doi : 10.17596/0003789

Cruise Report

CK09-01 *Chikyu* training cruise Leg. 1

Kumano Mud-Volcano Drilling: A Window to the Deep-Biosphere

Mar. 8-18, 2009

JAMSTEC

Shipboard scientific party

Expedition Project Managers (EPMs): Nobu Eguchi (EPM-chief), Kan Aoike, Yusuke Kubo, and Sean Taczko
Program Operation Group,
Center for Deep Earth Exploration (CDEX), JAMSTEC

Chief scientist: Fumio Inagaki (geomicrobiologist)
Geomicrobiology Group,
Kochi Institute for Core Sample Research, JAMSTEC
e-mail: inagaki@jamstec.go.jp

Juichiro Ashi (geologist)
Ocean Research Institute,
The University of Tokyo, Japan
e-mail: ashi@ori.u-tyokyo.ac.jp

Akira Ijiri (geochemist)
Institute for Research on Earth Evolution (IFREE),
Research Program for Paleoenvironment, JAMSTEC
e-mail: ijiri@jamstec.go.jp

Hiroyuki Imachi (microbiologist)
Subground Animalcule Retrieval (SUGAR) Program,
Extremo-biosphere Research Center (XBR), JAMSTEC
e-mail: imachi@jamstec.go.jp

Yuki Morono (microbiologist)
Geomicrobiology Group,
Kochi Institute for Core Sample Research, JAMSTEC
e-mail: morono@jamstec.go.jp

Ko-ichi Nakamura (geologist)

National Institute of Advanced Industrial Science and Technology (AIST)
e-mail: koichi.nkamura@aist.go.jp

Takeshi Terada (microbiologist)
Marine Works Japan, Co., Kochi, Japan
e-mail: teradat@jamstec.go.jp

Tomohiro Toki (geochemist)
Department of Chemistry, Biology, and Marine Science,
Faculty of Science, University of the Ryukyus, Japan
e-mail: toki@sci.u-ryukyu.ac.jp

Yasuhiko Yamaguchi (biogeochemist)
Ocean Research Institute,
The University of Tokyo, Japan
e-mail: y-t-yamaguchi@eps.s.u-tokyo.ac.jp

JAMSTEC Core Curator: Hideaki Matchiyama (sedimentrogist)
Kochi Institute for Core Sample Research, JAMSTEC
e-mail: bucci@jamstec.go.jp

Acknowledgement

The science party of the CK09-01 Leg 1 (Kumano mud volcano) wishes to thank all crews of the deep-earth research drilling vessel "*Chikyu*" and drilling staffs of Mantle Quest Japan (MQJ), Co., for providing an opportunity to study the subsurface of the Kumano mud-volcano no. 5 during the CK09-01 *Chikyu* training cruise. We are also grateful to the technical and curational staffs of Marine Work Japan (MWJ) for their supporting efforts on the core processing and laboratory experiments.

Introduction

The marine subsurface sediments harbor the largest microbial biomass that comprises one-tenth of all living biota on Earth (Whitman et al., 1999). The microbial population generally decreases with increasing burial depth because of the limitation of bio-available nutrient and energy substrates as well as the compaction of habitable space with depth burial (Parkes et al., 1994, 2000). The metabolic activity depends on the flux of nutrient and energy mainly from either the overlying seawater or the underlying basaltic aquifer (D'Hondt et al., 2004); hence, the primary photosynthetic production in the water column and the fluid flow regimes play significant roles in the nutrient and energy transport, affecting the population, activity and diversity of sub-seafloor microbial community. The recent biogeochemical studies demonstrated that the heterotrophic archaeal cells dominate the sub-seafloor microbial community, which abundance shows a parallel trend with organic contents in sediments (Biddle et al., 2006; Lipp et al., 2008). The sub-seafloor microbial community is generally composed of phylogenetically diverse, yet-to-be-characterized archaeal and bacterial components, whose genetic and physiological characteristics are largely unknown, and hence their ecological and biogeochemical roles remain elusive (Inagaki et al., 2003, 2006; Inagaki and Nakagawa 2008).

One of the key challenges in the scientific drilling is to comprehend the extent of life and biosphere (D'Hondt et al., 2007). Recent study in the Ocean Drilling Program Leg 210 off the Newfoundland margin showed that microbial populations over 10^5 cells/ml are present down to 1626 meters below the seafloor (mbsf) sediments that are 111 My old and 60º to 100ºC (Roussel et al., 2008). However, it needs a repeated clarification by the third party because the cell enumeration was performed by the manual count based on the conventional direct eye-discrimination of acridine orange (AO) stained-cells, which often causes serious overestimates of cell number because of the high fluorescent backgrounds. Recently, the improved cell detection and enumeration technique using computer-image calculation has been established, providing more precise, objective, and hence reliable data of cell abundance in cored samples (Morono et al., 2009). The new cell-enumeration technique is expected to be highly useful

for the future explorations of the deep sub-seafloor biosphere, including this CK09-01 cruise.

During the CK09-01 Leg 1 *Chikyu* training cruise, we tested to drill a mud volcano no. 5 in the Kumano forearc basin of the Nankai Trough, Japan. This provides us an unprecedented opportunity to expand our knowledge on the previously inaccessible deep and hot sub-seafloor biosphere because the mud is originally derived from the deep realm down to a few thousands of meters below the seafloor. The mud volcanoes are widely distributed in the prelate convergent continental margins (Milkov 2000; Kopf 2002). The formation process of mud-volcano is so called “diapirism”, in which a lower density, high fluid content, deformable deep material intrudes upward into the stratified overlying sediments along the deeply rooted fault since the density contrast acts as a force of buoyancy. The diapiric mud typically contains consolidated to semi-consolidated breccias trapped in the mud matrix during the upwelling intrusion. The mud fluids typically have low chloride (Cl) and high boron (B) and lithium (Li) concentrations (Hensen et al., 2007), indicating the depth-pressurized dehydration of clay minerals (smectite-illite reaction) at high temperature around 100ºC. The deep-sourced dehydrated water can be distinguished from the seawater by deuterium (δD) and oxygen (δ^{18}O) isotopic compositions of the pore water. One of the other characteristics of the mud diapir is the presence of a lot of hydrocarbon gasses including methane, sometimes oil materials, produced by either or both microbial and/or thermogenic processes (see a review of Etiope et al., 2009).

Although piston or gravity coring down to a few meters below the surface of mud volcano has been demonstrated many times in multiple locations, the deep scientific drilling has only been performed in the Ocean Drilling Program (ODP) Leg 160 at Site 970, the East Mediterranean Sea off Italy. At Site 970 Hole D, approximately 45.3 m cores were successfully retrieved using an advance piston core (APC) barrel from the summit of the mud-volcano. The pore water chemical analyses showed very low chloride concentrations (e.g., 61 mM at Core 4H-5, 30.7 mbsf), indicating two possibilities: 1) methane hydrate dissolution or 2) mineral dehydration upon increasing pressure (De Lange and Brumsack, 1998). The δD-δ^{18}O isotopic study of the pore water conclusively

suggested that the fluids are originally derived from clay mineral dehydration (mainly smectite-illite transformation), corresponding to a depth range of 3.5-7 km and an elevated temperature of about 120-160 ºC (Dählmann and De Lange, 2003). The deeply rooted hydrothermal imprint of clay-dehydrated low salinity water has also been supported by Li-B enrichment (Hensen et al., 2007), providing us an important implication to the occurrence of the deep, hot biosphere that can be seen through the diapiric mudflow.

In general, the activity of sub-seafloor microbes on the continental margin sediments largely depends on the organic matter buried from the overlying seawater column; however, the young diapiric mud volcano, which formed at an episodic geologic event such as faulting, has not been thickly covered by fleshly buried sediments; hence, the ecosystem in the deep mud volcano is expected to be different to the surrounding stratifying sedimentary environment and to still maintain the deep sub-seafloor microbial ecosystem, even in the relatively shallow part of the mound. In the Kumano basin of the Nankai Trough, ten or bit more diapiric mound-like structures have been observed by the seismic and sea-bottom surveys, among which the no. 5 mud volcano is thought to form in relatively young period because of the absence or very little coverage of overlying sediments. Furthermore, the bottom survey using the manned submersible *Shinkai 6500* revealed the presence of chemosynthetic *Calyptogena* colony along the rim of the crater, indicating the active seepage of hydrocarbons along the outer surface of the intrusion pathway.

The surface sediments of mud-volcano have been well studied where the microbes strongly mediate an anaerobic oxidation of methane (AOM) at methane seep sites of the volcanic summit. For example, at the Haakon Mosby mud volcano in the Barents Sea, anaerobic methanotrophic archaea (ANME-2 & 3) and sulfate reducing bacteria (*Desulfosarcina, Desulfococcus, Desulfobulbus*) consume <40% of the total flux of methane in the surface sediments close to the sulfate-methane interface (Niemann et al., 2006). Geochemical characteristics in the surface sediments of the Haakon Mosby mud volcano were studied down to approximately 300cm below the seafloor (Ginsburg et al., 1999). However, the microbiological and biogeochemical characteristics in deep realms have

remained elusive.

Methane hydrates were sometimes observed in or near the mud volcano. For example, the presence of methane hydrates associated with mud volcano was reported from the Barbados accrationary wedge (Martin et al., 1996), and the Middle American Trench, offshore the Costa Rica (Milkov, 2000) with gravity and/or piston coring. Massive hydrates were retrieved from 2m-gravity core sediment of the mud diapir no. 11 on off Costa Rica (Schmidt et al., 2005). Hensen et al. studied δD and δ^{18}O isotopic compositions of pore water from the hydrate-bearing mud samples and estimated the temperature range of the clay dehydration from 85 ºC to 130 ºC (Hensen et al., 2004). Methane hydrates were also reported from the mud volcano in the Gulf of Gaidz, in which pore waters contained low chloride concentrations potentially caused by the hydrate formation (Mazurenko et al., 2003). The presence of methane hydrates was widely observed in the Kumano forearc basin in the Nankai Trough. The seismic survey around the mud volcano no. 5 showed the presence of a bottom-simulating reflector (BSR) at around 340 mbsf in the flank sediments; however, no BSR or hydrate-indications have been observed in the body of mud volcano.

Despite the importance of diapiric environment as "**A Window to the Deep-Biosphere"**, there is no demonstration to study microbiology and biogeochemistry in the deep realms of the mud diapir, probably because of the limited opportunity to obtain the drilled core samples. During the CK09-01 *Chikyu* training cruise (Leg. 1), we have an opportunity to study the diapiric materials down to approximately 20 mbsf from the mud-diapir center (Site C9004) and the crater rim (Site C9005) close to *Calyptogena* colonies.

