Improvement of eutrophic coastal bottom environments by using an optical fiber and effective psychrophilic bacteria

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SUMMARY: Eutrophic coastal bottom environments are often under serious oxygen depletion in a stratified summer season, and the heterotrophic activities in the environment decreased significantly. For improving such anoxic conditions and stimulating self-purification ability, we supplied light into the bottom water through an optical fiber and stimulated the photosynthetic activities of phytoplankton. Results indicate that $15 \mu\text{E m}^{-2} \text{s}^{-1}$ of irradiation always resulted in net production of dissolved oxygen (DO). In winter, on the other hand, when DO was sufficient but low temperature was the limiting factor for the heterotrophic activities, addition of psychrophilic bacteria stimulated the microbial activities in the bottom environments. These results indicate that the combination using optical fiber and psychrophilic bacteria is promising strategies for the bioremediation of eutrophic bottom environments.

KEY WORDS: DO depletion, DO supply, bioremediation, optical fiber, psychrophilic bacteria

INTRODUCTION

The excessive discharge of organic nutrients from intensive fish farms in cage culture have resulted in serious eutrophication of water column and sediment in enclosed coastal areas. Such bottom environments are often under serious oxygen depletion in a stratified summer season, and the heterotrophic activities in the environment, and consequently the self-purification ability, decreased significantly. The increment of anoxic condition would cause the accumulation in toxic substance such as hydrogen sulfide.¹⁾ Therefore, to break down such anoxic conditions and to supply dissolved oxygen (DO) are indispensable for keeping environment sustainable. In winter, on the other hand, DO is sufficient throughout the water column but as temperature is too low, the heterotrophic activity in the environment are again limited.

It was already demonstrated that there were enough amount of nutrients and chlorophyll *a* (phytoplankton biomass) in the bottom layer.²⁾ However, as there is no solar light reaching to the bottom water, irradiance is one of the most important limiting factors in summer. In fact, Fukami et al.²⁾ reported that the irradiation of 500 lux to bottom waters resulted in net increases in oxygen concentration, probably due to stimulation of photosynthesis, especially in summer, when the DO concentration was minimum. Therefore, one possible way to increase of DO concentrations in the bottom

environments is to supply light energy and to stimulate photosynthetic activity.

The objective of this study is to evaluate the effects of irradiation through an optical fiber on oxygen production and of adding psychrophilic bacteria on the heterotrophic activity in the bottom water-sediment systems.

MATERIALS AND METHODS

Sampling site

Bottom water of 16 m depth and sediment samples were collected monthly from Uranouchi Inlet, Kochi, Japan. This small inlet is heavily eutrophic due to the intensive aquaculture and we often have red tides in summer.³⁾ Bottom environments are usually anoxic in a stratified season from May to the end of September.^{2),4),5)} Special attention was paid to minimize the oxygen contamination.

Oxygen supply by optical fiber

One hundred grams of wet sediments were added to dark plastic bottles containing 10 l bottom water. These bottom water-sediment systems were incubated under in situ temperature either in the dark, or under light conditions of 7 or $15 \mu\text{E m}^{-2} \text{s}^{-1}$ by using a halogen lamp (Aqua Beam AQHR-100, La Foret Engineering) through an optical fiber with 14:10 of L:D cycles. Duplicate samples for each treatment (dark, 7 and $15 \mu\text{E m}^{-2} \text{s}^{-1}$) were incubated for about 20 days, and changes in DO were measured

by using DO-probe (HORIBA). Concentrations of chlorophyll *a* (chl. *a*) at the beginning and end of experiments^{6,7} and inorganic nutrients during the incubation (Bran-Luebke) in the water were determined.

Addition of psychrophilic bacteria

Several strains of psychrophilic bacteria were isolated from the Antarctic. After preincubation in a FeTY liquid medium⁸, cell suspension was centrifuged and rinsed twice with aged seawater (ASW). Cells of bacteria resuspended in ASW were then added to the sediment core samples collected in winter. Density of bacteria added in the seawater was ca. 2×10^5 cells ml^{-1} , which was about 10 % of natural planktonic bacteria in bottom water. Effects of adding bacteria on the releasing rates of inorganic nutrients from sediment to overlying water were evaluated.

RESULTS

The initial DO concentrations in samples collected in winter were about 7 mg l^{-1} . During the incubation, DO concentrations in all treatments (dark, 7, and $15 \text{ uE m}^{-2} \text{ s}^{-1}$) decreased to ca. 5 mg l^{-1} in the first 5 days. In the dark control systems, DO concentrations decreased continuously to the level of 2 mg l^{-1} , while in the light conditions, both 7 and $15 \text{ uE m}^{-2} \text{ s}^{-1}$, DO started to increase after the first 1 week (Fig. 1). Final DO concentrations in two replicates of light supply with $15 \text{ uE m}^{-2} \text{ s}^{-1}$ were more than 6 mg l^{-1} . Effects of light supply on the DO production were remarkable when the samples collected in summer stratified season were incubated under in situ temperature (Fig. 1). Although DO concentrations at the beginning of the experiments were less than 2 mg l^{-1} , those in the light supply of both 7 and $15 \text{ uE m}^{-2} \text{ s}^{-1}$ increased dramatically. Increases in DO concentration were immediate and there was no acclimatizing period, which was observed in winter samples. In summer samples, even the light supply of $7 \text{ uE m}^{-2} \text{ s}^{-1}$ performed significant oxygen production, and maximal DO concentrations under 7 and $15 \text{ uE m}^{-2} \text{ s}^{-1}$ after 15 days incubation were ca. 5.4 and 6.7 mg l^{-1} , respectively (Fig. 1).

