The deep subseafloor biosphere: The microbial world under the sea

What kind of world lies under the sea? Does anything live there?



Drilling vessel CHIKYU. Length: 210 m, Hight: 130 m, Gross tonnage: 56,752 tons

Tatsuhiko Hoshino and Fumio Inagaki

Japan Agency for Marine-Earth Science and Technology, Yokosuka, Japan

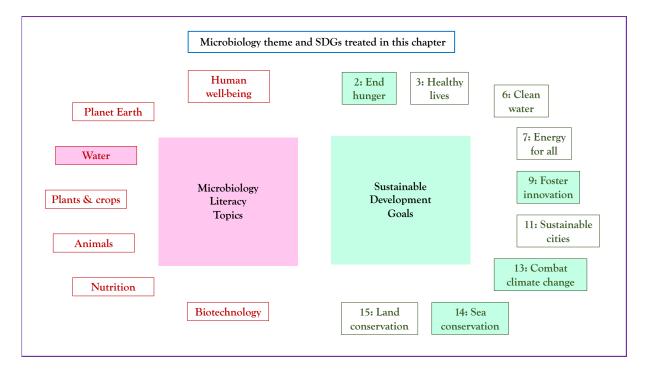
The deep subseafloor biosphere:

Storyline

The sea covers almost 70% of our planet. It is home to beautiful coral reefs, a wide variety of fish, mammals such as whales and dolphins, and jellyfish. We often focus on the beauty of the oceans and their recreational activities, like swimming and sailing. However, what lies beneath the sea? The seabed consists of a sediment of mud and sand that has been carried in from the land, and marine snow from dead marine life, mainly marine plankton. The average thickness of marine sediment is about 700 m, but it can be up to 10 km thick in some areas on Earth. No large creatures like fish or whales can live in these sediments, but many tiny microorganisms live in them. Their numbers are known to be comparable to those in seawater and soil. And these subseafloor microorganisms may be sitting there for millions of years, and have long played important roles in maintaining the health of the overlying ocean and the atmosphere.

The Microbiology and Societal Context

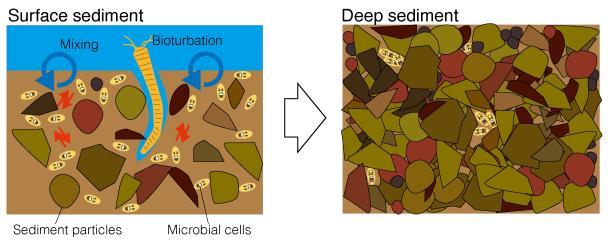
The microbiology: sediment microbes; anaerobic metabolism; sulfate reduction; methanogenesis; anaerobic methane oxidation; denitrification. *Sustainability issues*: hunger; innovation; climate change; sea conservation.



The deep subseafloor biosphere: the Microbiology

1. *What is the habitat under the sea?* What is the subseafloor like? How does it differ from our terrestrial and marine environments? On land where we live there is sunlight and green trees. In these environments, plants convert energy from the sun into organic matter, food for humans, cows, pigs, and birds. Similarly, in surface waters, aquatic plants and microbes convert energy from the sun into organic matter that supports the aquatic food web. In other words, the surface world is supported by the abundant energy supplied by the sun.

However, sunlight can only reach depths up to about 200 m in the ocean, so most of it is dark. The seawater is also cold, with a temperature of only about 4°C. Therefore, in a typical deep-sea sediment, the only food available is that produced in surface waters and that drifts down to the seafloor. This food is used up by organisms in the surface layer of the sediment, so the food supply is depleted as the sediment gets deeper. Also, as the sediment gets deeper, it is compacted so that what was once mud and sand gradually becomes rocky. In this way, the deeper the subseafloor sediment gets, the harder it is for life to live in it.



· Food and energy are abundant.

• The high pore space and adequate energy supply allow microbes to move.

Depletion of available food and energy.

• Microbes are trapped between sediment particles.

2. *What organisms live in the subseafloor?* Food arriving from the upper water column is metabolized in the surface layer of the seabed using oxygen. Because oxygen diffuses poorly through sediments, it cannot be replaced. The majority of subseafloor sediments therefore lack oxygen and are anoxic environments. Most eukaryotic organisms, including humans, cannot survive without oxygen because they live on the energy obtained using oxygen to oxidize organic matter.