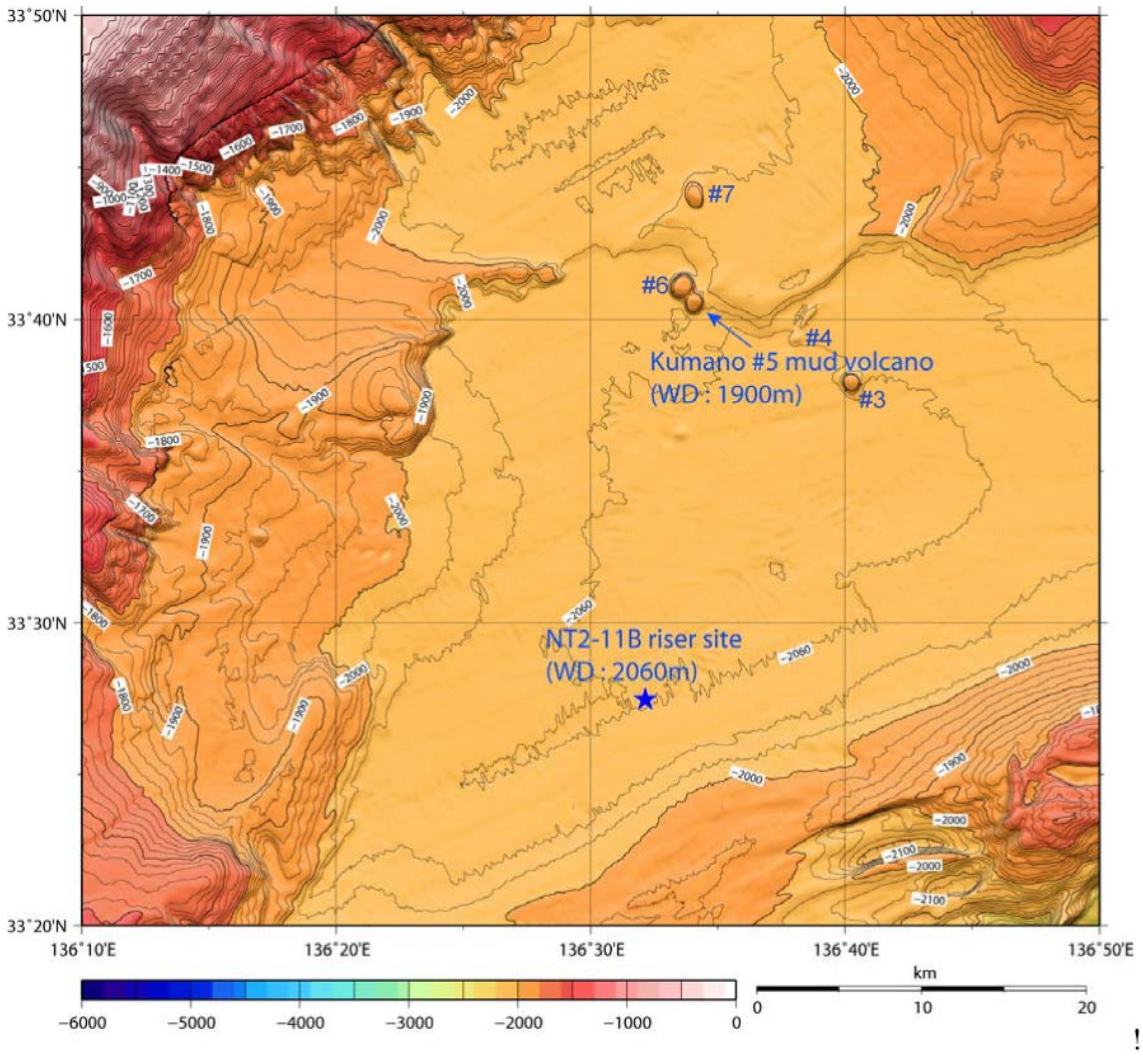
Our major scientific goals during this exploration are:

1) to understand the carbon and nitrogen biogeochemical cycles,
2) to understand the microbial population, community structure and diversity,
3) to understand the microbial metabolic rates and networks,
4) to retrieve the geochemical, biological, geological signatures reflecting the deep, high temperature zone close to the mud-diapiric origin,

5) to understand the formation process of the mud-volcano no. 5, and
6) to discuss the habitability of life in the deep realm associated with dehydration process.

To accomplish these scientific objectives, we perform multidisciplinary study including geology, organic and inorganic geochemistry, microbiology, and molecular biology using the cored sediments, and try to comprehend the unique environment as a window to the deep sub-seafloor biosphere.

Core Handling Flow and Site Summary



Location map of the mud volcanoes in the Kumano basin of the Nankai Trough.

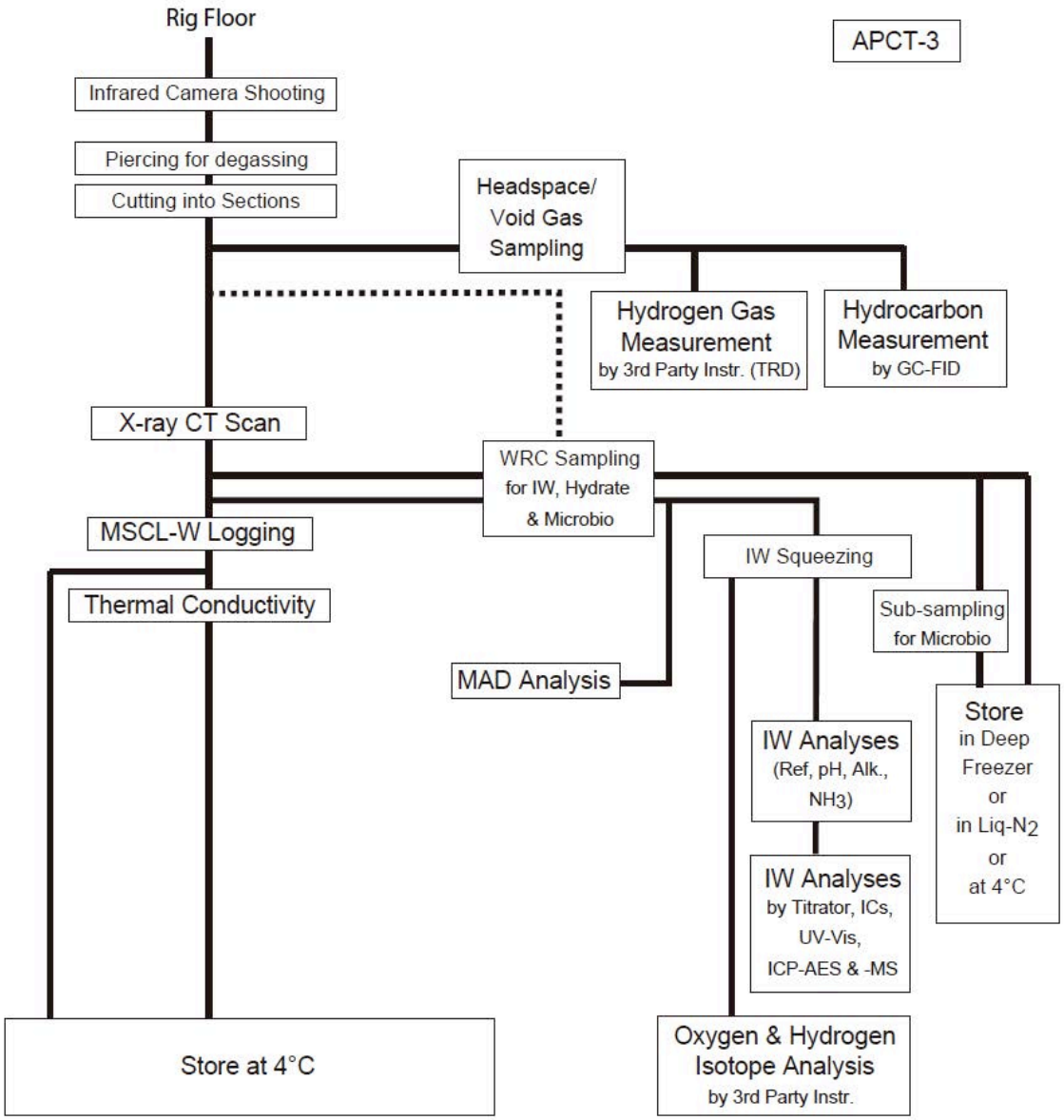
Core handling flow

Since the sediment core contains a lot of breccias with mud matrix, the vertical core splitting into working half and archive half is expected to be difficult to perform during the limited shipboard time. It may need a special tool or technique to make an undisturbed cutting surface, and time-consuming, thus we decided not to split core during the expedition time, instead, will transport the whole round core to the Kochi Core Center for the post-cruise processing. During the CK09-01 Leg 1 cruise at the Kumano basin, we tested coring with hydraulic piston coring system (HPCS). Since the surface sediments might contain high concentration of hydrogen sulfide (H_2S), we put cascade musk for processing safely on the core cutting area and rig floor, and released high-pressure gas by making small hales on the plastic liner to prevent core expansion. After core recovery on the cutting area, we immediately monitored temperature anomalies caused by methane hydrate-dissolution using thermo-view IR camera, resulting in the successful identification of the location of methane hydrates. After core sectioning and curational work on the core cutting area, we took samples directory from core-section ends for the headspace gas measurement (903HS), microscopic work (903FISH), and moisture and density measurement (903MUD). Then, we took XCT images of all the core sections and checked lithological features of the non-destructive WRCs, providing useful information for the subsequent WRC processing for geochemistry and microbiology. The WRC samples were collected in the QA/QC lab. During the WRC cutting, all cores were stored at reefer room to keep the cool temperature for living materials (i.e., microbial activities). The WRC samples were then distributed in geochemists and microbiologists for further sub-sampling steps and onboard analyses. The cores were then subjected to non-destructive routine measurements using MSCL-W (multi-sensor core logger), and finally stored at 4 ºC reefer. The cores will be sent to Kochi Core Center for further processing, MSCL measurements of the split cores, and sub-sampling for the additional geochemical and geological studies.

Core Flow & Measurement Plan for CK09-01 (Exp. 903)

Kumano Mud Volcano HPCS Coring

Science Party: Inagaki (KCC) *et al.*



Core processing on the core cutting area. All scientists and MWJ technicians working on the core cutting area put an air cascade on face because of the production of toxic hydrogen sulfide (H_2S) from the core materials.

