

Life in the Deep Sub-seafloor Biosphere

Bo Barker Jørgensen

Max-Planck-Institute for Marine Microbiology, Celsiusstr. 1, D-28359 Bremen

The interest in the origin of life on earth and the prospects of life on other planets have accelerated in recent years and have gained great public awareness. Whereas life has been explored and found everywhere on the surface of our own planet, the deep sub-surface was until recently considered to be influenced only by abiotic processes. The discovery of micro-organisms in several million year old sedimentary deposits and even in basement rock has profoundly changed our perspective of the limits of living organisms. It is now clear that processes in the geosphere may provide the driving force for life and that, vice versa, the sub-surface biosphere has a large impact on present geological processes. The emerging fluids of cold seeps in subduction zones along continental margins are chemically modified by deep bacteria, just like the fluids of hydro-thermal vents along mid-oceanic ridges are modified by geo-thermal processes. Both fluid flows have a major influence on modern ocean chemistry. The slow biological degradation of organic carbon in the deep sub-surface and the formation of huge quantities of methane have a large-scale impact on sea-floor processes, such as the accumulation of gashydrates or the formation of carbonate mounds.

There is abundant evidence that prokaryotic cells live deep beneath the sea floor. Prokaryotic cells have been found in surprisingly high numbers in buried sediments at every site that has been assayed for their presence. The abundance of those cells varies in a systematic and fairly predictable manner. For example, deeply buried shelf sediments from the Peru Margin (high surface-ocean productivity and shallow water depth) contain 10^8 – 10^9 cells per cm^3 , and sediments from the eastern equatorial Pacific (low surface-ocean productivity and abyssal water depth) contain only 10^6 cells per cm^3 (Parkes et al., 2000). The current database on the distribution of bacteria in sediment cores obtained from the Ocean Drilling Program (ODP) indicates that this newly discovered biosphere may comprise 10% of all living biomass on earth. The observed population densities of 10^5 – 10^7 cells per cm^3 down to >750 sediment depth are greater than those found in ocean water. The organisms in this deep environment have applied significance, for example, for the formation and exploitation of fossil fuels (oil, gas, gashydrates), deep burial of toxic wastes, and as a source of unique bacteria for biotechnology.

Pore water chemical data from hundreds of DSDP and ODP sites document the occurrence of subsurface catabolic activity in sediments throughout most of the deep ocean (D'Hondt et al., 2002). Microbial sulfate reduction, methane production, and methanotrophy are common processes in deeply buried marine sediments. Other catabolic processes are known to occur in subsurface marine sediments but have been studied very little (such as manganese and iron reduction).

Despite these recent advances, very little is known about the nature and activity of

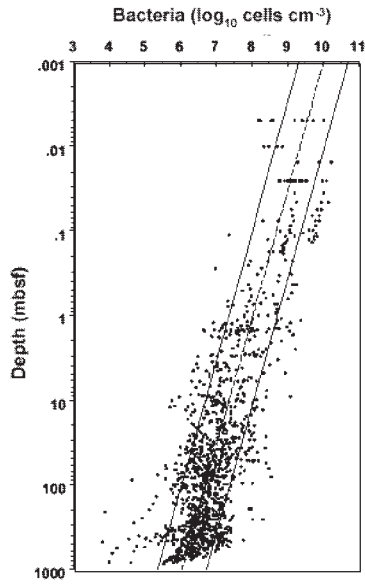


Figure 1. Depth distribution of bacterial cells in ocean sediments. Data based on microscopic cell counts from many legs of the Ocean Drilling Program. From Parkes et al. (2000) *Hydrogeol. Rev.* 8: 11–28



Figure 2. The German microbiologist, Heribert Cypionka, working on the enrichment of deep sub-seafloor bacteria at the anaerobic glove box

life in deep marine sediments. In particular, we know almost nothing about (1) the continuity of subsurface life from one oceanographic region to another; (2) the specialized metabolic properties, if any, that are required to survive in deeply buried marine sediments; or (3) the conditions under which subsurface microbes are active or inactive, living or dead.

ODP Leg 201 in spring 2002 was the first ocean drilling expedition dedicated to the study of life deep beneath the seafloor (Shipboard Scientific Party, 2002). Seven sites in the eastern tropical Pacific were selected to represent the general range of subsurface environments that exists in marine sediments throughout most of the world's oceans. In water depths as great as 5300 metres and as shallow as 150 metres, the expedition drilled up to 420 metres into oceanic sediments and the underlying rocky crust. The sediments ranged in temperature from 1°C to 25°C and in age from 0 to almost 40 million years. Leg 201 scientists found evidence of active microbial respiration throughout the sediment column at every site.

