Evaluating the contingency treatment performance of advanced electro-catalysis oxidation processes for marine bacteria in ballast water

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ABSTRACT

Effects of ballast water treatment by advanced electro-catalysis oxidation processes (AEOP) on abundance, activity, and diversity of marine bacteria were examined in a full-scale ballast water management system (BWMS) at Yangshan Port, Shanghai, China. Water samples were collected immediately after treatment and at discharge to evaluate the contingency treatment performance of the BWMS for bacteria. After treatment, the total viable count reduced to $0.7 \times 10^4$ CFU·mL$^{-1}$, and both \textit{Escherichia coli} and enterococci decreased to 10 CFU·100 mL$^{-1}$, which satisfied the D-2 Standard of the International Maritime Organization. AEOP can be as an effective contingency reception facility.

Sequencing of 16S rRNA gene amplicons demonstrated the declining trend in bacterial diversity, and while the treatment did not completely eliminate the risk of bacterial dispersal, potentially pathogenic bacteria survived in treated and discharged samples. Bacterial diversity is of greater concern when evaluating effects of ballast water treatment on microorganisms because the bacteria which can develop adaptive mechanisms to environmental change will have a greater potential for invasion in the new environment.

Key words: advanced electro-catalysis oxidation processes, bacterial diversity, bacterial invasion, ballast water contingency reception treatment

HIGHLIGHTS

- Using high-throughput sequencing based on V4-V5 region of 16S rRNA genes.
- AEOP can be considered as effective ballast port contingency reception equipment.
- The treatment cannot completely eliminate the risk of pathogen spread.
- Bacteria that develop adaptive mechanisms have a greater potential for invasion.
- Diversity is better than density of bacteria in indicating effects of treatment.

1. INTRODUCTION

Marine bioinvasion is defined as damage to the environment when marine organisms disperse outside their native ecosystems, and ballast water is a major vector for biological invasion (Anil \textit{et al.} 2002; Dobbs \& Rogerson 2005). With the development of international trade in recent years, more than 10 billion tons of ballast water have been transferred among ports all over the world (Wang \textit{et al.} 2020). Ships globally could potentially carry about $3 \times 10^{21}$ bacteria in their ballast tanks (Brinkmeyer 2016). The risk of bacteria invasion, can cause disease in plants, animals, and humans, and also lead to ecological imbalance (Drake \textit{et al.} 2007; Simberloff 2011; Galil \textit{et al.} 2014).

The International Maritime Organization (IMO), to help prevent the spread of potentially harmful aquatic organisms and pathogens in ships’ BW, has adopted an International Convention for the Control and Management of Ships’ Ballast Water and Sediments (the Ballast Water Management Convention or BWMC) (IMO 2004). The convention requires that ballast water is treated at uptake and/or discharge to reduce the number of organisms to meet the ballast water discharge standard (Gollasch \& David 2019). It was found (National Research Council 1996) that the most effective way to control the transport of organisms in ship ballast tanks would be by including a ballast water management system (BWMS). Since then, techniques such as the addition of chemicals, electrochlorination of seawater, ultraviolet (UV) radiation, ozone, deoxygenation, and heating have been applied to reduce the content of microorganisms in BW (Goncalves \& Gagnon 2012).
Treatment with these approaches has been found to be efficient in the reduction of bacteria (Tryland et al. 2010; Rubio et al. 2013; Wennberg et al. 2013). However, according to the International Association of Classification Societies (IACS), less than 10% of IACS ships have BWMS, and a certain percentage of installed BWMS have malfunctions or usage problems (Chang & Liu 2019). These problems not only bring risks to the ecological environment in the nearshore waters but may also cause shipping delays and port congestion, reducing the efficiency of port navigation. Therefore, the feasibility of a BWMS based on shore-based equipment has become a focus of research in recent years (IMO 2006). In addition, IMO has developed guidance on contingency measures (IMO 2017), stating that when ballast water discharged by ships does not meet the requirements of the BWMC, it can be discharged into barges or shore-based reception and treatment facilities for contingency reception. Therefore, an efficient BWMS is urgently needed to be applied to the contingency reception of the port.