Site C0004

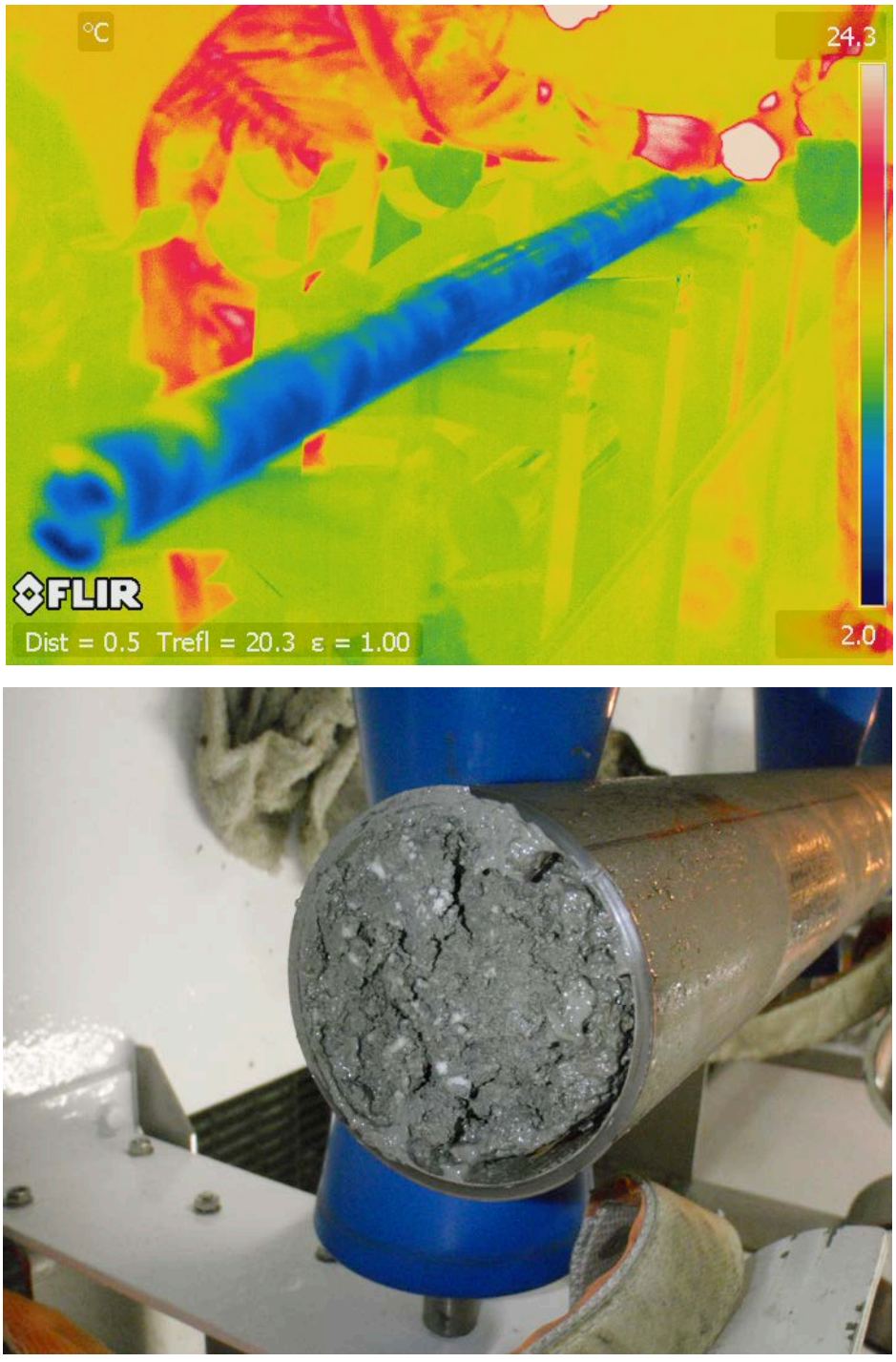
Site C0004 is located in the diapiric crater center of the mud-volcano no. 5. The water depth was 1896.7 m, and the site position was 136º 34.0851'E, 33º 40.5486'N. We started drilling on March 08 and recovered sediments down to 19.7 mbsf (Hole A, Core 1H-3H) by using hydraulic piston coring system (HPCS) during one-day coring operation. Since the production of H_2S from the surface sediments was expected, all the processing on the cutting area was performed with putting on the air-cascade musk. The core sediments were composed mainly of clay mud including consolidated to semi-consolidated sedimentary breccias. The core contained a lot of hydrocarbon gases; hence the cores were generally expanded with many small voids and some sediment portions were blowout from the core liner during the recovery. The temperature monitoring using a thermo-view IR camera revealed the presence of methane hydrates in several horizons at Site C0004. Several whitey massive particles of methane hydrates as well as a short WRC section were immediately stored in PFA cups with liquid nitrogen on the core cutting area, and processed to onboard geochemical analyses. The pore water chemical and dissolved gas analyses

revealed that chloride concentrations of the massive methane hydrates were extremely low (~4.7 mM) and C1/C2 values were notably higher than those in mud matrix sediments (<200 mM: see **Geochemistry**). The sediments were mostly sulfidic with low sulfate concentrations, indicating the occurrence of microbial anaerobic oxidation of methane (AOM) coupled to strong sulfate reduction according to the following equation: $CH_4 + SO_4^{2-} \rightarrow HCO_3^- + HS^- + H_2O$. Preliminary data of δD and $\delta^{18}O$ isotopic compositions of pore water (H_2O), which were measured onboard by an infrared isotopic mass spectrometry, indicated that, although the hydrate formation may somewhat disproportionate the isotopic values, the fluids are originally derived from the deep realm via the pressure-dehydrated clay minerals.

Site C0005

Site C0005 is located in the southwestern rim of the mud-volcano where the chemosynthetic *Calyptogena* colonies are spotty inhabiting sulfidic surface sediments associated with methane seepage. First, we tried to make Hole A close to a big *Calyptogena* colony at the water depth of 1886.7m (33º 40.5579' N, 136º 33.9396' E). However, the core barrel was broken presumably by the pipe swelling, thus the sediment recovery was unfortunately null. Then, we moved a few meters northeast and made Hole B at the water depth of 1887.7 m (33º 40.5615' N, 136º 33.9401' E), where several *Calyptogena* mussels dispersedly inhabited. Although we could have successfully recovered the surface core from Hole B, some upper portions of the surface sediments was unexpectedly blowout from the corer on the rig floor, potentially because of the dissolution of massive methane hydrates during the core recovery and pipe handling. Approximately 6m-sediment core was left on the core liner; however, the sediments were appeared to be terribly destructed by the gas explosion. Then, we slightly moved east and made Hole C again (1887.7 m in water depth, 33º 40.5615' N, 136º 33.9406' E), and retrieved the cores at almost the same point down to 13.2 mbsf using a combination of drilling and HPCS coring. The cored sediments were overall gassy with methane. We observed visible massive methane hydrates in the sediment matrix down to 12.7 mbsf (Hole D, Core 1H-5). The thermo-view IR camera revealed that the entire sediments in Hole D,

especially in Core 1H-4 and 5, widely contained methane hydrates. The two sections, 1H-4 and 1H5 in the depth range from 11.8 to 12.8 mbsf, were immediately transferred to the XCT-room and scanned the images before the hydrates melt. As far as we know, this is probably the first direct XCT analysis of natural hydrates in the mud diapir and other environments. The sediment cores down to 12 mbsf produced a lot of H_2S as the sensor always indicated red, suggesting the active microbial sulfate reduction coupled with methane (i.e., AOM) in the hydrate-bearing muddy sediments. Several pieces of massive hydrates and several hydrate-bearing sediment samples were quickly frozen by liquid nitrogen on the core cutting area, and preserved in PFA cup at -150 ºC for the subsequent geochemical and microbiological analyses. Interestingly, the core sediments below 13 mbsf from Hole E did not contain methane hydrates and sulfide. The XCT-scanned image showed a mass gradient structure of breccias, indicating the overflowing mud with breccias during the diapir formation process, which is a different sedimentological feature to the diapiric center at Site C0004 (see **Geology** section). The sulfate concentrations in the upper sedimentary unit above 13mbsf were very low or absent while the Hole E sediments contained over 25 mM, which is similar to the seawater. (see **Geochemistry** section) These results indicate that the outcrop of the mound top is highly permeable because of the mud-matrix-poor breccias, and hence secondary seawater circulation occurs, which may continuously supply oxidants (electron acceptors) such as oxygen or sulfate to microbes inhabiting the adjacent muddy environments associated with methane hydrates.



Thermo-view IR camera monitoring of negative temperature anomaly caused by the dissolution of methane hydrates. The photos show Core 1H-5 at Site C9005, Hole D, in which visible whitey massive hydrates were widely distributed.

Microbiology and Biogeochemistry

During the CK09-01 Leg 1 cruise at the Kumano mud volcano, we have processed core samples to store under appropriate conditions for shore-based microbiological and biogeochemical analyses. The quick sample processing is extremely important because most microbes in marine subsurface are most likely very sensitive to oxygen and high temperature, and once the microbes are dead the fragile bio-molecules such as RNA or intact polar lipids may be enzymatically degraded. Except for 903FISH and 903GH samples collected on the cutting area, all samples were collected as whole round core (WRC) in the QA/QC lab on the *Chikyu*, immediately after the XCT scanning. On behalf of the scientific party, the chief scientist made quick decisions for the WRC sampling location according to the core recovery, sampling depth interval, and lithology based on the XCT-scanned image. The sub-sampling procedure and the shore-based experimental plans for each sample are described as follows;

Sample Code: 903FISH. For microscopic works including cell enumeration and fluorescent in situ hybridization (FISH), 5 cm^3 of the innermost-core sediments was collected by a tip-cut sterilized syringe from all core section-ends and 903ACT samples. The sediments from the core section-end were collected immediately after the core splitting on the cutting area. The FISH samples from 903ACT were obtained in a lamina-flow clean bench of microbiology lab. 1 cm^3 of sediment was mixed with 9ml of 4% paraformaldehyde in phosphate buffer saline (PBS: pH 7.6), vortexed vigorously, and then incubated for 6-8 hours at 4ºC. If there are some consolidated sediment particles during the vortex mixing, we used an ethanol-wiped spatula for breaking the structure for the complete slurry. For 903FISH slurry, we used 15 ml round-end plastic centrifuge tube for easy mixing. After the fixation, the sediment slurry was centrifuged at 3,500*xg* for 10 min at 4ºC, discarded the supernatant, and added 9ml PBS for washing sediments (i.e, removal of excess paraformaldehyde). The sediments were voltexed again, and centrifuged at 3,500*xg* for 10 min at 4ºC. We performed this washing step twice. The final volume was adjusted at 10ml with PBS:ethanol solution (1:1), vortexed, and then stored at -20ºC. The sediment residue

(approximately 4 cm^3) was separately put into 15 ml plastic tube and stored at -80 ºC as the reference.

Sample Code: 903ACT. To study microbial metabolic activities, 20 cm WRC samples were collected in the QA/QC lab. 1 cm^3 of the sediments was first taken for microscopic works, and fixed as described above. The rest sediments were replaced into Ar-flushed autoclaved 1L glass bottle with ethanol-wiped spatulas. After the replacement, we additionally flushed the bottle with Ar for a few minutes to completely remove oxygen from the bottle. The reason why we use Ar for flushing instead of nitrogen is to prevent microbial nitrogen assimilation, which might be critical for subsequent isotope-labeled experiments. The sample bottle was sealed with a black butyl rubber stopper and a screw cap, and then stored at 4 ºC.

As for the shore-based experiments to measure the potential activity rates, we will perform some incubation experiments using ^{14}C and ^{35}S-labeled radiotracers for organic-consuming sulfate reduction rate, methane-consuming sulfate reduction rate, homo(CO_2-reducing)-methanogenesis rate, aceticlasitc methanogenesis rate, homo-acetogenesis rate, and acetate-oxidation rate. After the incubations with radiotracers, metabolic products such as sulfide, methane, and acetate will be extracted from the incubation slurry and the radioactivity will be measured by scintillation counter. We also study the potential assimilation activities using stable isotope (^{13}C and ^{15}N)-labeled substrates such as methane, formate, acetate, bicarbonate, glucose, pyruvate, ammonia, nitrate, nitrite, and amino acids for carbon and nitrogen sources. All the incubation experiments for the metabolic activities will be conducted using 903ACT sediment slurries, supplemented with anaerobic artificial water close to *in situ* salinity and pH conditions. After the incubations, substrate incorporation rates, population ratios, and the phylogenetic identifications will be studied using NanoSIMS (nano-scale secondary ion mass spectrometry) at single cell level as well as at biomarker level.

Sample Code: 903CULT. For the cultivation experiments, 5 cm to 10 cm WRC samples were collected in the QA/QC lab, immediately transferred to a clean

bench, and then the innermost-core sediments were put into autoclaved 250 ml glass bottle with continuous supply of nitrogen. The bottle was sealed with butyl rubber stopper, and then stored at 4 ºC. The trimmings of core surface were separately put into a zip-loc vinyl bag, and stored at -80 ºC, which will be used for the extraction of co-nutrient sources from the mud.

Cultivation provides direct evidences for the existence of active microbial components and sometimes results in isolation of new species, hence microbiologically very important. Previous cultivation studies of sub-seafloor microbes have suggested that the predominant microbial components, most of which are phylogenetically distinct from any previously known isolates, are highly resistant to the conventional batch-type cultivation technique or need long time for the growth. Using the 903CULT samples from the mud-volcano no. 5, we try to make enrichment cultures by using a flow-through-type reactor system and to reproduce *ex situ* acetate/bicarbonate-based microbial ecosystem. In addition, we try to cultivate thermophilic to hyperthermophilic microbes including archaea from the mud diapir samples, since the diapiric mud is derived from deep, hot sub-seafloor environments.