Fig. 2 illustrates changes in the amount of chl. *a* and %-pheopigments in the samples collected in July 2000 during the incubation under dark or light conditions. The initial value of chl. *a* was 0.64 ug l^{-1} and %-pheopigments was almost 100. After 20 days incubation, although no increasing in chl. *a* concentration was observed in the dark control, significant increases to 2.5–3.7 ug l^{-1} were obtained in the light supply of 7 and $15 \text{ uE m}^{-2} \text{ s}^{-1}$. Percentages of

pheopigments in the light supply treatments decreased to as low as 20% while those in the dark were more than 80% (Fig. 2). The initial amount of DIN was 15 uM . In the systems under light supply of $15 \text{ uE m}^{-2} \text{ s}^{-1}$, DIN concentration reduced rapidly and almost no DIN remained after 20 days incubation, probably due to the consumption by phytoplankton (data not illustrated).

Fig. 1. Changes in DO concentrations during the incubation of bottom water-sediment systems under dark or light condition of 7 or $15 \text{ uE m}^{-2} \text{ s}^{-1}$.

As results of incubation experiments described as above, it was revealed that the irradiation of $15 \text{ uE m}^{-2} \text{ s}^{-1}$ to the bottom water-sediment systems always performed net oxygen production throughout the year, whereas in the dark always showed decreases in DO.

When the sediment core samples collected in winter were incubated in situ temperature of 12°C

under dark condition, concentrations of DIN and PO_4 in the overlying water increased from ca. 2 μM to 40 μM , and from 0.2 μM to 2 μM , respectively (Fig. 3), due to the mineralization of sediment organic matter by microorganisms. Addition of a psychrophilic bacterium strain AN-1 to the system stimulated significantly both the releasing amounts and rates of inorganic nutrients (Fig. 3). Amounts of DIN and PO_4 regenerated and released into the water phase were about 1.8 and 2 times greater than those without adding bacteria, respectively (Fig. 3). On the contrary, the DO consumption by adding bacteria was not significant (Fig. 3).

Fig. 2. Initial and final concentrations of chl. a (open and dark columns) and %-pheopigments ($\square$, $\bullet$) in the water during the incubation of bottom water-sediment systems under dark or light condition of 7 or 15 $\text{uE m}^{-2} \text{s}^{-1}$.

DISCUSSION

From the results obtained in this study, irradiation of 15 $\text{uE m}^{-2} \text{s}^{-1}$ in the bottom water-sediment systems could increase DO concentration to the level of more than 5 mg l^{-1} (Fig. 1), which is the optimum concentration for living organisms. Light supply of 7 $\text{uE m}^{-2} \text{s}^{-1}$, on the other hand, did not show consistent results, sometimes not effective for increasing in DO, however, it had clear oxygen production effects (Fig. 1). The DO increasing under 7 $\text{uE m}^{-2} \text{s}^{-1}$ was as much as that under 15 $\text{uE m}^{-2} \text{s}^{-1}$ in a stratified summer season. These results suggest that 7 $\text{uE m}^{-2} \text{s}^{-1}$ was nearly the compensation light intensity in the bottom

Fig. 3. Changes in concentrations of DIN, PO_4 , and DO during the incubation of sediment core samples with ($\bullet$) or without ($\circ$) addition of a psychrophilic bacterium AN-1 strain. Samples were collected in winter and incubation temperature was 12 $^{\circ}\text{C}$.

environments, and the irradiance would be the most important limiting factor in summer for the photosynthesis of phytoplankton in the bottom water. In fact, amounts of both nutrients and chl. a were relatively abundant and temperature was high in summer, while in other season, either of nutrients or temperature was the limiting factor for the growth of phytoplankton²). Another possibility to explain why effects of irradiance were more evident in summer stratified season is the photoadaptation of phytoplankton. It is said that phytoplankton cells living in low light intensity are acclimated to such environments by such as increasing in intracellular chlorophyll content and/or in photosynthetic rates^{9),10)}. Therefore, phytoplankton in the summer bottom water would respond more quickly to the irradiance supplied through an optical fiber.

During the incubation of sediment-bottom water systems collected in summer, amounts of chlorophyll a in a water phase increased 5-13 times and proportion of pheopigments decreased under light conditions (Fig. 2). These results indicate that the irradiance, even such a low light intensity as 7 $\text{uE m}^{-2} \text{s}^{-1}$, stimulated not only the phytoplankton biomass but also its activity. As a result of preliminary

experiments, most of phytoplankters stimulated by light supply, grown, and predominating after incubation were diatom species, such as *Chaetoceros* spp. and *Skeletonema costatum*. These results strongly suggest that light supply is effective way for DO production in the oxygen depleted bottom environments in summer.

Although the amount of psychrophilic bacteria added to the system was only 10 % of naturally occurring planktonic bacteria, and moreover, they were added into the water phase, the effect was still evident in cold temperature (Fig. 3). However, addition of psychrophilic bacteria performed almost nothing when the system was incubated in summer temperature of over 20°C (data not illustrated). These results of experiments using psychrophilic bacteria indicate that addition of some bacterial strains, preferring low temperature, to the bottom environments stimulate the microbial activity in winter season.

From the results obtained in the present study, light supply through an optical fiber for DO production and addition of psychrophilic bacteria for stimulating degradation of sediment organic matter are effective to enhance the self-purification ability, and applications of optical fiber in summer and psychrophilic bacteria in winter are promising strategies for the bioremediation of eutrophic bottom environments.

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