Because the ballast water contingency reception facilities are not limited by space, energy consumption, or other factors, the range of process selection is quite extensive. Compared with the BWMS on board, the most obvious difference in the process of the contingency reception facilities is that the ballast water after instantaneous treatment meets the D-2 Standard (A/SCowi 2012; Ryan et al. 2016). Therefore, it is advisable to choose efficient instantaneous processing technology to meet the requirement that the ballast water can be discharged immediately after treatment.

Advanced electro-catalysis oxidation process (AEOP) is a newly developed oxidation technology and has been the focus of much attention in the environmental field for its high efficiency, simple operation, and environmental compatibility (Fan et al. 2009). AEOP, used boron-doped diamond (BDD) membrane electrode, hydroxyl radicals are directly or indirectly generated by the electrode reactions at normal temperature and pressure, by which the nonbiodegradable pollutants are finally degraded. BDD film electrode is an innovative material with a wide electrochemical window and an excellent stability in an acid or alkali solution, the current efficiencies of more than 90% for water body purification (Yu 2008). At present, there are few studies on the application of AEOP technology in the treatment of ballast water, and there is no relevant report on the effectiveness of AEOP for bacterial treatment. As for bacteria in ballast water, most research has focused on the survival of pathogens after treatment, with limited knowledge on the diversity of bacterial communities, in addition, there are gaps in the understanding of the modification in the structure and function of bacterial communities after treatment. Therefore, to fully evaluate the effect of treatment with high efficiency ballast water management system (AEOP) on the bacterial community, we analyzed the activity, diversity, and metabolic function expression of bacteria in ballast water after emergency treatment.

Understanding microorganism biodiversity of ballast water and function of bacterial communities could improve risk assessment for the port and the propague pressure of imported pathogens (Bradie et al. 2013). To our knowledge, this is the first study to combine the abundance, activity, and diversity of bacterial communities to examine the performance of contingency reception of AEOP.

2. MATERIALS AND METHODS

2.1. Challenge water preparation

Initially, at least 500 m$^3$ of seawater (approximately 19 practical salinity unit, PSU) was pumped from the harbor into a 500 m$^3$ feeding tank and slowly agitated with a submerged propeller. To ensure the BWMS is widely used in natural water bodies, the IMO and the US Coast Guard carried out a BWMS type approval land-based experiment of water dissolved organic carbon (DOC), particulate organic carbon (POC), and total suspended solids (TSS) configuration requirements. To comply with both the IMO and the US Coast Guard requirements, the following minimum values must be achieved in the challenge water: (1) 6 mg DOC·L$^{-1}$ and 5 mg POC·L$^{-1}$; (2) 50 mg TSS·L$^{-1}$. The challenge water used for approval testing of BWMS was prepared in the feeding tank by augmentation of ambient seawater with additions of calcium lignosulphonate, corn starch, and kaolin (US 2012; IMO 2016) (Table 1). The physical parameters at sampling ports 2, 3, 4, and 5 are given in supplementary data.

2.2. Treatment and sampling

During the ballast operation of BWMS, the electrocatalysis ultra treatment unit as the key part of the system can produce high concentrations of hydroxyl radicals of high concentration. Hydroxyl radicals have strong oxidizing properties and their oxidation capacity is similar to that of fluorine. Hydroxyl radicals can survive for a very short period of time, they react instantly with microorganisms and other organisms to kill and remove them. The target dose of total residual oxidant (TRO) was kept
around 2 mg·L⁻¹ and the average flow was about 250 m³·h⁻¹. Because the land-based type approval test set up the treatment group and control group at the same time, after preparing the challenge water, 250 m³ water was transferred from the feeding tank and treated in the BWMS route to the 250 m³ treatment tank. The remaining 250 m³ water remained untreated and was transferred to a similar 250 m³ control tank and served as control water (Figure 1). The whole ballast process took 2 h, because of the automatic backflushing of the BWMS reduced instantaneous flow. The typical TRO content in the treated water was 1.5–2.5 mg·L⁻¹, which was neutralized to 0–0.03 mg·L⁻¹ by sodium thiosulfate (Na₂S₂O₃) before discharge. The tests were performed according to the requirements for type approval testing by the IMO and US Coast Guard (US Coast Guard 2012; IMO 2016).