Sample Code: 903MBIO. For molecular (DNA and RNA) and biomarker analyses of the microbial community in the mud samples, 10 cm to 15 cm WRCs were collected in the QA/QC lab. The WRC was put into a zip-loc vinyl bag, and immediately stored at -80 ºC. For the better preservation of RNA, 50 cm^3 of innermost-core sediments were taken by an ethanol-wiped spatula and autoclaved 50 ml tip-cut syringe, put the sediment into an autoclaved 125 cc PFA (perfluoroalcoxyalkane) cup, added 50 ml RNA later solution (Ambion), and then stored at -80 ºC. The frozen WRC samples will be aseptically cut without melting using a newly developed electric scope saw system at JAMSTEC-Kochi for sharing the samples at exactly the same horizon between molecular and biomarker experiments.

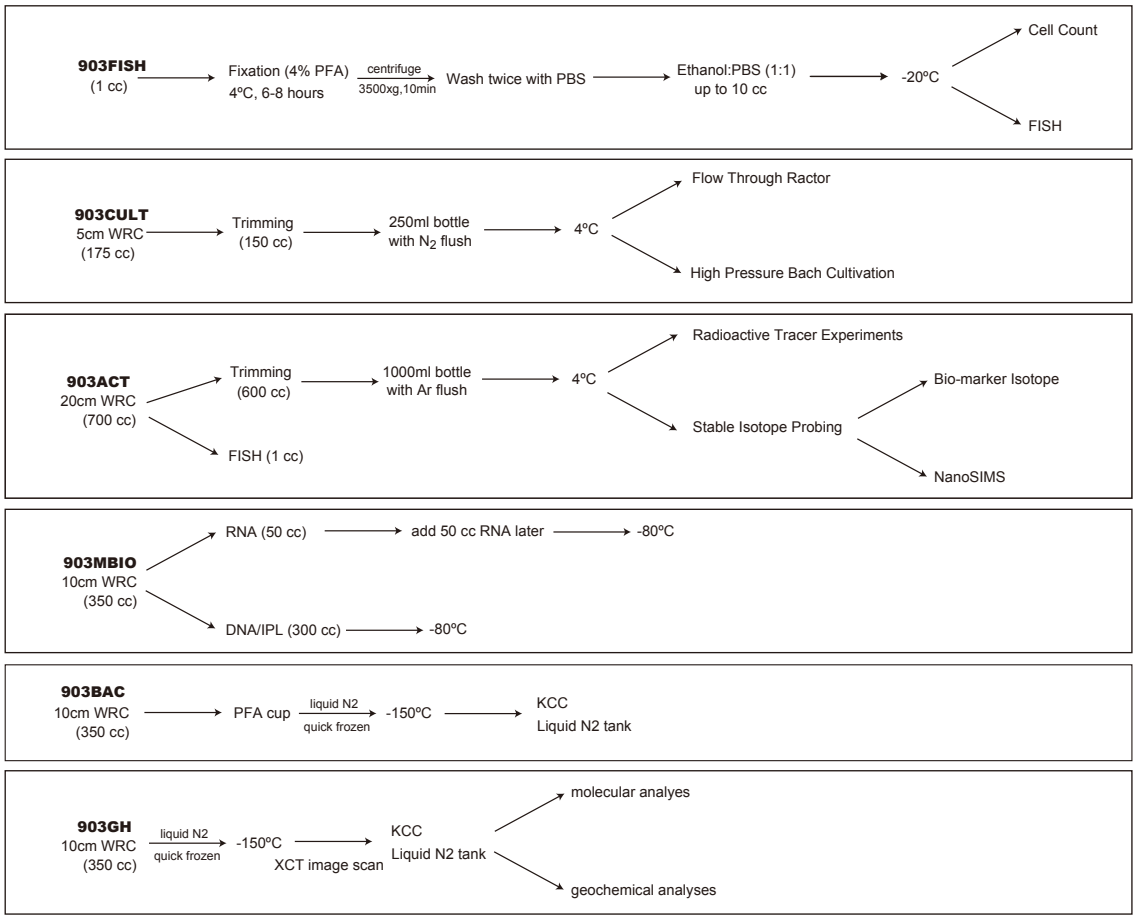
Our recent study of extracted DNA and intact polar lipids (IPLs) suggested that the sub-seafloor stratified sediments harbor a large population of heterotrophic

Archaea (Lipp et al., 2008). However, the microbial community structure and its metabolic functioning in the mud diapiric habitat from shallow to deep have remained completely unknown. Using 903MBIO samples, we extract bulk DNA/RNA and IPLs and quantify the amount of archaeal and bacterial 16S rRNA gene copy numbers and IPLs. The extracted DNA/RNA will be amplified with phi29 polymerase with careful contamination monitoring with real-time PCR. Using amplified environmental genomes, the copy numbers of some key functional genes will be surveyed (Inagaki et al., 2004): e.g., methyl-co-enzyme M reductase (*mcrA*) gene for methanogens and methanotrophs, dissimilatory sulfite reductase (*dsrAB*) gene and dissimilatory adenosine-5'-phsphosulfate rudcatase (*apsA*) gene for sulfate reducers, and formyl tetrahydrofolate synthetase (*fhs*) gene for acetyl-coA pathway. The genetic information will be useful to construct the potential metabolic network and ecosystem in the mud diapiric environment. If RNA is successfully extracted from metabolically active microbial assemblages, we try to study for the first time the functioning structure of whole amplified messenger-RNA (so called "metabolome" analysis) in sub-seafloor environment, if possible.

Sample Code: 903BAC. A few samples of 10cm WRC were collected in the QA/QC lab for the purpose of "bio-archive". To test the better preservation of RNA, we used 903BAC sample from the top 10 cm of Core 1H-1 at Site 9004 Hole A, and stored 25cm^3 surface sediments at different conditions as follows: 1) store at 4ºC, 2) store at -20 ºC, 3) store at -80 ºC, 4) store at -80 ºC with an equal volume of RNA later, 5) store at -20 ºC with an equal volume of acetone, 6) snap-freezing in liquid nitrogen (-196 ºC) and store at -80 ºC, and 7) snap-freezing in microwave freezer and stored at -80 ºC. The several other 903BAC samples were stored at -80 ºC in zip-loc vinyl bag. The bio-archive samples will be aseptically sub-sampled with an electric scope saw, and stored in liquid nitrogen tank (-160 ºC) according to the policy of JAMSTEC core curation.

Sample Code: 903GH. Because the existence of methane hydrates was expected based on the physical and geochemical condition of the mud-volcano

no. 5, we monitored the temperature anomalies caused by the dissolution of hydrates with Thermo-View IR camera on the cutting area as described above. We found over 20 horizons of the lower temperature anomalies at both Sites C0004 and C0005, indicating the widespread existence of methane hydrates in the mud volcano no. 5. We collected several hydrate horizons as WRC, put the hydrate-bearing sediment into the autoclaved PFA (perfluoroalcoxyalkane) cup, and then pored liquid nitrogen directory on the samples on the cutting area. The frozen hydrate samples were then stored at -150 ºC, which will be used for geochemical and molecular biological analyses.



A summary flow chart of the sample processing and the shore-based analyses for microbiology and biogeochemistry.

Geochemistry

A total of 20 and 4 WRC samples (**903IW**) were collected in the QA/QC lab for onboard interstitial water analyses at Sites 9004 and 9005, respectively. After the XCT-scan and WRC sampling, interstitial waters were squeezed from WRCs (10 to 55 cm in core length), and salinity, pH, alkalinity, chlorinity, Br, SO_4, Na, K, Mg, Ca, Sr, Ba, Mn, Li, B, Mn, Fe, Si, Sr, Ba, NH_4, V, Cu, Zn, Rb, Mo, Cs, Pb, and U concentrations as well as hydrogen and oxygen isotopic composition of water were analyzed onboard. Fore shore-based analyses such as iodine analyses, ~18.1 ml water sample was sub-sampled from each IW sample and stored under the appropriate conditions.

For the analyses of hydrocarbon and hydrogen gases, a total of 18 and 14 sediment samples (3 cm^3) were collected by tip-cut sterilized syringes at Sites 9004 and 9005, respectively, immediately after the core recovery on the core cutting area. Approximately 3 cm^3 sediment was placed in a 50 cm^3 glass vial with a degassed $HgCl_2$ solution, and then the vial was sealed with a Teflon coating butyl septum and a metal crimp cap.

At both Site C0004 and C0005, the cores contained gas hydrates at several horizons. 2 and 3 massive whitey gas hydrate samples (ca. 5-cm^3) stored in liquid nitrogen were used for chemical analyses at Sites 9004 and 9005, respectively. We thawed the hydrate sample in a 1000 cm^3 glass bottle filled with nitrogen inside and sealed with a butyl rubber septum. Then, the melted water and released gases in the bottle were extracted by a pipette and a gas-tight syringe, respectively.

Explanatory Notes for Onboard Chemical Analyses on Chikyu

Salinity was determined by a reflect meter. pH was determined by an electrode. Alkalinity and chlorinity were determined according to the routine titrimetric methods in the IODP. Br, SO_4, Na, K, Mg, and Ca were determined by an ion chromatography. Sr, Ba, Mn, Li, B, Mn, Fe, Si, Sr, and Ba were determined by

ICP-AES. V, Cu, Zn, Rb, Mo, Cs, and Pb were determined by ICP-MS. Hydrogen and oxygen isotopic compositions of water were determined by a laser (infrared) adsorption spectroscopy (Los Gatos Research, Inc.). Concentration of hydrocarbon and hydrogen gases was determined by GC-FID and GC-TRD, respectively.

The sub-sample processing and storage conditions for the onshore analyses were as follows:

1) Hydrogen isotopic composition of H_2 (Subsample Code: **903IWH**); as a pretreatment, $HgCl_2$ regent was supplemented into 20-cm^3 glass vial that was sealed with a Teflon-coated butyl septum and metal crimp cap. Then, 3ml water sample was placed into the vial via the septum by using a plastic syringe. The vial was stored at -20 ˚C.
2) Concentration of trace gases (CH_3Cl, COS, etc) (Subsample Code: **903IWTG**); $HgCl_2$ regent was supplemented into 20-cm^3 glass vial that was sealed with a butyl septum and metal cap. Then, 3 ml water sample was placed into the vial via the septum by using a plastic syringe. The vial was stored at -20 ˚C.
3) Concentrations and $\delta^{13}C$ of CO_2 and CH_4 (Subsample Code: **903IWC1**); 2 ml water sample was placed in 2-cm^3 glass vial with amidosulfuric acid and $HgCl_2$. The vial was sealed with a butyl septum and metal crimp cap and stored at room temperature.
4) Concentrations and $\delta^{13}C$ of DOC and acetate (Subsample Code: **903IWC2**); 4 ml water sample was placed into 10-cm^3 screw top glass vial. The glass vial was stored at -20 ˚C.
5) Concentration of iodine (Subsample Code: **903IWI1**); 1 ml water sample was placed into 4 ml high-density polyethylene screw top vial. The vial was stored at 5˚C.
6) Isotopic composition of iodine (Subsample Code: **903IWI2**); 5 ml water sample was placed into 8 ml high-density polyethylene screw top vial. The vial was stored at 5 ˚C.

