Life and Ecosystem in Deep Biosphere and Subseafloor Aquifers

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Abstract

The deep subseafloor biosphere is substantially associated with fluid flow regime and biogeochemical elements cycle. To understand the ecological nature of the deep subseafloor biosphere, an interdisciplinary approach involving microbiology, geochemistry, hydrogeology and geology is required. As the result of the domestic deep subseafloor biosphere workshop in Japan, we suggest five major themes that remain to be clarified in the future scientific drilling; 1) Extent of the Biosphere, 2) Ecological roles of geo-fluids and thermal energy, 3) Biogeochemical functions, fluxes and ecological roles, 4) Modern proxies for the past biosphere and ecosystem, 5) Analytical and technological development. To achieve these scientific goals, the utilization of *Chikyu* riser-drilling system and integration of other scientific communities is highly recommended.

1. Introduction

One of the key challenges of the future scientific drilling is to comprehend the geosphere-biosphere interaction in the modern earth's system and the past and future co-evolution of life and planet. Previous studies demonstrate that subseafloor populations and activities depend mainly on the supply of nutrie nts and energy substrates from the overlying surface world (i.e., land and ocean) and/or the underlying lithosphere (i.e. earth's crust and mantle). Hence, fluid flow regimes and geophysical conditions must play significant roles in nutrient and energy transport, affecting habitability and ecosystem existence. The subseafloor microbial community is generally composed of phylogenetically diverse Archaea and Bacteria, which genetic and physiological characteristics are largely unknown, and hence the ecological and biogeochemical roles and functions remain largely elusive. Future expeditions of the Integrated Ocean Drilling Program (IODP) will provide unprecedented opportunities for addressing these fundamentally important scientific objectives concerning deep subseafloor life and the biosphere, and the explorations are thus capable of expanding our knowledge of the co-evolution of life and planet.

2. Important themes of deep-biosphere research

In the IODP Initial Science Plan (ISP: 2002), "The Deep-biosphere and Subseafloor Ocean" is described as an important scientific target that should be addressed within the current phase of IODP expeditions. Although our knowledge concerning this environment has greatly expanded since publication of this ISP in 2002, newly posed or unsolved fundamental questions have yet to be addressed. Here, resulting from discussions during the domestic deep subseafloor biosphere workshop in Japan, we report on five major scientific themes of deep-biosphere research that should be directly addressed during the next phase of the IODP beyond 2013.

1) *Extent of the Biosphere*

The marine subsurface environment is currently considered to be a largest part of the biosphere, probably harboring one-tenth of the living biota on Earth. The ubiquitous microbial community there represents the energetic and geophysical constraints to habitability and the dispersal of microbes. Scientific ocean drilling using the *Chikyu* and other drilling platforms in the deep subsurface realm will provide unique opportunities for defining the transition between habitable and uninhabitable zones, or biotic and abiotic processes. The physiological states that microbes can adopt (i.e., alive, dormant, or dead) are crucial for determining their survival of the fittest in constant or transient nutrient and energy supplies to deep subseafloor habitats**.** It is important to determine the indigenous locations of metabolically active microbial habitats associated with ideal geophysical and geochemical settings in the deep biosphere, where microbial cell abundance generally decreases with increasing depth. Through deep drilling ventures using *Chikyu*, valuable sampling opportunities will allow biomass measurements and characterization of both the physiological state of subseafloor life and habitable conditions in diverse oceanographic and tectonic settings. These studies will provide new insights into the nature and extent of the earth's biosphere.

Potential drilling sites: various ocean seafloors with various oceanographic settings, deep continental margin sediments, deep lithosphere drilling sites (e.g., mohole project)

2) *Ecological roles of geo-fluids and thermal energy*

In the dark subseafloor biosphere, geologically produced nutrient and energy substrates may support some naturally occurring microbial populations. The substrates production and transportation are closely related to tectonic movement and thermal energy, which provide the primary driving force of regional fluid flow systems. Furthermore, the depth-pressured dehydration process that occurs in deep sedimentary realms of convergent plate boundaries provides essential water for dehydrated deep subseafloor microbial life. This so-called "geo-fluid" or "geo-pressured fluid" flow is sometimes observed in active faults and fractures in an accretionary wedge as well as in diapiric intrusion of high-buoyancy mud or salt-captured aquifers. Thermal energy from the earth's mantle directly affects widespread fluid circulation in ridge flank and back-arc hydrothermal systems. The supply of mantle-derived and/or thermogenically produced nutrients and energy via geo-fluid circulation potentially controls deep subseafloor microbial activity, population sizes, and community compositions, and plays an important role in biogeochemical cycles. This important issue is highly relevant to the geosphere-biosphere connectivity, and thus should be addressed with multidisciplinary studies in the future IODP.

