interpret this parameter as a measure of heterotrophic potential. This is reinforced by the significant positive relation between potential nitrate-reduction and total anaerobic count ($r = 0.73$, $p < 0.01$).

The maximum rates of sulphate-reduction of 0.464 and 0.491 nmol cm^{-3} d^{-1} at 1.7 and 2.0 m respectively are low but within the broad range reported for both inshore and continental shelf sediments (inshore 0.2–2800 nmol cm^{-3} d^{-1}; Senior *et al.* 1982; Parkes & Taylor 1985; Parkes & Buckingham 1986, continental shelf surface sediments 0.000 1–46 nmol cm^{-3} d^{-1}; Sorokin 1962; Tsou *et al.* 1973; Goldharber & Kaplan 1975; Jorgensen 1983; Edenborn *et al.* 1987). The rapid decrease in sulphate-reduction below 2 m is associated with an increase in methanogenesis to its maximum rate of approximately 0.18 nmol cm^{-3} d^{-1} between 2.5 and 3.3 m, followed by a slow decrease in rate to approximately 10 m. Rates of methanogenesis are low and approximately 2.6 times lower than the maximum rate of sulphate-reduction (figure 7), but they are comparable with previously published rates (e.g.

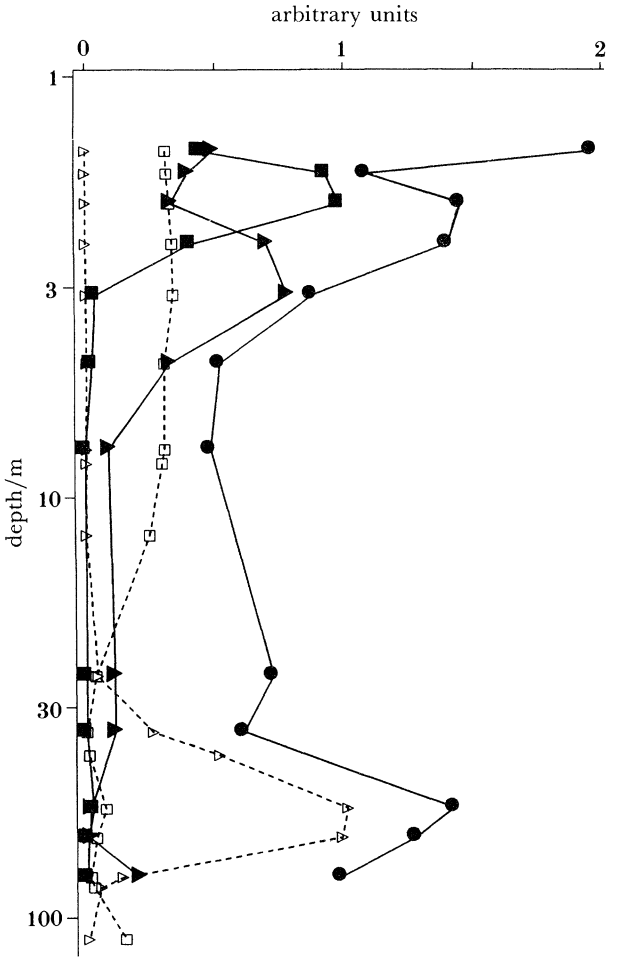

FIGURE 8. Depth distribution of rates of sulphate-reduction × 2 (■); rates of methanogenesis × 4.25 (▶) nmol cm^{-3} d^{-1}; pore water sulphate concentrations ÷ 50 mmol dm^{-3} (□); dissolved methane concentrations × 1 mmol dm^{-3} (▷); log (numbers of total anaerobic viable bacteria) ÷ 2.8 (●).