Preliminary Results: Interstitial Water and Gas Hydrate

Site 9004

One of the unique characteristics of interstitial water in the mud volcano's core is its significantly low chlorinity relative to seawater (Fig. Geochemi-1a). The chlorinity values were ranging from 242 mM at 0.7 mbsf as maximum to 150 mM at 3 mbsf. Bellow the depth of 3 mbsf, the chlorinity showed little change, and averaged 130 mM. This value is 23% of chlorinity in seawater. Preliminary results of the stable isotopic compositions of the interstitial water showed ^{18}O-enriched and D-depleted values relative to those in seawater, indicating that the most fluids were derived from the dehydrate reaction of clay minerals in deeply buried marine sediments. The high concentrations of B (~13 mM) were observed in 903IW samples, consistently indicating that the fluids were derived from clay mineral dehydration at a temperature range from 60˚C to 150˚C (Haese et al., 2006). The SO_4 concentrations were overall very low (<1.5 mM), even in the shallowest depth of 0.7 mbsf, and decreasing in parallel with depth down to 3 mbsf, 0.5 mM (Fig. Geochemi-1b). The overall low concentrations of SO_4 indicate the occurrence of microbial sulfate reduction in the cored samples.

Chlorinity of the formation water of massive gas hydrate samples (903GH) was found to be extremely low as the minimum value at 4.7 mM (Fig. Geochemi-1a). The stable isotopic compositions of the water exhibited both ^{18}O- and D-enriched values, indicating that the gas hydrates are composed of the deeply rooted interstitial water in the sediment matrix because it is known that gas hydrates preferentially incorporate the heavier oxygen and hydrogen isotopes as its formation water (Kvenvolden and Kastner; 1990; Martin et al, 1996; Matsumoto and Browski, 2000).

Site 9005

The chemical characteristics of the interstitial waters at Site C0005 were identified as low chlorinity, high $\delta^{18}O$ and low δD, and high B contents, which were similar to those at Site C0004 and consistently indicated that the most fluids in the southeastern rim of the mud diapiric crater were derived from the deep sedimentary column. The chlorinity of the shallowest 903IW sample was

382 mM and fell down to 170 mM at 9.15 mbsf of Hole D (Fig. Geochemi-1a), in which the existence of gas hydrates was observed by the visual inspection and the Thermo-View IR camera. In clear contrast to the upper hydrate-bearing mud layers, the deeper core section at 12.92mbsf, Hole E (1H-1) contained high chloride (537mM) and SO_4 (28.8mM) concentrations in the interstitial water close to those in seawater (Fig. Geochemi-1b). The cored materials in Hole E were mainly composed of silty grains and breccias, hence the seawater may infiltrate into this sedimentary unit through the large pore spaces.

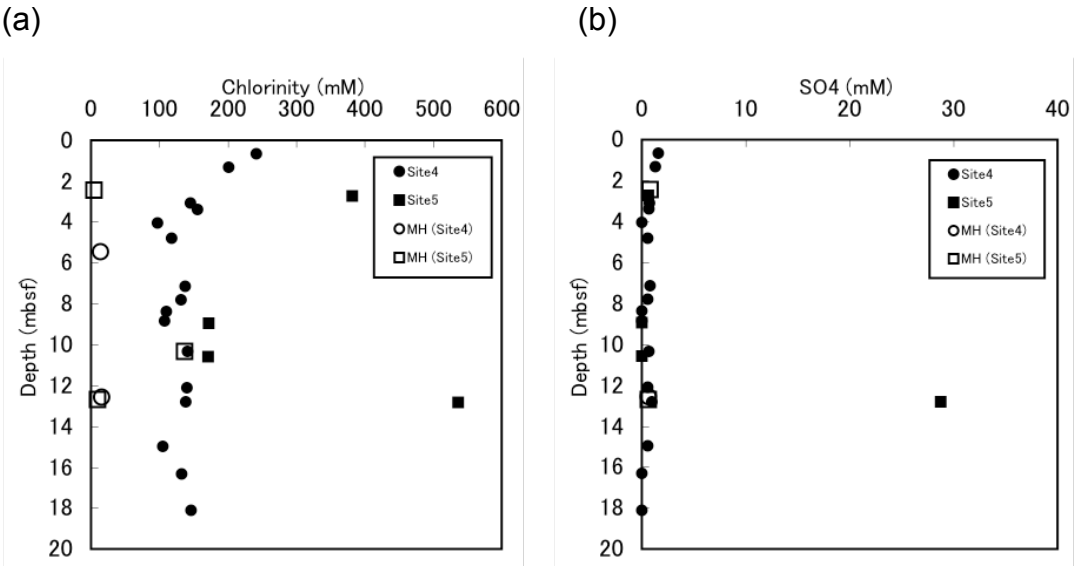


Fig. Geochemi-1. Vertical profile of (a) chlorinity and (b) SO_4 concentrations at Sites 9004 and 9005.

Preliminary Results: Gas compositions

Concentrations of CH_4, C_2H_6, H_2, and CO in the gas-phase of sediment samples (903HS) and gases from dissociated gas hydrate samples (903GH) were measured in the geochemistry lab of *Chikyu*. The presence of ethane and the low to intermediate C1/C2 ratios of 130 to 1500 for all the samples (Fig. Geochemi-2) suggests that the gasses at Sites C0004 and C0005 are the mixture of microbial and thermogenic origins. Noteworthy, a very low C1/C2 ratio less than 100 was observed in the gas hydrate sample at Site C0004, indicating a strong contribution of thermogenic gas in the hydrate sample; however, it is

unknown why such extremely low C1/C2 ratio occurs in the hydrate sample specifically and what is the effect of microbial contribution (i.e., ethanogenesis) on the gas composition associated with the hydrate sample. Conclusively, the results of gas-analysis indicate that the pore fluids are generally enriched in thermogenic gas transported from greater depths with some contributions of localized microbial activities in shallow habitats.

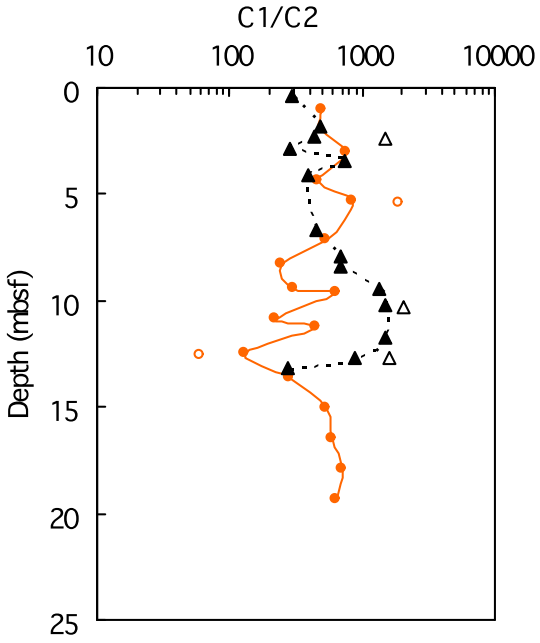


Fig. Geochemi-2. Vertical profiles of CH_4 to C_2H_6 (C1/C2) molecular ratios in headspace gas (903HS) at Site C0004 and C0005. Trends with depth for C1/C2 ratios at Site C0004 and Site C0005 are indicated by circler and triangle plots, respectively. X- and Y-axis represents C1/C2 ratio and sediment depth, respectively. The data for the gas from dissociated gas hydrate samples (903GH) are also plotted in the profile by open circles and open triangles for Site C0004 and Site C0005, respectively.

Shore-based geochemical study

The results from onboard analyses indicated that most fluids were derived from the dehydrate reaction of clay minerals in deeply buried marine sediments. It highly supports our expectation that the mud volcano is "**A Window to the Deep-Biosphere"**. To better understand the biogeochemical processes in the deep biosphere, we plan to do isotopic analyses for various materials such as acetate, CH_4, and ΣCO_2 etc., which play important roles for biogeochemical

carbon cycling naturally occurring in the Kumano mud volcano no. 5. In 2006, we retrieved a short piston core (1.5 m length) from around summit of the mud-volcano no. 5 during KH06-03 cruise of R/V Hakuho in 2006. The carbon isotopic compositions of acetate and DIC, DOC, and methane showed that the $\delta^{13}C$ values of acetate increased with increasing depth (from -41 to -21‰). Interestingly, the $\delta^{13}C$ values of ΣCO_2 consistently increased from -19 to +33‰. The parallel correlation of $\delta^{13}C$ values between acetate and ΣCO_2 is most likely caused by the acetogenesis, in which anaerobic bacteria (i.e., homo-acetogens) produce acetate via the reductive CO_2-pathway (i.e., acetyl-coA pathway). The extremely heavier shift of $\delta^{13}C$ of ΣCO_2 may indicate *in situ* consumption of CO_2 by autotrophic microbes such as methanogens and homo-acetogens; however, the CO_2-consuming microbiological characteristics have remained elusive. Since the 1.5 m-core recovered during the KH06-03 cruise was too short for the deep-biosphere research, the CK09-01 Leg 1 coring expedition provides us an unprecedented opportunity to study the continuing isotopic trends of key substrates for the carbon biogeochemical cycles in the deeper area of the mud volcano no. 5, which is our on going experimental effort as the shore-based geochemical experiments.

Geology

Moisture and Density Measurements (MAD) and Whole Round Multi-Scan Core Logger (MSCL-W) Measurements

Method: MAD measurement

Index properties of sediments were determined by measuring wet mass, dry mass, and dry volume. The method was followed by the standard procedure of the IODP MAD measurement (see Method chapter of IODP Proceedings 315). Approximately 5 cc of samples were taken for MAD from each section bottom. Sediment samples for headspace gas analysis were also obtained from the same locations. The water content data measured by MAD were shared with geochemists for the headspace gas measurement.

Index property measurements were conducted on 32 samples. After measurement of wet sediment mass, dry sediment mass and volume were measured after drying in a convection oven for 24 hours at a temperature of 105 ± 5 °C. Wet and dry masses were weighed using paired electronic balances and dry volume was measured using a helium-displacement pycnometer (Quantachrome Penta-Pycnometer). A constant 1.024 g/cm^3 was used on calculation for pore fluid density.

Method: MSCL-W measurements

MSCL is composed of the following four logging systems for unsplit core: Gamma Ray Attenuation (GRA) Density, Magnetic Susceptibility (MS), Natural Gamma Radiation (NGR) and P-Wave Velocity (PWV) tools. These measurements are conducted routinely as a standard measurement item for IODP. The method used on this study is followed by the standard procedure of the IODP MSCL-W measurement (see Method chapter of IODP Proceedings 315).

Method: Thermal Conductivity measurement

All recovered cores from mud volcanoes are highly disrupted due to expansion of gassy sediments. We abandoned thermal conductivity measurements

because a large number of tiny bubbles severely affect on thermal conductivity values.

Results of MAD and MSCL-W measurements

Physical property of sediment is one of the important factors recording states of compaction and digenesis. Mud volcanoes, targets of our study, are basically formed by buoyancy of under-consolidated muddy sediment with reversed density to the surrounding formation. Therefore, physical properties of erupted sediments are significant for understanding formation processes of mud volcanoes. We obtained whole round MSCL data and index properties of discrete samples onboard.

Muddy matrix sediments were mainly collected from section ends using 12 ml syringe for MAD measurements although core samples include various sized clasts. 18 samples were measured down to 20 mbsf at Site C9004. One sample is exceptionally a semi-consolidated mud clast. At Site C9005, MAD data were obtained on 14 samples. We observed a small piece of methane hydrate on one sample at about 6.5 mbsf. The sample from the Hole E bottom at about 13 mbsf is characterized by clast-bearing muddy sediment, Bulk density, porosity and grain density from MAD measurements are shown in Figures MAD-1 and MAD-2. These figures also show results of bulk density derived from gamma ray attenuation and magnetic susceptibility by MSCL measurements.