Potential drilling sites: deep subduction zone including geological and goehydological complexities, and mud or serpentine diapir (e.g., Nankai Trough, Lau Basin, Mariana Forearc), ridge crustal system (e.g., Indian Ocean, Mid Atlantic Ridge), back-arc hydrothermal system (e.g., Okinawa Trough, Rau Basin)

3) *Biogeochemical functions, fluxes and ecological roles*

The activities of subseafloor microbes presumably play significant roles in the earth's biogeochemical cycles in geologic time. Long-term accumulation of extremely low metabolic products affects the circulation of carbon, nitrogen, oxygen, sulfur and other elements in the subseafloor environment. Complex metabolic networks are most likely responsible for connecting this food/chemical web in energy-starved microbial ecosystems. However, it is currently unknown what kinds of microbes play major roles in particular biogeochemical reactions in the food/chemical chains; it is also not known how they metabolize intermediates from buried recalcitrant organic materials or other abiogenic substrates in the final feeding processes in the ecosystem. Biogeochemical fluxes are regionally diverse relative to differences in oceanographic, hydrogeologic, and tectonic settings: for example, to primary organic production and redox status in the overlying water columns, sedimentation rates, and fluid flow regimes. However, our knowledge of the metabolic function, fluxes, and ecological roles of each biogeochemical process's key player is very limited. This must be clarified during the future IODP.

Potential drilling sites: hydrocarbon systems of the continental margin, including methane hydrates and free gas zones (e.g., northwestern Pacific off the Shimokita Peninsula of Japan, Nankai Trough, Gulf of Mexico), various oceanographic settings

4) *Modern proxies for the past biosphere and ecosystem*

During the evolution of life over the past 3.5 billion years or some more, ancient environments hosted primordial microbial life under anoxic and high-temperature conditions. These physical and chemical constraints spurred the adaptation and evolution of life forms during the earth's history (e.g., oxygen mass-production via photosynthesis). Previous scientific ocean drilling program has successfully revealed paleoenvironmental records related to drastic climate changes and geologic events during the earth's history. However, it is currently unknown how subseafloor life and the biosphere accommodate environmental changes via adaptive evolution and how they occupy ecological niches. Conducting scientific drilling to find modern proxies for the past biosphere and ecosystem, it is highly recommended for the future IODP. To discriminate modern (living or surviving) and past (dead or fossilized) biological signatures from extant core materials, method development is in high demand, because genomic and proteomic progresses may provide decipherable records of evolutionary history. Moreover, the expanding knowledge in this sphere and simulation analysis promote the understanding of both habitability and possible evolution of life forms on virtual or real other planets. This is an entirely new concept that has not been described in previous ISPs and is hence one of the challenges of the future IODP.

Potential drilling sites: hydrothermal fields, polar subseafloor, anoxic water column subsurface, sapropels, estuaries

3. Analytical and technological development

To promote the achievement of the major scientific objectives described above, analytical and technological development of onboard and shore-based experiments are very much required. These techniques can then be subsequently applied to drilled cores and fluids in case studies. In this regard, bio-archived core samples and their storage (i.e., frozen cores, fixed slurries for microscopic analyses, anaerobic sediment samples) are very important, because they provide opportunities to repeatedly study previously cored sites of interest with newly developed analytical techniques.

As for onboard studies, we must make every effort to generate additional data while aboard the research vessels. For example, a newly developed non-destructive scanning tool for the biological signatures using deep UV wavelengths may be applicable to the half-cut core surface. Cell enumeration should be performed using a high-throughput computer image analysis with high depth-resolution. The most important onboard work is proper preparation of frozen, fixed, or anaerobic samples for promising shore-based experiments. In shore-based laboratories, molecular and isotopic analyses at the single cell level are rapidly progressing with nanotechnology development. 'Omics' studies to approach gene and protein directly have been poorly represented in deep-biosphere research, including statistical and simulation analyses of pooled molecular and geochemical data.

One of microbiological ultimate goals is to cultivate dominant microbial components and isolate them for growth and metabolic characterizations. High-pressure incubation or flow-through reactor cultivation experiments may improve culture of slowly growing subseafloor microbes in laboratory conditions. We should consider not only the components of archaea and bacteria but also viruses and eukaryotes, including their relics and gene transfer, in terms of their ecological roles and functions.

Organic geochemicals and trace elements are crucial targets to investigate biogeochemical process and microbial physiology of deep biosphere. A series of state-of–art analytical techniques, e.g. X-ray microscopy and spectroscopy, enable to distinguish micro-heterogeneity in small spot. We should consider how such advanced techniques apply to drilled core samples.

Subseafloor life forms and biogeochemical process in basaltic aquifers has been poorly explored in ocean drilling projects because of the difficulty of sampling the rubble crusts and aquifer. The borehole observatory using CORK-like system and the installed sensors is expected to provide excellent opportunities for studying microbiology, biogeochemistry, and hydrogeology in previously inaccessible subseafloor aquifers within the basaltic lithosphere or deep sediment layers. Borehole utilization has great potential for developing a new *in situ* research system for harvesting actively growing subseafloor microbes, trace elements, and intermediate chemicals in biogeochemical process.