A sampling of water for bacteriological analyses included (Figure 1): (a) initial challenge water (untreated, S.P.1); (b) treated water without holding time, neutralized in the sample bottle by adding Na₂S₂O₃ directly to ensure the authenticity (treated T₀, S.P.2); (c) discharge (treated dis, S.P.3); (d) untreated challenge water (control, S.P.4); (e) discharge (control dis, S.P.5). In each sampling point, 100 mL of sample water was used for the culture of heterotrophic bacteria, 500 mL for detection of *Escherichia coli*, 500 mL for detection of enterococcus, and 1 L for detection of *Vibrio cholerae*. Additionally, 1 L of water samples were collected at S.P.1, S.P.2, and S.P.3, respectively for high-throughput sequencing. To ensure the accuracy and scientifi city of the data, two sets of samples were collected at the same time (the article uses _1,_2 to distinguish groups). All water samples were kept in sterile flasks in coolers within ±1 °C of the water temperature for up to 2 h until analysis in the laboratory.

The WTW Multi 3630 portable water quality analyzer was used to determine the in situ analysis (water temperature, salinity, dissolved oxygen, and pH) of the ballast water samples. The turbidity and TSS were measured by the turbidity meter and electronic balance, respectively. The total organic carbon (TOC) analyzer (Shimadzu TOC-L CPN; Shimadzu, Japan) TOC-L was used to determine the DOC and POC in the ballast water sample.

### Table 1 | The average of physical and chemical parameters in natural seawater and challenge water

| Parameter                | Natural seawater | Challenge water |
|--------------------------|------------------|-----------------|
| Temperature (°C)         | 29               | 29 (n = 3, s.d. = 0) |
| Salinity (PSU)           | 19               | 18.8 (n = 3, s.d. = 0) |
| pH                       | 8.1              | 8.3 (n = 3, s.d. = 0) |
| DO (mg·L⁻¹)              | 7.2              | 8.3 (n = 3, s.d. = 0.3) |
| "TSS (mg·L⁻¹)            | 33               | 55.4 (n = 3, s.d. = 0.3) |
| "POC (mg·L⁻¹)            | 0.33             | 7.3 (n = 3, s.d. = 0.4) |
| "DOC (mg·L⁻¹)            | 1                | 9.3 (n = 3, s.d. = 0.4) |

*Indicates augmentation of the parameters by challenge water preparation. s.d. standard deviation.

Figure 1 | Schematic representation of the effectiveness of BWMS using filtration & AEOP. S.P.1-S.P.5 denote sampling points. T₀h represents holding time = 0 h.
2.3. Determining total viable count and culturable indicator bacteria

2216E agar and thiosulfate citrate bile salts (TCBS) agar was used to assess the effectiveness of a treatment for bacteria. The abundance of culturable E. coli and enterococci was determined by Protocol For The Verification Of Ballast Water Treatment Technology (ETV 2010).

2.4. Bacterial diversity

2.4.1. Sample pretreatment

All samples were stored in refrigerated containers and sent to the laboratory, testing was performed within 2 h after the end of sampling. Each 1-L water sample of untreated, treated T0, and treated discharge was filtered through 0.45-micron microporous filter membranes, and each membrane was transferred to the 1.5-mL Eppendorf tube and stored in a refrigerator at -80 °C.

2.4.2. Extraction of genomic DNA

DNA Isolation Kit V2.2 was used to extract the DNA in the membrane, and the concentration and purity of DNA were evaluated using the ultra-micro spectrophotometer (Nanodrop 2000c; Thermo). The qualified DNA was applied for the next analysis.