0–0.028 nmol cm^{-3} d^{-1} in salt marsh sediments (Senior *et al.* 1982), 0.01–20 nmol cm^{-3} d^{-1} for Baltic Sea sediments (Lein *et al.* 1981)). The succession from sulphate-reduction to methanogenesis has been previously observed within shallow water sediments and this is explained by the ability of sulphate-reducing bacteria to out compete methanogenic bacteria for common substrates (Kristjansen *et al.* 1982; Schonheit *et al.* 1982; Parkes & Senior 1988) and sulphate-reduction eventually becoming limited by the decreasing sulphate concentrations within the sediment, thus releasing substrates for methanogenesis to increase. However, this explanation is not valid for our sediments as sulphate is still present (Suess & von Huene1986) in non-limiting concentrations (16 mmol dm^{-3}, figure 8). Another possibility is that the type of metabolic intermediates being produced by the heterotrophic bacteria changed from a substrate that sulphate-reducing bacteria can use to substrates that are predominantly used by methanogens (e.g. methanol, methylamine, trimethylamine (Oremland & Polcin 1982; King *et al.* 1983; King 1984)). However, as the increase in methanogenesis was due to methane production from hydrogen, a substrate that sulphate-reducing bacteria can use, this explanation is also unsatisfactory.

After about 50 m heterotrophic potential (figure 7), FDC (figure 2), bacterial viability (figure 5) and bacterial numbers (both direct counts, figure 1 and anaerobic viable counts figure 8) increase again. At approximately the same depth there is a small increase in the sulphate concentration (figure 8) and an equally small stimulation in sulphate-reduction (figure 7). This sulphate-reduction lasts for about 10 m and probably is sulphate limited allowing methanogenesis to develop once again at 80 m (figure 7). Just above this increase in methanogenesis is a broad peak in methane (figure 8, Suess & von Huene 1986), some of which may be consumed by sulphate-reduction higher in the sediment (Iversen & Jorgensen 1985), although this will be restricted due to sulphate limitation. This dramatic stimulation of bacterial activity so deep in the sediment is probably due to brine incursion from below (Suess & von Huene 1986) supplying nutrients and electron acceptors (e.g. sulphate) that were previously limiting bacterial activity. There is also an increase in depositional organic carbon at this depth (Patience *et al.* 1990), which might contribute to this enhanced activity, although it is probably relatively recalcitrant to bacterial hydrolysis (see below). It is tempting to speculate that sulphate-reduction would again increase below 80 m where sulphate concentrations begin to steadily increase, and thus bacterial interactions in the lower part of the sediments would be a mirror image of the changes observed in the upper part of the sediment (figure 8), although reduced in magnitude. It is highly unlikely that the stimulation in bacterial activity towards the bottom of the core is due to contamination from the more active surface layers as bacterial stimulation occurs in a number of independent bacterial measurements and the zone immediately above is extremely low in bacterial activity and viable bacteria.

The reasons for the marked and rather unexpected changes in bacterial activity and biomass in the top 10 m are far from clear, but one possible explanation might be changes in the quality or bioavailability of the organic matter to bacteria with sediment depth, and we would like to present this as a working hypothesis. It seems likely that the bulk of sulphate-reduction was concentrated in the top 15 cm or so of the sediment (for example, Parkes & Buckingham 1986) and was decreasing rapidly before our first sample at 1.5 m. At this depth the bulk of organic carbon readily available to bacteria had probably already been removed and the heterotrophic bacteria were having to utilize recalcitrant organic polymers with resulting slow rates of carbon degradation and energy production. Anaerobic degradation of organic matter characteristically involves a mixed population of bacteria with either methanogens or sulphate-reducing bacteria conducting the essential role of terminal oxidizers, which allows further degradation to proceed (Parkes & Senior 1988). It may be that, when recalcitrant polymers are being degraded, a synergistic coupling of heterotrophic bacteria and methanogens is favoured rather than coupling with the potentially faster-growing sulphate-reducing bacteria. This would explain why sulphate-reduction decreased before sulphate depletion and why there were sufficient substrates for methanogenesis (figure 7). If the coupling between heterotrophic bacteria and sulphate-reducing bacteria was becoming stressed this would ultimately be reflected in a decrease in the heterotrophic population, as was observed between 1.5 and 2.5 m (figure 3), and where there was a similar decrease in heterotrophic potential (figure 7). After methanogenesis had increased, the numbers of heterotrophic bacteria and heterotrophic potential also increased. The methane produced in this zone then could diffuse upwards and provide the substrate to produce the zone of sulphate-reduction. Secondary peaks in sulphate-reduction attributed to anaerobic methane oxidation have been demonstrated previously

