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Enrichment and immobilization of sulfide removal microbiota applied for environmental biological remediation of aquaculture area☆

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ABSTRACT

To remove sulfide in the deteriorating aquaculture sediment and water, sulfide-oxidizing microbiota was enriched from Jiaozhou Bay, China, by using sulfide-rich medium. Composition and structure of microbial communities in the enrichments were investigated by 16S rDNA molecular biotechniques. Results showed that microbial community structure continuously shifted and the abundance of sulfate reducing bacteria, i.e., *Desulfobacterium*, *Desulfococcus* and *Desulfobacca* apparently declined. Several halophile genera, *Vibrio*, *Marinobacter*, *Pseudomonas*, *Prochlorococcus*, *Pediococcus* and *Thiobacillus* predominated finally in the microbiota. The enriched microbiota was capable of removing a maximum of 1000 mg/L sulfide within 12 h with 10% inoculum at pH 7.0, 20–30 °C. After immobilized, the microbiota presented excellent resistance to impact and could completely remove 600 mg/L sulfide in 12 h. Moreover, the immobilized microbiota recovered well even recycled for five times. In conclusion, the immobilized sulfide-removing microbiota showed a quite promising application for biological restoring of sulfide-rich aquaculture environment.

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1. Introduction

Sulfide, an important water pollutant widely found in petrochemical, pharmaceutical, tannery and aquaculture wastewater, has been demonstrated to have interference and inhibition on metabolism and health of plants and animals in sulfide-rich environment (Wu et al., 2016). In marine sediment, the sulfide (S^{2-}, HS^-) content is significantly and positively correlated with total organic carbon (Gao et al., 2013). Discharges of anthropogenic organic materials and redundant bait feeding in marine aquaculture area often lead to high sulfide concentration in sediment, even in overlying water (Chen et al., 2014). This will further result in massive death of aquatic life and even damage to the local ecosystem (Reese et al., 2008). The removal of sulfide or inhibition of sulfide production in aquaculture environment is commonly applied by coal ash (Asaoka et al., 2009) or ferric hydroxide (Sun et al., 2014). However, these physical or chemical measures are costly and easy to produce secondary pollution, and the most important is they are hard to achieve long-term and permanent efficiency. Comparatively, biological sulfide removal by indigenous microorganisms provides an environment-friendly, effective, and permanent strategy; therefore is more promising in sulfide removal in the deteriorating aquaculture water.

Sulfur-oxidizing bacteria (SOB) are a group of high diverse microorganisms with the capacity of oxidizing sulfide or sulfur (Tang et al., 2009). SOB application in sulfide biological removal in industries has been widely reported (Tang et al., 2009). Moreover, indigenous bacterial species always have higher suitability and a better environmental friendliness than allochthonous species during practice of ecological restoration (Li et al., 2013; Ortiz et al., 2015). Thus, the marine sediment-originating sulfide-oxidizing microbiota is more suitable for sulfide biological removal in marine aquaculture environmental remediation. Moreover, research showed that microbial community with mixed strains perform more resistant to external interference such as disinfection treatment than single strain (Stijn van der Veen, 2011), and the immobilization of microorganisms would further protect them from

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shock load application and toxicity of chemicals (Sekaran et al., 2013). Although the application of immobilized SOB on sulfide removal has been reported in gas desulfuration in biotrickling filter (Tang et al., 2009), rare studies have been addressed in sulfide-rich aquaculture environmental remediation.

In this study, we enriched indigenous sulfide removal microbiota from the sediment of aquaculture environment and immobilized them to apply in the artificial deteriorating aquaculture environment to remove sulfide in sediment and water. The shift of microbial community structure during enrichment and the microbial composition and abundance of the enriched microbial community were investigated by culture-independent denaturing gradient gel electrophoresis (DGGE) and high-throughput sequencing techniques. Sulfide removal performance of the microbiota under different sulfide concentration and the reusable ability of the immobilized microbiota were also assessed.

2. Materials and method

2.1. Sampling sites and inoculum

As shown in Fig. 1, Jiaozhou Bay is a semi-enclosed shallow bay located in the southern part of Shandong Peninsula, China (Shi et al., 2011) and its surface sediments in the eastern coast are eutrophic due to overload aquaculture (Chen et al., 2014). The sediment used to be incubated in this study was picked out from sites A, B, C and D of Jiaozhou Bay. The properties of sediment at the four sampling sites were listed in Table S1. Site A is a shellfish aquaculture area for 30 years and site B is a new cage aquaculture area for sea cucumber. C is a transition area from aquaculture to non-aquaculture and D is far away from aquaculture area. Vertical columnar sediment samples were obtained from the four sites by using sediment grab tubes in October, 2014, and were immediately transported to laboratory and stored at 4 °C. Surface sediments of 0–10 cm were carefully collected from the bulk sediments, mixed and used as the inoculum for the enrichment of sulfide removing microbiota.