Lastly, the deep riser-drilling system of *Chikyu* will provide excellent opportunities to address the limit and extent of subseafloor life. The riser-drilling system using blowout preventer (BOP) will enable us to study high-pressure hydrocarbon systems in shallow to deep continental margin sediments. We should consider how X-ray CT scan could be used for onboard sampling and characterization of microbiology and biogeochemistry. The high pressure-coring technology including the X-ray monitoring sample transfer system without depressurization is highly recommended to deploy on the riser-drilling system of *Chikyu*.

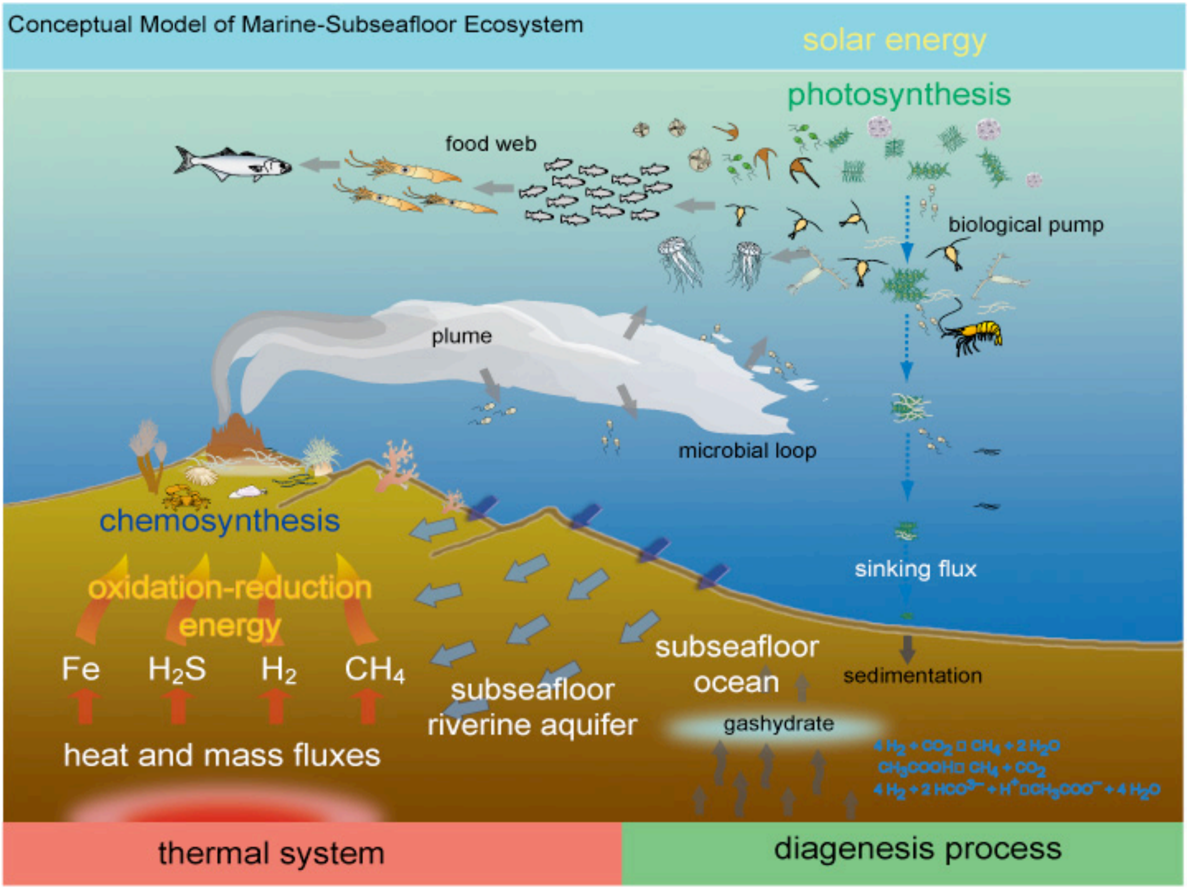

Figure 1. Schematic diagram of marine-subseafloor ecosystems.

The finding of a diverse and active subseafloor microbial community in deep subseafloor sediments has changed the concept of life's habitat. However, the extent of biosphere and the nature of subsurface life remain largely unknown. Subseafloor ocean and riverine aquifer are also largely unknown, although these environments are highly relevant to the fluid flow and biogeochemical cycling as well as the subseafloor life. Photosynthesis-based heterotrophy is predominant in upper sedimentary environments while chemosynthesis may predominantly occur in deep and/or rocky aquifers near the ridge systems. Linkages between past- and modern-earth's environments, and interactions between seafloor and subseafloor ecosystems are of significant scientific themes that should be addressed during the future IODP expeditions.