2.4.3. PCR amplification and high-throughput sequencing

Our target was the V4–V5 hyper-variable region of the bacterial 16S rRNA gene. Polymerase chain reaction (PCR) was started immediately after the DNA was extracted. Two universal bacterial 16S rRNA gene amplicon PCR primers (polyacrylamide gel electrophoresis purified) were used: 515F (5'-GTGCCAGCMGCGG-3') and 907R(5'- CCGTCAATTCMTT-TRAGTTT-3'). The reaction was set up as follows: microbial DNA (10 ng·μL⁻¹) 2.5 μL; amplicon PCR forward primer (5 μm) 0.8 μL; amplicon PCR reverse primer (5 μm) 0.8 μL. The plate was sealed and PCR performed in a thermal instrument (Applied ABI GeneAmp® 9700) using the following program: 1 cycle of denaturing at 95 °C for 3 min, followed by 25 cycles of denaturing at 95 °C for 30 s, annealing at 55 °C for 30 s, elongation at 72 °C for 45 s, and a final extension at 72 °C for 10 min. The PCR products were checked using electrophoresis in 2% (w/v) agarose gel sand sent to Shanghai Meiji Biomedical Technology Co. Ltd for high-throughput sequencing. Amplification results: the target band size is correct and the concentration is appropriate, which can be used for subsequent sequencing experiments.

2.4.4. Data analysis after high-throughput sequencing

PCR products were sequenced on the Illumina platform of Shanghai Meiji Bio-medical Technology Co. LTD. After quality control calibration, the high-quality sequences were divided into operational taxonomic units (OTU) at 97% similarity level, and then the Ribosomal Database Project classifier Bayesian algorithm was used for taxonomic analysis by aligning the Silva 11.9 database (Quast et al. 2013).

2.4.5. Inferring the functions of the microbiome

Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) was used to infer the gene functional spectrum of their common ancestor based on the 16S rRNA full-length sequence of the tested bacterial genome, to infer the gene functional spectrum of other unmeasured species in the Greenenes database, to construct the gene functional prediction spectrum of the whole spectrum of Archaea and bacteria domain, and finally to ‘map’ the flora composition obtained by sequencing to the database to predict the metabolic function of the flora.

3. RESULTS

3.1. Density analysis of total viable count and indicator bacteria

The total viable bacteria in the untreated water and the control water were similar (about 0.5 × 10⁵ CFU·mL⁻¹) (Figure 2(a)). After the AEO, the CFU number in the treated water was reduced to 0.7 × 10⁴ CFU·mL⁻¹. Therefore, the treatment significantly reduced the total viable count, whereas in the control group, there was no significant difference in bacterial density between inflow and discharge. Additionally, before discharge, the inactivation rate of total viable bacteria was as high as 99% under the effect of residual TRO.

We inspected indicator bacteria specified in the IMO standards (IMO 2004). No V. cholerae were found in any samples. The density of E. coli in the untreated water and control water was about 520 CFU·100 mL⁻¹ (Figure 2(b)); while enterococci was about 830 CFU·100 mL⁻¹ (Figure 2(c)). Significantly, the original density of E. coli and enterococci in the untreated water was different—there far more enterococci than E. coli, about twice as many. After treatment, their density fell to very low
concentrations (E. coli and enterococci, about 10 CFU·100 mL⁻¹), which was reduced to below the D-2 discharge standard. Conversely, in the control samples, there was no significant change in either and a slight increase was detected.

3.2. Diversity index of high-throughput sequencing

The diversity and abundance of bacteria in water treated by AEOP decreased obviously. The diversity index is listed in Table 2. The 49483–72140 sequence was obtained for all samples, and those sequences were clustered into 300 (treated dis_1) -606 (untreated_1) OTUs by aligning the sequence length of 375 bp, and the Good’s coverage values of all samples were greater than 99% (Table 2), suggesting that the obtained sequences could reflect most of the bacterial community in this study.

The Shannon diversity index provides the distribution of each species in an ecosystem. The Shannon index of the six samples ranged from 2.98 to 4.01 and untreated_2 exhibited the highest diversity among the six communities. The predicted OTUs (Sobs) by Chao and Ace exhibited a similar trend for each sample. The numbers of OTUs estimated with the Chao suggested that untreated_1 had the maximum OTUs, as validated by Ace indices (Table 2). Moreover, all samples in the discharge had smaller values than those without holding time.