2.2. Medium

To enrich sulfide removal microorganisms, a selective liquid medium with sulfide (Lee et al., 2006) was adopted. The chemical ingredients contained: 2.0 g $NaHCO_3$, 1.2 g KH_2PO_4, 1.2 g K_2HPO_4, 0.4 g NH_4Cl, 0.2 g $MgCl_2 \cdot 6H_2O$ and 0.01 g $FeSO_4 \cdot 7H_2O$ in 1 L of 0.45 μm membrane-filtered and autoclaved sea water. During enrichment, Na_2S was added to the fresh liquid medium with a gradient concentration of 600 mg/L, 800 mg/L, and 1000 mg/L.

The minimal medium used for monitoring biological sulfide removal under different conditions contained the same composition with the selective liquid medium. Because S^{2-} will convert into

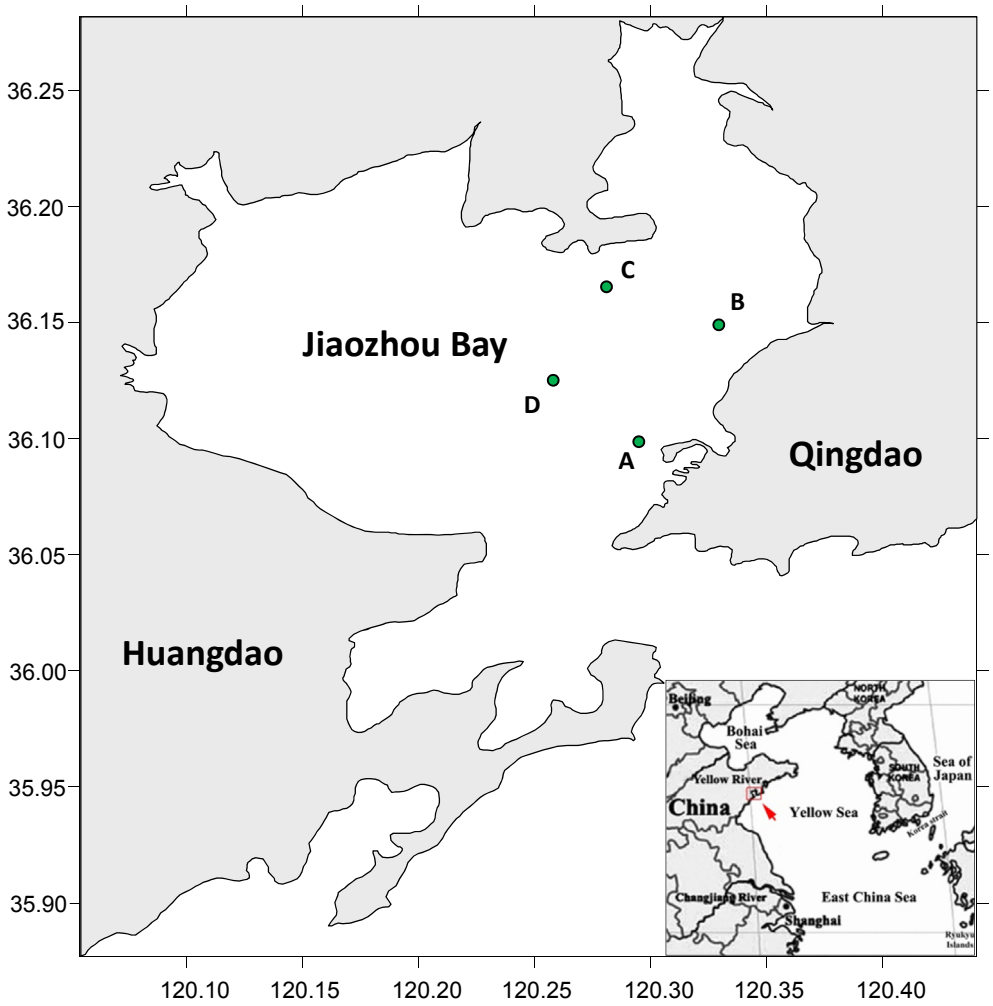

Fig. 1. Maps show the sampling sites (A, B, C and D) of Jiaozhou Bay (larger) and the Yellow Sea (bottom, adapted from reference (Dang et al., 2010) with revision).

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