Sub-seafloor respiration was supported by the diffusion of sulfate down from the overlying ocean, as well as by the dissolution of iron- and manganese-bearing minerals. Methanogenesis occurred everywhere but was dominant only in the absence of sulfate and other electron acceptors for bacterial respiration. At the open Pacific sites, respiration deep beneath the seafloor is also supported by the transport of sulfate, nitrate and oxygen diffusing up from water circulating through the underlying basaltic crust. A large number of samples from Leg 201 are currently being analyzed in laboratories around the world to explore the types of micro-organisms and biogeochemical processes occurring in the deep subsurface.

Among the critical questions addressed during Leg 201 was the potential risk of contamination of the deep sediment samples by bacteria from the surface world. Two types of contamination tests were therefore conducted throughout the coring operations: A) a perfluorocarbon tracer was continuously injected into the drilling fluid,

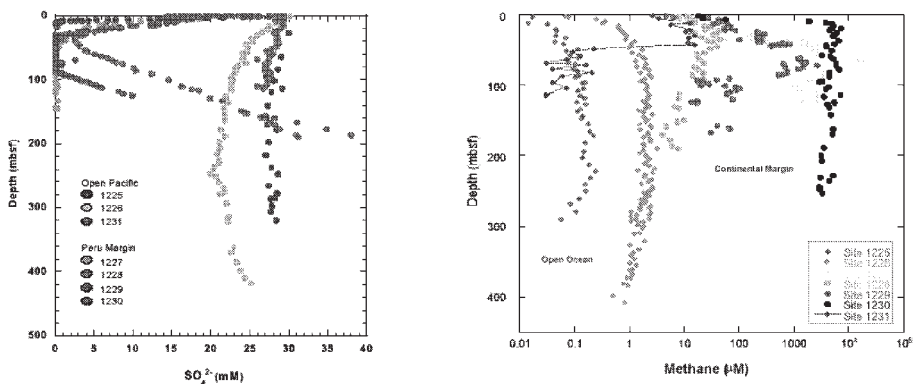


Figure 3 (a and b). Pore water chemistry of deep cores from Ocean Drilling Program Leg 201, Equatorial East Pacific. Open ocean sites show only weak gradients indicating low microbial activity, while continental margin sites show high activity.

At all the sites, both sulfate reduction and methane production take place.

and B) a suspension containing 2×10^{11} bacterial-sized fluorescent beads was released by the impact of all piston cores used for microbiological sampling (Smith et al., 2000). The results of these tests showed that uncontaminated sampling of deep sediments is indeed possible, provided that microbiologically clean techniques are applied.

It remains an enigma how the large populations of prokaryotic organisms in the deep biosphere can be sustained by the extremely low energy flow in million-year old sediments. For example, by comparison of the number of bacterial cells in open Pacific sediments with the modelled rates of sulfate reduction it can be estimated that the average metabolic rate is ca. 10^{-20} mol per cell per day. This is at least 10^5 -fold below the metabolic rate of sulfate reducing bacteria in laboratory cultures. Assuming that the deeply buried bacteria have a similarly high growth yield as their cultured relatives, this metabolic rate should theoretically allow a generation time of 10,000 years. At this point we cannot explain such low activity. Is most of the population inactive, or dead, or are these prokaryotic cells adapted to a cryptic growth for which we have no comparison from current laboratory studies?

Future research will require a multi-disciplinary cooperation in order to use and further develop advanced technological facilities for, e.g. mapping, coring and sampling of the sea floor. Novel concepts and new analytical and experimental methods are required for the study of these microscopic prokaryotes, for which we do not even know what are the dominant organisms, how they maintain basic life processes, or what the relevant physical-chemical properties of their habitat are.

Among the main objectives of future research efforts should be:

- to explore the limits of the subsurface biosphere with respect to depth, temperature, energy availability, and other properties of the habitat;
- to study unique habitats such as cold seeps, gas hydrates, oil reservoirs, deep brines, mud volcanoes and carbonate mounds;
- to identify the dominant organisms and their biomass, diversity, and eco-physiology;
- to study microbe interactions with minerals and other potentially energy-providing interfaces; and
- to search for novel types of prokaryotes with unique properties.

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