Living organisms on Earth can be divided into three main groups. Eukaryotes, Bacteria, and Archaea. Humans (*Homo sapiens*) are eukaryotes. Other eukaryotes include elephants, lions, dogs and cats, fish and insects, and smaller ones such as molds and yeasts. The remaining two

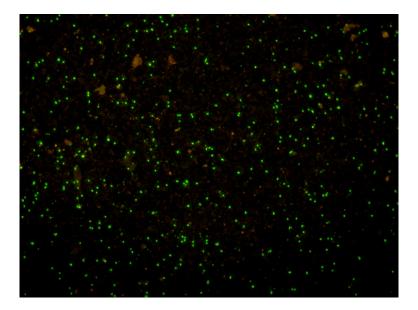
types, Bacteria and Archaea, include many organisms that are sometimes grouped together as prokaryotes. Surprisingly, some prokaryotes can live in extreme environments, such as in water hotter than 100°C or highly alkaline, environment far too hostile for humans.

While eukaryotic organisms such as gobies and mussels live on sediment surfaces where seawater mixes with the surface layer of the seafloor, only Bacteria and Archaea are known to live in deeper sediments. In the absence of oxygen, what do they 'breathe': what do they use to metabolise organic matter to obtain energy? In fact, some Bacteria and Archaea can breathe different substances, such as sulfate ions, which are abundant in seawater, while others can use nitrate ions or even metals such as iron and manganese.

3. *The subseafloor is the largest microbial biosphere on Earth!* Until recently, it was thought that organisms could not live under the seafloor: in 1955, scientists studied 8 m-deep marine sediment samples but could not find living microbes and concluded "we have reached the lower limits of the biosphere".

Since then, numerous scientific drilling expeditions to subseafloor sediments using the US drilling vessel *JOIDES Resolution* and the Japanese drilling vessel *Chikyu* have taken many sediment core samples over several hundred meters in length. Scientists counted the number of microbial cells in those sediment samples with microscopes. As a result, the number of microbes living in the subseafloor sediment is estimated to be as high as 10²⁹ cells on Earth. Compared to the number of stars in the universe, 10²³, there are a million times more microbes beneath the ocean than stars in the universe. This number is greater than the number of microbes in all the seawater on Earth, almost equal to the number of microbes living in all the land soil, and almost half the number of microbes living on Earth.

And, despite the fact that the environment beneath the seafloor is harsh and foodstarved, it is inhabited by a rich diversity of microbes, comparable to that of the ocean and land soils.



4. *A million years for one cell to grow into two!* Even though many microbes are living beneath the ocean, their metabolic activity is not very high and decreases with depth. To reproduce, microbes must make all the components of a new cell by taking up elements from the environment. Laboratory measurements of the rate of carbon uptake by microbes collected from the subseafloor sediment, and calculations of the time required to produce new cells, suggest several hundred years for sediments 20 cm below the surface, thousands of years for depths of 3 m, and millions of years for depths of 100 m. Results from laboratory experiments must be viewed with caution, since the experimental conditions may not completely reflect the conditions in nature, but there is no doubt that life is slower in deep sediments below the seafloor.

5. *What are microbes doing in such a harsh environment?* What are all the different microbes doing beneath the ocean? The process by which fallen leaves and animal remains are returned to the soil through microbes, for example, in forest soils, is well known. In general, similar processes occur in the subseafloor sedimentary environment. The most common of these processes is the oxidation of organic matter by so-called sulfate-reducing microbes using sulfate ions in seawater.

One of the most important biological reactions in subseafloor sediment is methane production, known as "methanogenesis". While methane is known to be produced by the thermal decomposition of buried organic matter, which is a physico-chemical process, methanogenesis is primarily carried out by methanogenic archaea. The ability to produce methane is unique to archaea, and as yet no bacteria are known to produce methane.

Other important microbial reaction processes discovered in marine sediments include the anaerobic oxidation of methane (AOM), in which methane is oxidized, again using sulfate. This AOM reaction does not occur in a single microorganism but is carried out by a specific consortium of methane-oxidising (methanotrophic) archaea and sulfate-reducing bacteria.

6. What is the role of subseafloor microbial communities in the global environment? Microbial communities in subseafloor sediments play important roles in the biogeochemical cycling of elements beneath the ocean. This has a significant impact on the elemental cycles in the overlying ocean and the atmosphere, and contributes to the stability of the global environment. For example, the consumption of sulfate ions during decomposition of organic matter and the resulting formation of iron sulfide in sediments, contribute to an increase in the alkalinity of seawater. The alkalinity of seawater has a significant impact on the Earth system, including temperature, as it controls how much atmospheric carbon dioxide – an important greenhouse gas – can be dissolved in seawater.