Bulk density at Site C9004 shows almost constant values from the seafloor to 20 mbsf (Figure MAD-1). Grain density also indicates no systematic trend with depth. Variations of porosity basically well correspond to changes of bulk density. The MAD data from a clast sample were characterized by high density and low porosity. This suggests that the clast was derived from nearby deeper semi-consolidated host-rock.

Bulk density and porosity at Site C9005 indicate constant values from the seafloor to 9 mbsf (Figure MAD-2). Data from 9 to 13 mbsf show considerably lower bulk density and higher porosity values than those from the shallower sequence. Such soupy sediments indicate either the dissociation of methane hydrate or the severe mixing with seawater by coring disturbance although hydrate itself was not recognized in these MAD samples. Grain density indicates

no systematic trend with depth. Clast-bearing mud form the bottom was characterized by high density and low porosity due to existence of consolidated host-rock pieces.

Bulk density values estimated from MSCL at Sites C9004 and C9005 are much lower than those from MAD measurements (Figures MAD-1 and MAD-2). This discrepancy was caused by insufficient sediment fillings of core liners because these data were calculated as full sediment fillings in core liners. Magnetic susceptibility data from MSCL, which have similar patterns to bulk density from MSCL, also didn't exhibit correct changes with depth, but just filling ratios in core liners. We need volume correction on MSCL data for further discussion on bulk density and magnetic susceptibility.

Voids in core liners severely affect on P-wave velocity measurements on MSCL. Heterogeneity of voids in core liner make impossible to obtain correct electrical resistivity by MSCL. Therefore, we took no account of results from these measurements.

In conclusion, there is no systematic trend on muddy matrix samples by MAD measurements at both drilling sites. This result suggests the diapirism of low-density mud and little modification by compaction after the diapirism. MAD data of clasts are limited, but significant for estimating depth of host rock formation. One of important shore-based studies is further MAD measurement of clasts from split cores.

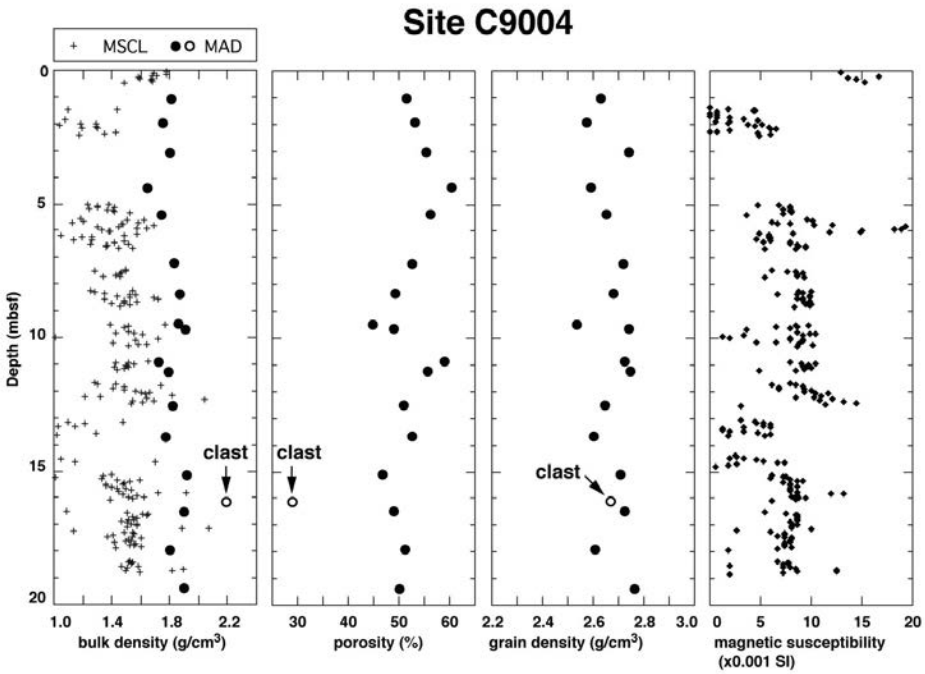


Fig. MAD-1. Bulk density, porosity and grain density from MAD measurement, and bulk density derived from gamma ray attenuation and magnetic susceptibility by MSCL measurement at Site C9004.

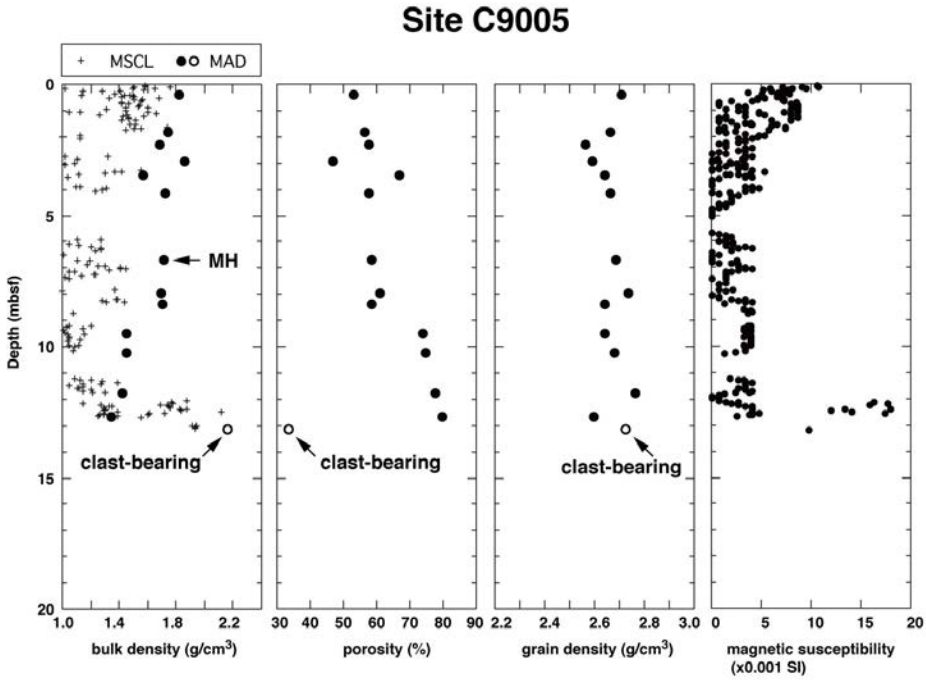


Fig. MAD-2. Bulk density, porosity and grain density from MAD measurement, and bulk density derived from gamma ray attenuation and magnetic susceptibility by MSCL measurement at Site C9005.

Visual and X-ray CT (CAT) scan geological observation

During the CK09-01 (Leg 1) cruise at the Kumano mud volcano no. 5, all of the cores were scanned by the General Electric Healthcare Light Speed Ultra 16 X-ray computer tomographic scanner installed in the core laboratory of *Chikyu*. The supplemented protocol no. 6.6 was used throughout the scanning works. The main specifications of this protocol were as follows:

Scan type: Helical Full scan by 0.5 sec rotation time

Helical thickness: 0.625 mm, SFOV: small, DFOV: 9.6 cm

X-ray source tube voltage: 120 kV, current: 100 mA

We tentatively analyzed the XCT-scanned data using OsirX ver. 3.3.2 software connected to the XCT-DICOM on the core processing deck of *Chikyu*.

Site C0004

The cores at Site C0004, the central part of summit crater of the Kumano mud volcano no. 5, were composed of consolidated to semi-consolidated mud rock fragments within the matrix mud. Several millimeter-sized spherical voids were predominant throughout the core images, indicating the gas separation from interstitial water at and near the surface. Thermo-View IR camera imaging of the core liner surface on the core cutting area clearly indicated the presence of gas hydrates, which are presumably composed of methane. The CT images showed that the size and fragments of mud rock clasts become bigger as the depth increased. Below the bottom of the core 2, the piston cored off some bigger mud fragments, which filled the entire core in the section images. These are the evidence that the expelled mud was composed of not only fluidal mud but also harder fragments of mudstone, which were the main components of building cone-shape structure under the water. Hence, it was assumed that the fluidal mud could not produce convex upward structure on the seafloor without the harder mud rock fragments.

Site C0005

The main constituents (i.e., mud rock fragments and matrix mud) of the cores at Site C9005 were similar to those at the C9004, as well as spherical voids in

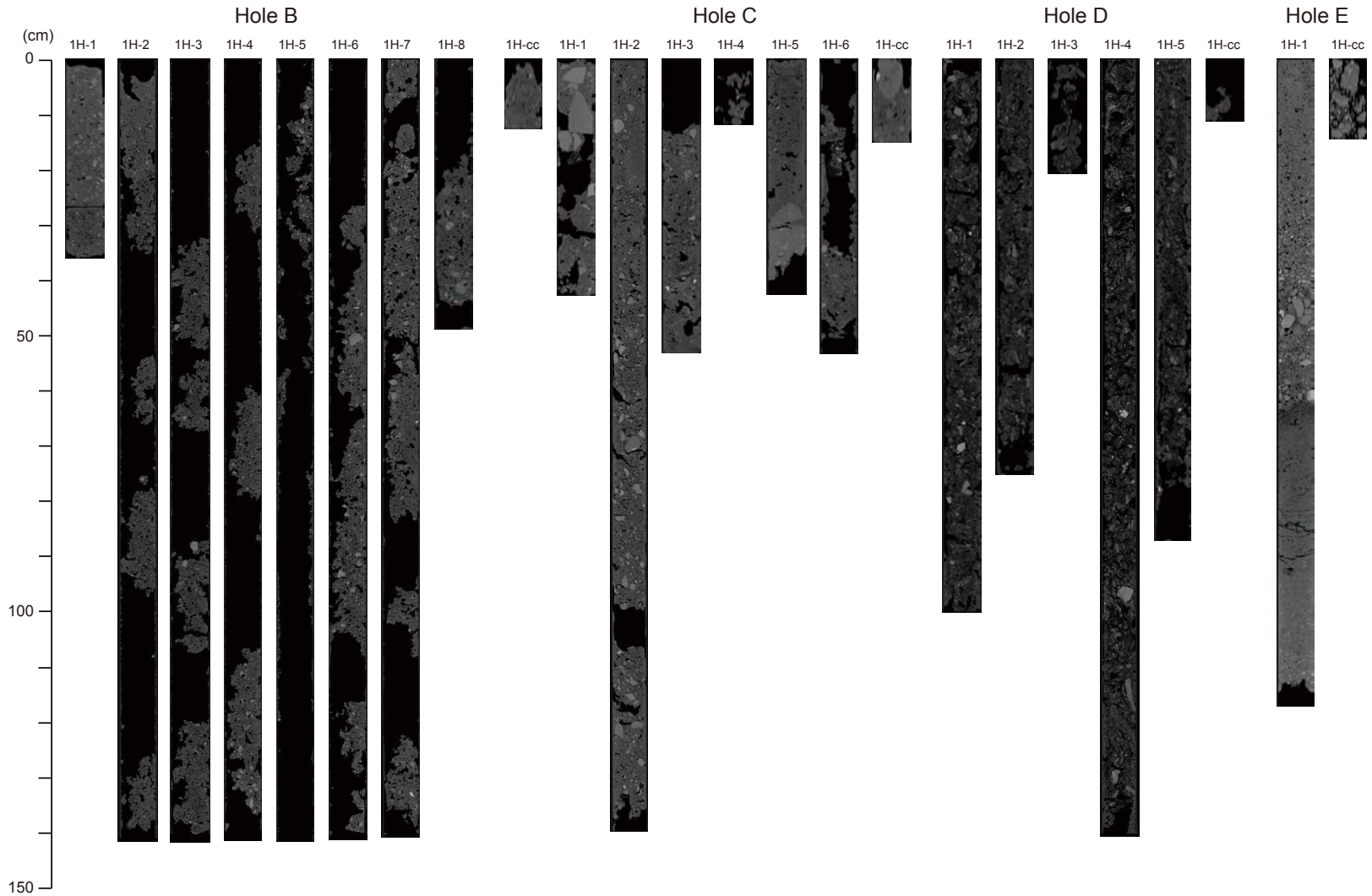
various degrees. The mud rock fragments at Site C9005 were relatively smaller than those at Site C9004. Gas-hydrates were abundant at Site C0005, enabled us to make a visual inspection of gas-hydrate texture on the CT images. In Core 1 of Hole E, two sequences of graded bedding of fine sand to cobble size mud clasts and fragments were observed on the CT images. The upper sequence of graded bedding contained some mollusk shell fragments. Both graded bedding and shell fragments indicated that these units in the Hole E were reworked sediments of chemosynthetic colonies on the expelled mud.