(Iversen & Jorgensen 1985). Although the maximum rates of sulphate-reduction are much higher than methanogenesis (figure 7), the rates integrated with depth are almost identical (0.44 and 0.49 mmol m^{-2} d^{-1} respectively), because of the broader peak in methanogenesis, and hence completely consistent with the above hypothesis. In addition anaerobic methane oxidation would explain the apparent contradiction of a zone of methanogenesis without the presence of significant amounts of methane gas (figure 8).

Gradually methanogenesis also decreases, reflecting the continued decrease in heterotrophic bacteria supplying their substrates. After about 7 m there is very little bacterial activity (figure 7), in addition the total viable bacterial population (figure 8), bacterial viability (figure

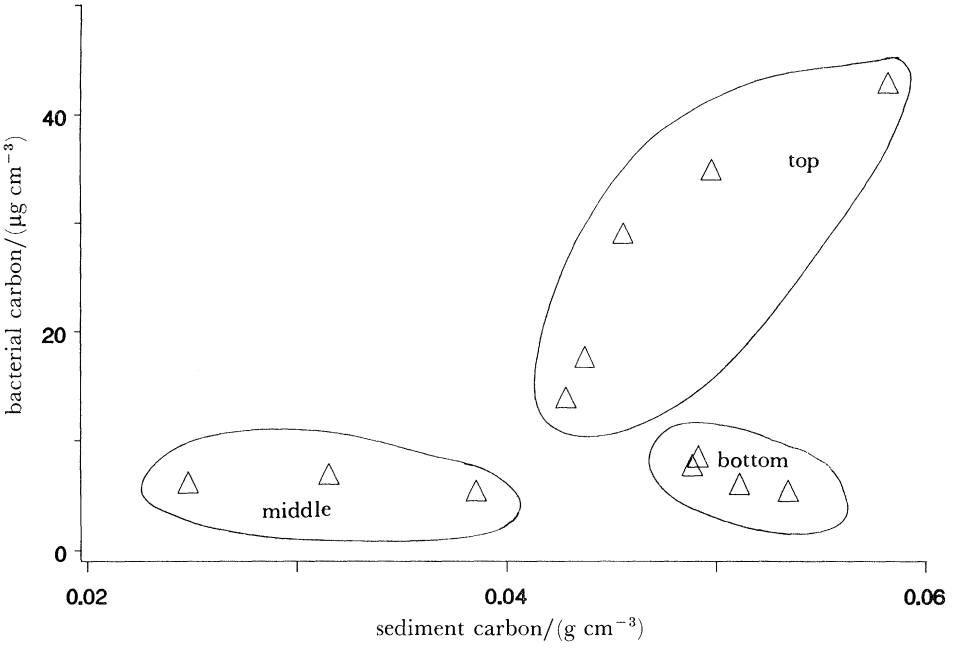

FIGURE 9. The relationship between bacterial carbon (μg cm^{-3}) and sediment organic carbon (g cm^{-3}) at the same depth within the sediment. Top (1.5–3.3 m), middle (4.8–26.7 m), bottom (36.2–80.2 m).

5) and FDC (figure 2) are all very low indicating that the bulk of the organic carbon is predominantly unavailable to the bacteria. The very low rates of sulphate-reduction below 3 m (figure 7), when integrated with depth, are sufficient to account for the continued removal of sulphate (figure 8).