Denitrification – the production of nitrogen gas from nitrate and nitrite ions – in anoxic sediments is another reaction that only microbes can carry out, and is very important in controlling biomass in seawater. This is because nitrate is often the limiting factor in marine biomass production. Without denitrification reactions in sediment, there would be more nitrate and thus more growth of algae and other organisms, some of which cause toxic red and blue tides. As a result, regions of the ocean may become anoxic and the many large organisms that live in it may die.

In this way, microbes that live quietly beneath the seabed, which we do not normally see, play an essential role in all life on Earth.

It should also be mentioned that some of the organic matter produced in the surface layers of the ocean, and arriving at the sea floor, is not metabolized and becomes buried more profoundly in the sediment. Deep seabed sediments therefore act as a sink for carbon derived from carbon dioxide, and therefore help to reduce greenhouse gas levels.

Relevance for Sustainable Development Goals and Grand Challenges

- Goal 2: Zero hunger. Microbes in the subseafloor sedimentary biosphere have played essential roles in element cycling through the oceans and seafloor during almost 4 billion years of Earth's history. For example, in the organic matter decomposition process, microbial communities help maintain a healthy environment in the ocean so that we can sustainably obtain the marine gifts we eat in our daily lives, such as fish, shellfish, and shrimp.
- Goal 9: Industry, Innovation and Infrastructure. New microbial reactions have been discovered in the subseafloor sedimentary biosphere, which are not found in other environments. Further research could lead to the discovery of new reaction processes, which could lead to the discovery of enzymes that could be useful to industry.
- Goal 13: Climate action. The activity of subseafloor microbial communities has a significant impact on elemental cycles in marine sediment. This strongly affects carbon and nitrogen cycling between the ocean and the atmosphere and therefore is relevant to climate change.
- Goal 14: Life Below Water. Without the activity of microbial communities on the seafloor and below, the marine environment will be drastically altered. For example, eutrophication of the ocean and loss of oxygen in large parts of the ocean, could lead to diversity loss and ecosystem collapse.

The Evidence Base, Further Reading and Teaching Aids

https://youtu.be/QWj3tFQnl5o

Kallmeyer, J., Pockalny, R., Adhikari, R. R., Smith, D. C., & D'Hondt, S. (2012). Global distribution of microbial abundance and biomass in subseafloor sediment. Proceedings of the National Academy of Sciences, 109(40), 16213-16216. doi.org/10.1073/pnas.1203849109 Flemming, H. C., & Wuertz, S. (2019). Bacteria and archaea on Earth and their abundance in biofilms. Nature Reviews Microbiology, 17(4), 247-260. doi.org/10.1038/s41579-019-0158-9 Hoehler, T. M., & Jørgensen, B. B. (2013). Microbial life under extreme energy limitation. Nature Reviews Microbiology, 11(2), 83-94.doi.org/10.1038/nrmicro2939

Hoshino, T., Doi, H., Uramoto, G. I., Wörmer, L., Adhikari, R. R., Xiao, N., ... & Inagaki, F. (2020). Global diversity of microbial communities in marine sediment. Proceedings of the national academy of sciences, 117(44), 27587-27597. doi.org/10.1073/pnas.191913911

Orsi, W. D. (2018). Ecology and evolution of seafloor and subseafloor microbial communities. Nature Reviews Microbiology, 16(11), 671-683. doi.org/10.1038/s41579-018-0046-8

Glossary

Sediment: A deposit of fine gravel, sand, mud, remains of organisms, volcanic ash, etc., which has not yet formed into rock. In this article, it means the deposits that have accumulated on the seafloor.

Microbes: In a broad sense, it is a general term for organisms that cannot be seen with the naked eye. In this article, it refers to Bacteria and Archaea.

Organic/inorganic matter: Organic matter is a general term for compounds containing carbon produced by animals and plants in nature; inorganic matter is a substance not produced by other living organisms and includes minerals. Carbon dioxide, however, is an inorganic matter even though it contains carbon.

Oxic and Anoxic: Oxic means containing molecular oxygen (O_2) while anoxic does not. The seafloor surface is oxic because seawater contains oxygen. Oxygen is generally used up in the oxidation of organic matter in the sediment surface layers. In open ocean sediments, where there is little organic matter, oxygen is not consumed and remains present throughout sediment.

Abiotic: Meaning that no living organisms are involved. The high temperatures in deep sediments result in abiotic reactions in which buried organic matter is thermally decomposed and methane is produced.