Because we did not cut the cores in half on board, the observation was limited and was far from full lithological description of the cores, which will be performed at the Kochi Core Center as the post-cruise study.

Additional remarks: The other drilled cores at Site NT2-11 (for Geotech study) during the CK09-01 *Chikyu* training cruise have lots of small partings and cracks in mud section at the interval of several mm to several cm along the cores, which were most likely produced by piston coring. This low core quality hinders digital data processing of CT images as well as most data analysis of physical property measurements such as gamma attenuation and P-wave velocity. The core quality should have to be improved for future science programs to maximize the usefulness of high-tech and modern instrumental facilities of *Chikyu* for advanced science.

CK09-01 Leg 1 Kumano Mud Volcano No. 5 Site C0004 XCT-Scan Image

CK09-01 Leg 1 Kumano Mud Volcano No. 5 Site C0005 XCT-Scan Image



References

Dählmann A, De Lange GJ. (2003) Fluid-sediment interactions at Eastern Mediterranean mud volcanoes: a stable isotpe study from ODP Leg 160. *Earth Plan. Sci. Lett.* **212**: 377-391.

De Lange GJ., Brumsack HJ. (1998) Pore-water indications for the occurrence of gas hydrates in Eastern Mediterranean mud dome structure. *Proc. ODP Sci. Res.* **160**: 569-574.

D'Hondt S, Jørgensen BB, Miller DJ, Batzke A, Blake R, Cragg BA, et al. (2004) Distributions of microbial activities in deep subseafloor sediments. *Science* **306**: 2216-2221.

D'Hondt S, Inagaki F, Ferdelman T, Jørgensen BB, Kato K, Kemp P, et al. (2007) Exploring subseafloor life with the Integrated Ocean Drilling Program. *Sci. Drilling* **5**: 26-37.

Etiope G, Feyzullayev A, Milkov AV, Waseda A, Sun CH. (2009) Evidence of subsurface anaerobic biodegradation of hydrocarbons and potential secondary methanogenesis in terrestrial mud volcanoes. *Mar. Petrol. Geol.* In press. (doi:10.1016/j.marpetgeo.2008.12.002).

Ginsburg GD, Milkov AV, Soloviev VA, Egorov AV, Cherkashev GA, Vogt PR, et al. (1999) Gas hydrate accumulation at the Haakon Mosby mud volcano. *Geo-Mar. Lett*. **19**: 57-67.

Haese RR, Hensen C, De Lange GJ. (2006) Pore water geochemistry of eastern Mediterranean mud volcanoes: Implications for fluid transport and fluid origin. *Marine Geology* **225**: 191-208.

Hensen C, Wallman K, Schmidt M, Ranero CR, Suess E. (2004) Fluid expulsion related to mud extrusion of Costa Rica-A window to the subduction slab. *Geology* **32**: 201-204.

Hensen C, Nuzzo M, Hornibrook E, Pinheiro LM, Bock B, Magalhães VH, Brückmann W. (2007) Sources of mud volcano fluids in the Gulf of Cadiz-indications for hydrothermal imprint. *Geochim. Cosmochim. Acta* **71**: 1232-1248.

Inagaki F, Suzuki M, Takai K, Oida H, Sakamoto T, Aoki K, et al. (2003) Microbial communities associated with geological horizons in coastal subseafloor sediments from the Sea of Okhotsk. *Appl. Environ. Microbiol.* **69**: 7224-7235.

Inagaki F, Tsunogai U, Suzuki M, Kosaka A, Machiyama H, Takai K, Nunoura T, Nealson KH, Horikoshi K. (2004) Characterization of C1-metabolizing prokaryotic communities in methane seep habitats at the Kuroshima Knoll, southern Ryukyu arc, by analyzing *pmoA, mmoX, mxaF, mcrA*, and 16S rRNA genes. *Appl. Environ. Microbiol*. **70**: 7445-7455.

Inagaki F, Nunoura T, Nakagawa S, Teske A, Lever M, Lauer A, et al. (2006) Biogeographical distribution and diversity of microbes in methane hydrate-bearing deep marine sediments on the Pacific Ocean Margin. *Proc. Natl. Acad. Sci. U.S.A.* **103**: 2815-2820.

Inagaki F, Nakagawa S. (2008) Spatial distribution of the subseafloor life: Diversity and biogeography. *In* Dilek Y, Furnes H, Muehlenbachs (eds.) Links Between Geographical Processes, Microbial Activities & Evolution of Life. Springer Science+Business Media B. V., pp. 135-158.

Kvenvolden KA, Kastner M (1990) Gas hydrates of the Peruvian outer continental margin. *Proc. ODP Sci. Res.* **112**: 517-526.

Kopf AJ. (2002) Significance of mud volcanism. *Rev. Geophys.* **40**: 1005 (doi:10.1029/2000RG000093).

Lipp JS, Morono Y, Inagaki F, Hinrichs K.-U. (2008) Significant contribution of Archaea to extant biomass in marine subsurface sediments. *Nature* **454**: 991-994.

Martin JB, Kastner M, Henry P, LePichon X, Lallement S. (1996) Chemical and isotopic evidence for sources of fluids in a mud volcano field seaward of the Barbados accretionary wedge. *J. Geophys. Res.-Solid Earth* **101**(B9): 20325-20345.

Matsumoto R, Browski WS (2000) gas hydrate estimates from newly determined oxygen isotopic fractionation ($a_{GH\text{-}IW}$) and $\delta^{18}O$ anomalies of the interstitial waters: Leg 164, Blake Ridge. *Proc. ODP Sci. Res.* **164**: 59-66.

Mazurenko LL, Soloviev VA, Gardner JM, Ivanov MK. (2003) Gas hydrates in the Ginsburg and Yuma mud volcano sediments (Moroccan Margin): results of chemical and isotopic studies of pore water. *Mar. Geol.* **195**: 201-210.

Milkov AV. (2000) Worldwide distribution of submarine mud volcanoes and associated gas hydrates. *Mar. Geol.* **167**: 29-42.

Morono Y, Terada T, Masui N, Inagaki F. (2009) Discriminative detection and enumeration of microbial life in marine subsurface sediments. *ISME J* in press (doi:10/1038/ismej2009.1).

Niemann H, Lösekann T, de Beer D, Elvert M, Nadalig T, Knittel K, et al. (2006) Novel microbial communities of the Haakon Mosby mud volcano and their role as a methane sink. *Nature* **443**: 854-858.

Parkes RJ, Cragg BA, Bale SJ, Getliff JM, Goodman K, Rochelle PA, et al. (1994) Deep bacterial biosphere in Pacific Ocean sediments. *Nature* **371**: 410-413.

Parkes RJ, Cragg BA, Wellsbury P. (2000) Recent studies on bacterial populations and processes in subseafloor sediments: A review. *Hydrogeol. J.* **8**: 11-28.

Roussel EG, Bonavita MC, Querellou J, Cragg BA, Webster G, Prieur D. et al. (2008) Extending the sub-sea-floor biosphere. *Science* **320**: 1046.

Schimidt M, Hensen C, Mörz T, Müller C, Grevemeyer I, Wallmann K, Mau S, Kaul N. (2005) Methane hydrate accumulation in “Mound 11” mud volcano, Costa Rica forearc. *Mar. Geol.* **216**: 83-100.

Whitman WB, Coleman DC, Wiebe WJ. (1998) Prokaryotes: The unseen majority. Proc. *Natl. Acad. Sci. U.S.A.* **95**: 6578-6583.

Appendix 1. Curational Section Summary

Expedition	903	Site	C9004A		
Expedition name	CK09-01				
Section Summary					
Section	Section curated length (m)	Top Depth [mbsf]	Bottom Depth [mbsf]	Top Depth [mbsf, CMP]	Bottom Depth [mbsf, CMP]
C9004A-1H-1	0.51	0	0.51	0	0.479
C9004A-1H-2	0.62	0.51	1.13	0.479	1.062
C9004A-1H-3	0.95	1.13	2.08	1.062	1.956
C9004A-1H-4	1.16	2.08	3.24	1.956	3.046
C9004A-1H-5	1.41	3.24	4.65	3.046	4.372
C9004A-1H-6	1.09	4.65	5.74	4.372	5.397
C9004A-1H-CC	0.11	5.74	5.85	5.397	5.5
C9004A-2H-1	0.74	5.5	6.24	5.5	6.091
C9004A-2H-2	1.41	6.24	7.65	6.091	7.217
C9004A-2H-3	1.41	7.65	9.06	7.217	8.342
C9004A-2H-4	1.425	9.06	10.485	8.342	9.48
C9004A-2H-5	0.235	10.485	10.72	9.48	9.668
C9004A-2H-6	0.13	10.72	10.85	9.668	9.772
C9004A-2H-7	1.41	10.85	12.26	9.772	10.897
C9004A-2H-8	0.465	12.26	12.725	10.897	11.269
C9004A-2H-CC	0.415	12.725	13.14	11.269	11.6
C9004A-3H-1	0.93	11.6	12.53	11.6	12.527
C9004A-3H-2	1.17	12.53	13.7	12.527	13.692
C9004A-3H-3	1.42	13.7	15.12	13.692	15.107
C9004A-3H-4	1.41	15.12	16.53	15.107	16.512
C9004A-3H-5	1.415	16.53	17.945	16.512	17.922
C9004A-3H-6	1.475	17.945	19.42	17.922	19.391
C9004A-3H-CC	0.31	19.42	19.73	19.391	19.7

Note: [mbsf, CMP] means a depth below sea floor by composite depth. In general, soft-sediment cores expand after retrieving, so that a depth of core-bottom often becomes deeper than that of the next core-top, defined by Operation group. So, the length of the former cores is recalculated to fit to the depth of next core-top.]