Bioavailability of organic matter is very difficult to quantify and hence it is difficult to provide data to substantiate the inferred changes in bioavailability of sediment organic carbon with depth. However, one measure of the availability of organic carbon for bacteria must be the amount of bacterial biomass produced per unit carbon. Therefore, the total direct bacterial count has been converted into carbon (Fry 1988) and plotted against the total organic carbon (Patience *et al.* 1989) at the same depth in the sediment (figure 9). This shows clearly three distinct depth horizons with different bioavailability of organic matter, consistent with the predicted changes in bioavailability described above. The organic carbon at the top of the sediment (1.5–3.3 m) is distinctly different from the other two sections and is much more bioavailable; as there is a positive correlation between bacterial carbon and sediment organic carbon but there is no such relation for the middle section (4.8–26.7 m). The bottom section

(36.2–80.2 m) has a much higher organic carbon than the middle section (probably reflecting changes in depositional carbon (Patience *et al.* 1990)), but a bacterial carbon content similar to that of the middle section and much lower than the upper section. Hence the organic carbon in the bottom section is much less bioavailable than in the other two sections. It will be interesting to see if these three groups relate to the three different types of organic carbon predicted by Berners' 3G model (Berner 1980) for organic matter degradation.

Conclusions

This work has comprehensively demonstrated the presence and activity of bacteria down to at least 80.2 m BSF with subtle interactions between different bacterial groups, which would not be anticipated from purely chemical data. Decreases in the bioavailability of organic carbon with sediment depth may have been an important factor influencing the rates and distribution of bacterial activity. Direct bacterial counts reduce relatively slowly with depth, with no indication of a more rapid decline in the deeper layers, while the FDC indicates that a portion of the bacteria population were growing rather than just surviving, even in the deepest samples. Bacteria were able to be cultured in laboratory media from all depths sampled further confirming the viability of these organisms *in situ*. Where numbers of viable bacteria and activity were decreasing the brine incursion dramatically stimulated further growth and activity, even in sediments approximately one million years old (Suess & von Huene 1986). It is highly probable that bacteria continue to survive and maintain activity to even deeper sediment layers than those examined here and therefore have a profound effect on diagenesis. The high porosity of these sediments imply that motile bacteria, which may be mobile in the sediment (Davidson & Fry 1987), could reach deep sediment layers by migration, although the extent of movement would be restricted by energy requirements. The different bacterial horizons, however, may be able to maintain relatively constant depth positions, despite the accumulation of sediment at the surface, and thus ensure an optimum physico-chemical environment for their activity and growth. Hence the presence of bacteria in these deep sediment layers does not necessarily indicate that they have been there since deposition.

We express our gratitude to G. Eglinton and K. Kuenvolden, B. Julson and M. Mefferd for collecting and handling the sediments on board ship. We are also pleased to acknowledge the technical assistance provided by Wil Buckingham, John Getliff and Mark James, and the general assistance of Edmund Nickless of the Natural Environment Research Council. This research was funded by the Natural Environment Research Council.

References

Belyaev, S. S. & Ivanov, M. V. 1983 *Environ. Geochem. Ecol. Bull.* **35**, 273–280.
Berner, R. A. 1980 In *Biogeochemistry of organic matter at the sediment–water interface*. CNRS Int. Colloq. pp. 35–44.
Bianchi, A. 1986 *C.R. Acad. Sci., Paris* **303**, 449–451.
Colwell, R. R. 1979 Native aquatic bacteria: enumeration, activity and ecology. *Proc. Am. Soc. Test. Mater.* **695**, 56–61.
Cragg, B. A., Parkes, R. J., Fry, J. C., Herbert, R. A., Wimpenny, J. W. T. & Getliff, J. M. 1990 *Ocean drilling program*, vol. 112. Washington: U.S. Govt. Printing Office. (In the press.)
Davidson, A. M. & Fry, J. C. 1987 *Microb. Ecol.* **13**, 31–45.
Davis, J. B. 1967 *Petroleum microbiology*. Amsterdam: Elsevier.
Edenborn, H. M., Silverberg, N., Mucci, A. & Sundby, B. 1987 *Mar. Chem.* **21**, 329–345.
van Es, F. B. & Meyer-Reil, L.-A. 1982 *Adv. microb. Ecol.* **6**, 111–170.

