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Citation	(2018-08-29)
Issue Date	2018-08-29
URL	<a href="http://hdl.handle.net/10069/38661">http://hdl.handle.net/10069/38661</a>
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This document is downloaded at: 2019-09-17T00:47:50Z

Studies on temporal changes in the sediment oxygen consumption and  
bacterial community structure in a seasonally hypoxic enclosed bay,  
Omura Bay

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Seasonal formation of oxygen-depleted water masses in bottom environments is a widespread phenomenon in coastal areas around the world. Dissolved oxygen (DO) depletion in bottom water is lethal to macrobenthic animals and eliminates sensitive species, while hypoxic conditions would enhance microbial heterotrophic activity and hence diversion of energy-flow into the microbial food web. In general, microbial respiration is responsible for the depletion of DO, and its availability in turn exerts fundamental changes in the respiratory metabolism, thereby drives shift in microbial community structure in aquatic ecosystem. Omura Bay, the study area of this investigation, is a shallow enclosed bay that experiences severe bottom water hypoxia (less than  $<3$  mg O<sub>2</sub>/L) every summer from mid-June through September. Strong wind force associated with a typhoon or low pressure system occasionally enhances vertical mixing and thus transiently increases DO level in the bottom water to a normoxic condition in the middle of hypoxia. However, the DO level would be often brought back to hypoxic condition fairly rapidly. Sediment oxygen consumption (SOC) mediated by sediment microbial community in the center region of the bay has been believed to play a fundamental role in the formation of basin-wide hypoxia (Wada *et al.*, 2012) and is also likely to contribute to buffering such a transient increase in DO level during hypoxia (Mori *et al.*, 2015). However, little has been known about temporal dynamics of SOC and the responsible microbial community. In order to gain quantitative insights into how sediment microbial community respiration and hypoxia interact with each other, and how seasonal bottom hypoxia would affect the sediment microbial (bacterial) community richness and structure, a series of sediment sampling and monitoring of bottom-water hypoxia were conducted in Omura Bay for three consecutive years (2011 – 2013).

An *in vivo* ETSA method (Wada *et al.*, 2012) was used to demonstrate potential SOC in the samples. This method is based on the measurement of reduction rate of a tetrazolium compound (INT) in samples in either the presence or the absence of a fixative (formalin) to infer the extent to which whole sediment oxygen consumption (WSOC) was mediated by reduced chemical compounds or living microorganisms (COC and BOC, respectively). Direct measurement of DO was first made in some selected samples with a fiber optic oxygen sensor to confirm oxygen consumption in conjunction with the INT reduction method. A significant positive correlation was found between WSOC and whole INT reduction (WIR) with a value of 29.1 for WSOC/WIR (R-w/ETSA-w) ratio. WIR remained stable within 24 hours under a laboratory condition. These results provide empirical evidence that (1) WIR rate can be used to obtain realistic estimate of WSOC in the sediment samples, and (2) that the relative contribution of COC and BOC to WSOC can be inferred by subtracting chemical INT reduction (CIR) from WIR (Chapter II). In the following chapters, WSOC, COC and BOC estimated from INT reduction are shown

as “WSOC<sub>INT</sub>”, “COC<sub>INT</sub>” and “BOC<sub>INT</sub>”, respectively.

In order to clarify seasonal dynamics of SOC and bottom-water hypoxia in Omura Bay, temporal changes in WSOC<sub>INT</sub>, COC<sub>INT</sub> and BOC<sub>INT</sub> were examined. Not only COC<sub>INT</sub> but BOC<sub>INT</sub> increased noticeably during hypoxia. Both WSOC<sub>INT</sub> and COC<sub>INT</sub> correlated with DO and temperature of bottom-water. This suggests oxygen and temperature dependence of sulfate reducing bacterial (SRB) activity. On the other hand, BOC<sub>INT</sub> was correlated only with DO. Preservation of labile organic matter in the sediment and/or increase in sediment bacterial abundance during hypoxia may have contributed to the increase in BOC<sub>INT</sub> during hypoxia (Chapter III).

In order to clarify how bacterial community composition at surface sediment would change during hypoxia in Omura Bay, diversity, richness and structure of the bacterial population were examined in the uppermost (0-5 or 0-7 mm depth) and the subsurface layers (5-10 or 7-14 mm depth). Automated ribosomal intergenic spacer analysis (ARISA) revealed a unimodal pattern in the diversity index with DO, peaking at suboxic (11  $\mu\text{M O}_2$ ) conditions. Shifts in the bacterial communities were also evident in response to the availability of DO. Changes in the operational taxonomic units (OTUs) that were less abundant accounted for a large part of the community dissimilarity. It was further demonstrated that the relative abundance of OTUs affiliated with *Gammaproteobacteria* was correlated positively with DO, while that with *Deltaproteobacteria* was inversely correlated with DO. Additional analysis of the 16S rRNA gene amplicon sequences conducted for uppermost sediment samples in 2011 confirmed these patterns of bacterial diversity in response to DO conditions. It was further demonstrated that *Desulfobacteraceae* within *Deltaproteobacteria* was the most abundant bacterial family across the sediment samples, and that *Woeseiaceae* was the most abundant family within *Gammaproteobacteria*. These results strongly suggest that DO availability of bottom water plays a fundamental role in shaping the bacterial community, and that *Woeseiaceae* may be a responsible bacterial member for BOC<sub>INT</sub> in the sediment surface (Chapter IV).

Temporal dynamics of the SRB community, which was thought to be largely responsible for COC<sub>INT</sub>, was further examined with a terminal restriction fragment length polymorphism (T-RFLP) analysis of *dsrA* genes. The SRB community was significantly different between the two sediment layers, while no significant shifts in the community structure were observed under varying DO conditions. Another batch of bacterial 16S rRNA gene amplicon sequences revealed *Desulfococcus*, a member of SRB with a high tolerance to oxygen, was the most predominant *Deltaproteobacteria* across the uppermost sediment samples. Considering the predominance of shared OTUs across the SRB community in the sediment (0–10 mm) regardless of the bottom-water DO, some SRB that are physiologically tolerant to a wide range of DO conditions may have dominated and masked the influence of other SRB in the sediment (Chapter V).

From these results, it was clearly demonstrated that DO availability of bottom water exerted fundamental impacts on potential SOC (WSOC<sub>INT</sub>) consisting of COC<sub>INT</sub> and BOC<sub>INT</sub>. It was further demonstrated that responsible microorganisms (at genera or family level) for COC<sub>INT</sub> and BOC<sub>INT</sub> in surface sediment of Omura Bay were *Desulfococcus* and *Woeseiaceae*, respectively. Future research should involve validation of the above-mentioned findings in different coastal areas, and integration of shifts in sediment microbial activities (respiration) and community composition into ecosystem modeling

under varying DO concentrations in bottom water in order to better predict the possible ecosystem consequences imposed by global trends in ocean deoxygenation.