Expedition	903	Site	C9005B		
Expedition name	CK09-01				
Section Summary					
Section	Section curated length (m)	Top Depth [mbsf]	Bottom Depth [mbsf]	Top Depth [mbsf, CMP]	Bottom Depth [mbsf, CMP]
C9005B-1H-1	0.365	0	0.365	0	0.232
C9005B-1H-2	1.41	0.365	1.775	0.232	1.129
C9005B-1H-3	1.41	1.775	3.185	1.129	2.026
C9005B-1H-4	1.41	3.185	4.595	2.026	2.924
C9005B-1H-5	1.41	4.595	6.005	2.924	3.821
C9005B-1H-6	1.41	6.005	7.415	3.821	4.718
C9005B-1H-7	1.405	7.415	8.82	4.718	5.612
C9005B-1H-8	0.49	8.82	9.31	5.612	5.924
C9005B-1H-CC	0.125	9.31	9.435	5.924	6.003

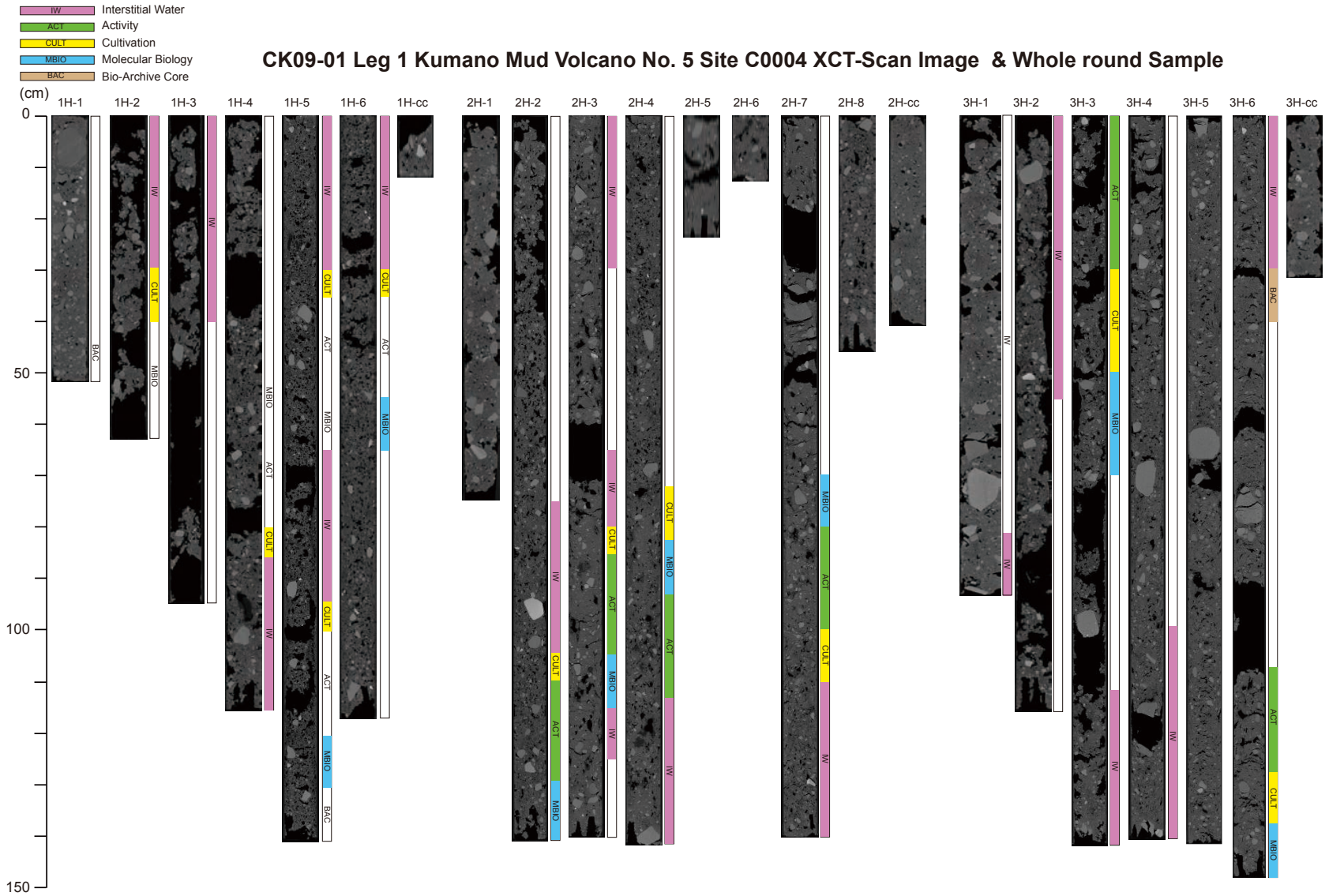
Note: [mbsf, CMP] means a depth below sea floor by composite depth. In general, soft-sediment cores expand after retrieving, so that a depth of core-bottom often becomes deeper than that of the next core-top, defined by Operation group. So, the length of the former cores is recalculated to fit to the depth of next core-top.]

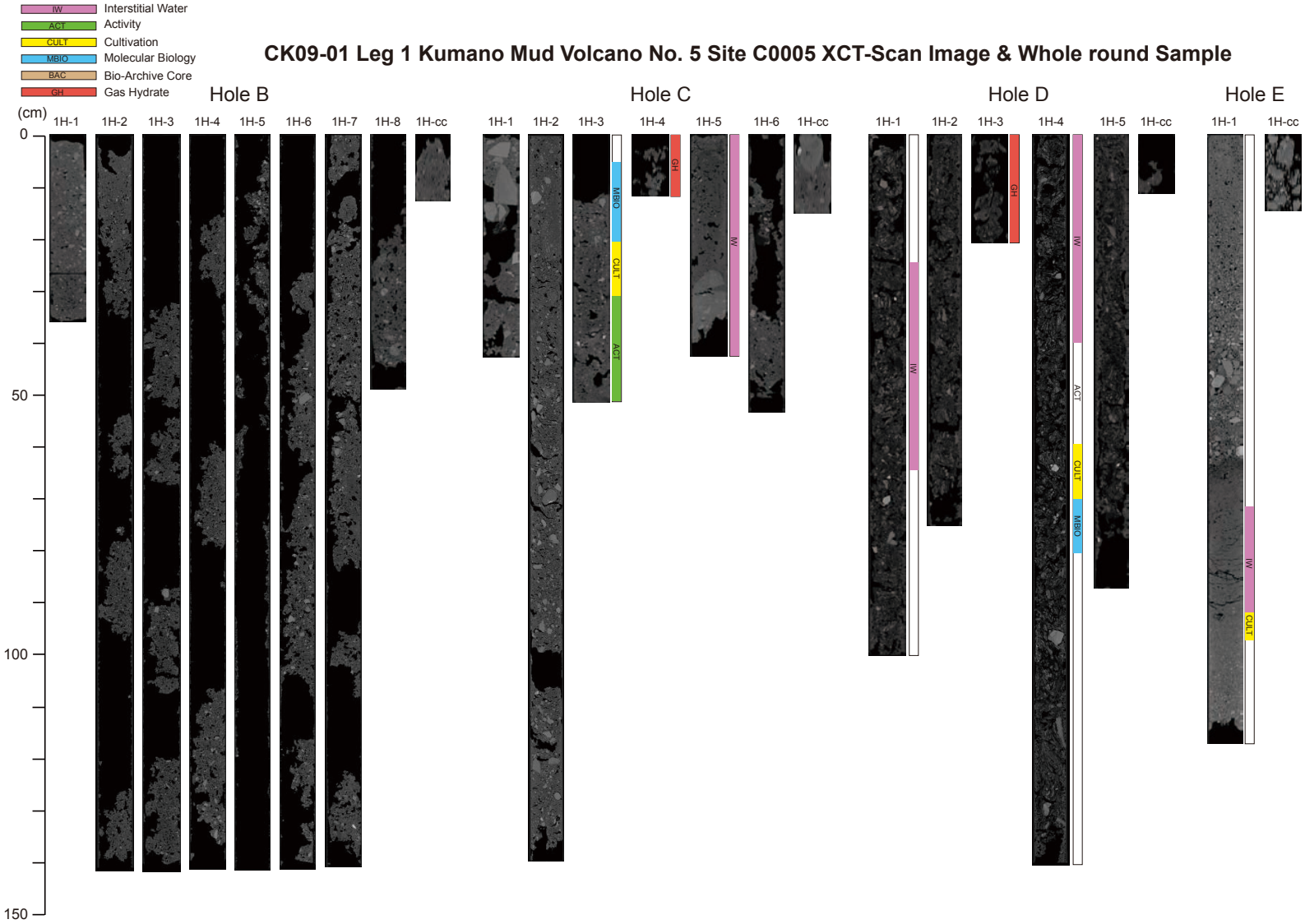
Expedition	903	Site	C9005C		
Expedition name	CK09-01				
Section Summary					
Section	Section curated length (m)	Top Depth [mbsf]	Bottom Depth [mbsf]	Top Depth [mbsf, CMP]	Bottom Depth [mbsf, CMP]
C9005C-1H-1	0.42	0	0.42	0	0.42
C9005C-1H-2	1.4	0.42	1.82	0.42	1.82
C9005C-1H-3	0.52	1.82	2.34	1.82	2.34
C9005C-1H-4	0.18	2.34	2.52	2.34	2.52
C9005C-1H-5	0.41	2.52	2.93	2.52	2.93
C9005C-1H-6	0.53	2.93	3.46	2.93	3.46
C9005C-1H-CC	0.14	3.46	3.6	3.46	3.6
Note: [mbsf, CMP] means a depth below sea floor by composite depth. In general, soft-sediment cores expand after retrieving, so that a depth of core-bottom often becomes deeper than that of the next core-top, defined by Operation group. So, the length of the former cores is recalculated to fit to the depth of next core-top.]					

Expedition	903	Site	C9005D		
Expedition name	CK09-01				
Section Summary					
Section	Section curated length (m)	Top Depth [mbsf]	Bottom Depth [mbsf]	Top Depth [mbsf, CMP]	Bottom Depth [mbsf, CMP]
C9005D-1H-1	1.01	8.5	9.51	8.5	9.51
C9005D-1H-2	0.745	9.51	10.255	9.51	10.255
C9005D-1H-3	0.13	10.255	10.385	10.255	10.385
C9005D-1H-4	1.405	10.385	11.79	10.385	11.79
C9005D-1H-5	0.88	11.79	12.67	11.79	12.67
C9005D-1H-CC	0.1	12.67	12.77	12.67	12.77
Note: [mbsf, CMP] means a depth below sea floor by composite depth. In general, soft-sediment cores expand after retrieving, so that a depth of core-bottom often becomes deeper than that of the next core-top, defined by Operation group. So, the length of the former cores is recalculated to fit to the depth of next core-top.]					

Expedition	903	Site	C9005E		
Expedition name	CK09-01				
Section Summary					
Section	Section curated length (m)	Top Depth [mbsf]	Bottom Depth [mbsf]	Top Depth [mbsf, CMP]	Bottom Depth [mbsf, CMP]
C9005E-1H-1	1.175	12	13.175	12	13.076
C9005E-1H-CC	0.14	13.175	13.315	13.076	13.205

Note: [mbsf, CMP] means a depth below sea floor by composite depth. In general, soft-sediment cores expand after retrieving, so that a depth of core-bottom often becomes deeper than that of the next core-top, defined by Operation group. So, the length of the former cores is recalculated to fit to the depth of next core-top.]


IW Interstitial Water
ACT Activity
CULT Cultivation
MBIO Molecular Biology
BAC Bio-Archive Core
CK09-01 Leg 1 Kumano Mud Volcano No. 5 Site C0004 XCT-Scan Image & Whole round Sample
(cm)
0
50
100
150
1H-1
1H-2
1H-3
1H-4
1H-5
1H-6
1H-cc
2H-1
2H-2
2H-3
2H-4
2H-5
2H-6
2H-7
2H-8
2H-cc
3H-1
3H-2
3H-3
3H-4
3H-5
3H-6
3H-cc


IW Interstitial Water
ACT Activity
CULT Cultivation
MBIO Molecular Biology
BAC Bio-Archive Core
GH Gas Hydrate
CK09-01 Leg 1 Kumano Mud Volcano No. 5 Site C0005 XCT-Scan Image & Whole round Sample
Hole B
Hole C
Hole D
Hole E
(cm)
0
50
100
150
1H-1
1H-2
1H-3
1H-4
1H-5
1H-6
1H-7
1H-8
1H-cc
MBIO
CULT
ACT
GH
IW