Fallon, R. D., Newell, S. Y. & Hopkinson, C. S. 1983 *Mar. Ecol. Prog. Ser.* **11**, 119–127.
Fry, J. C. 1988 *Methods in aquatic bacteriology* (ed. B. Austin), pp. 27–77. Chichester: John Wiley.
Goldhaber, M. B. & Kaplan, I. R. 1975 *Soil Sci.* **119**, 42–55.
Hagstrom, A., Larsson, U., Horstedt, P. & Normark, S. 1979 *Appl. environ. Microbiol.* **37**, 805–812.
Hurley, M. A. & Roscoe, M. E. 1983 *J. appl. Bact.* **55**, 159–164.
Iversen, N. & Jorgensen, B. B. 1985 *Limnol. Oceanogr.* **30**, 944–955.
Jannasch, H. W. & Taylor, C. D. 1984 *A. Rev. Microbiol.* **38**, 487–514.
Jorgensen, B. B. 1983 *The major biogeochemical cycles and their interactions* (ed. B. Bolin & R. B. Cook), pp. 477–515. Chichester: John Wiley.
King, G. M. 1984 *Appl. environ. Microbiol.* **48**, 719–725.
King, G. M., Klug, M. J. & Lovley, D. R. 1983 *Appl. environ. Microbiol.* **45**, 1848–1853.
Kristjansson, J. K., Schonheit, P. & Thauer, R. K. 1982 *Arch. Microbiol.* **131**, 278–282.
Krumbein, W. E. 1983 *Microbial Geochemistry*. Oxford: Blackwell.
Lein, A. Y., Namsaraev, G. B., Trotsyuk, V. Y. & Ivanov, M. V. 1981 *Geomicrobiol. J.* **2**, 299–315.
Lochte, K. & Turley, C. M. 1988 *Nature, Lond.* **333**, 67–69.
Nedwell, D. B. 1984 *Adv. microb. Ecol.* **7**, 93–131.
Newell, S. Y. & Fallon, R. D. 1982 *Microb. Ecol.* **8**, 333–346.
Oremland, R. S. & Polcin, S. 1982 *Appl. environ. Microbiol.* **44**, 1270–1276.
Oremland, R. S., Marsh, L. M. & Polcin, S. 1982 *Nature, Lond.* **296**, 143–145.
Parkes, R. J. & Buckingham, W. J. 1986 *Proc. 4th Int. Symp. Microbial Ecol.* (ed. F. Megusar & M. Gantar), pp. 617–624. Ljubljana: Slovene Society for Microbiology.
Parkes, R. J. & Taylor, J. 1985 *J. appl. Bact.* **59**, 155S–173S.
Parkes, R. J. & Senior, E. 1988 *Handbook of laboratory model systems for microbial ecosystems* (ed. J. W. T. Wimpenny), pp. 51–72, vol. 1. Florida: CRC.
Patience, R. L., Clayton, C. J., Kearsley, A. T., Rowland, S. J., Bishop, A. N., Rees, A. W. G., Bibby, K. G. & Hopper, A. C. 1990 *Ocean drilling program*, vol. 112. Washington, D.C.: U.S. Govt. Printing Office. (In the press.)
Revsbech, N. P., Sorensen, J. & Blackburn, T. H. 1980 *Limnol. Oceanogr.* **25**, 403–411.
Rheinheimer, G. 1985 *Aquatic microbiology*, 3rd edn. Chichester: John Wiley.
Rice, A. L., Biller, D. S. M., Fry, J. C., John, A. W. G., Lampitt, R. S., Mantoura, R. F. C. & Morris, R. J. 1986 *Proc. R. Soc. Edinb.* B **88**, 265–279.
Senior, E., Lindstrom, E. B., Banat, I. M. & Nedwell, D. B. 1982 *Appl. environ. Microbiol.* **43**, 987–996.
Schonheit, P., Kristjansson, J. K. & Thauer, R. K. 1982 *Arch. Microbiol.* **132**, 285–288.
Sorokin, Yu. I. 1962 *Microbiol.* **31**, 329–335.
Suess, E. & von Huene, R. 1986 In *Init. Rep.* DSDP 112. Washington, D.C.: U.S. Govt. Printing Office.
Tsou, J. L., Hammond, D. & Horowitz, R. 1973 In *Init. Rep.* DSDP 15. Washington, D.C.: U.S. Govt. Printing Office.
Whelan, J. K., Oremland, R., Tarafa, M., Smith, R., Howarth, R. & Lee, C. 1985 In *Init. Rep.* DSDP 96. Washington, D.C.: U.S. Govt. Printing Office.

Discussion

J. A. Allen (*University Marine Biological Station, Millport, Isle of Cumbrae, U.K.*). Because the Core Station in the Peru margin is an area of high productivity associated with major upwelling, could this be a factor in explaining why bacterial activity is found so deep within the sediment?

R. J. Parkes. The high productivity of the Peru margin sediments may contribute to the magnitude of bacterial activity in the deeper layers, but the presence of bacterial activity in these layers may not be dependent upon high organic matter input to the sediment surface. High organic matter input to the sediment surface will stimulate bacterial activity and hence rapid removal of the available organic matter, thus the quantity of organic matter entering the deeper sediments may not be that different from sediments with a much lower organic matter input. Therefore, the bacterial activity in the deeper layers of sediments with high and low organic matter inputs may be more similar than would initially be expected. The definitive answer to the question, however, is to study more sites with a range of inputs from the water coloumn, and this we intend to do.

M. Whitfield (*Plymouth Marine Laboratory, U.K.*). Dr Parkes indicates the need for very close integration of microbiological and geochemical studies; particularly in studies of diagenetic processes at considerable depths below the sediment surface. His incubation experiments show the propensity of bacteria sampled at depth to utilize the substrates that you provide for them. However, some of the results he shows, such as the capability for nitrate reduction at 100 m in the sediment core, indicate that great care must be taken in the interpretation of the experimental results. What chemical studies are available of the organic substrates in the sediment that can be utilized and of the by-products of the bacterial degradation?

R. J. Parkes. Studies using inhibitors specific for certain bacterial groups have provided information concerning the organic compounds that are bacterial fermentation–hydrolysis products and also the substrates for the terminal oxidising groups of bacteria such as sulphate-reducers. Unfortunately such studies have been restricted to surface sediments, but they do indicate that these metabolic intermediates vary considerably in different sediments and may be related to both the quantity and quality (bioavailability) of the sedimenting organic matter (Parkes *et al.* 1989). What organic matter is being degraded or modified in the production of these metabolic intermediates is a intriguing question, and although there are a large number of studies quantifying changes in organic matter with sediment depth, some of which might be attributable to bacterial activity, there is little direct evidence linking organic matter changes with bacterial activity. Now that we can quantify bacterial activity within deep sediment layers it opens exciting opportunities for integrated microbiological and geochemical studies.

Additional reference

Parkes, R. J., Gibson, G. R., Mueller-Harvey, I., Buckingham, W. J. & Herbert, R. A. 1989 Determination of the substrates for sulphate-reducing bacteria within marine and estuarine sediments with different rates of sulphate-reduction. *J. gen. Microbiol.* **135**, 175–187.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY A MATHEMATICAL, PHYSICAL & ENGINEERING SCIENCES