3.3. Taxonomic complexity of bacterial community

The bacterial community played important ecological and biogeochemical roles in the aquatic ecosystem as they could adapt to the environment. All the sequences fell into seven major lineages of the bacterial phylum, the Proteobacteria (74.5%–76.1%), Bacteroidetes (2.7%–5.7%), Actinobacteria (2.7%–3.5%), Cyanobacteria (6.3%–6.9%), Tenericutes (7.2%–7.4%) as well as unclassified_k_norank (0.12%–0.18%) and others (1.9%–2.2%) (Figure 3(a)). Proteobacteria accounted for the largest proportion in the untreated sample, while the remainder of the bacterial groups identified were only a small part of the sample. Tenericutes was another dominant phylum accounting for 7.2%–7.4% of total OTUs. In addition, Cyanobacteria was found to account for 6.3%–6.9% of OTUs in BW. After treatment, Proteobacteria (32.3%–40.2%) was still dominant,

Table 2 | Richness and diversity indices of the bacteria families in the ballast water

| Sample        | Ace   | Chao   | Coverage | Shannon | Simpson | Sobs  | Seq.num. | Length |
|---------------|-------|--------|----------|---------|---------|-------|---------|--------|
| Untreated_1   | 645.71| 642.54 | 0.9987   | 3.94    | 0.0643  | 606   | 60495   | 375.16 |
| Treated T0_1  | 561.67| 574.10 | 0.9976   | 3.56    | 0.0646  | 459   | 58981   | 374.27 |
| Treated dis_1 | 382.87| 362.88 | 0.9982   | 2.98    | 0.1146  | 300   | 49483   | 376.28 |
| Untreated_2   | 658.59| 637.44 | 0.9992   | 4.01    | 0.0543  | 585   | 72140   | 375.20 |
| Treated T0_2  | 607.00| 603.48 | 0.9982   | 3.90    | 0.0454  | 559   | 66217   | 374.77 |
| Treated dis_2 | 432.84| 418    | 0.9980   | 3.03    | 0.1107  | 323   | 54883   | 376.28 |
but Bacteroidetes (33.6%–42.4%) became the first dominant species in the community. In the discharge water, Proteobacteria (62.9%–63.6%) once again dominated, and Actinobacteria (30.9%–31.1%) became the second dominant species in the community.

Figure 3 | Barplot indicating percentages of bacterial community abundance of samples at the phylum level (a), at the class level (b), and the genus level (c).
Figure 3(b) shows the bacterial structure at the class level. In those BW samples, eight classes were identified and the Proteobacteria group primarily comprised species affiliated with α-Proteobacteria and γ-Proteobacteria on the class level. γ-Proteobacteria was the first dominant class in all untreated samples and accounted for 61.4%–62.9% of the total sequences. Moreover, the dominant classes contained α-Proteobacteria (12.9%–13%), Mollicutes (7.2%–7.4%), and Oxysphotobacteria (6.2%–6.8%). After treatment, it is obvious that Bacteroidia has increased and as the first dominant class accounting for 33.52%–42.46%, followed by α-Proteobacteria (20.42%–23.1%). Additionally, that Actinobacteria (31%–31.1%) was increased after treatment. Inversely, the proportion of γ-Proteobacteria (11.8%–16.9%) was significantly decreased. In the discharge water, α-Proteobacteria (38.9%–40%) was the first dominant class. Within α-Proteobacteria, the relative abundance of Rhodobacteraceae (35.3%–36.2%) was high (at the family level). Moreover, the dominant classes contained Actinobacteria (31%–31.1%) and γ-Proteobacteria (23.6%–24%).

The main dominant classes and their abundances varied significantly among the four BW samples. The treatment reduced the diversity of bacterial families, compared to the untreated water. The treatment increased the relative abundance of Flavobacteriaceae (24.1%–32.3%) overall, and Rhodobacteraceae (35.3%–36.2%) became the most abundant family.

The analysis on the genus level suggests the functions of bacterial communities in ballast water (Figure 3(c)). Thirty-four genera were detected in all of the untreated samples as the core community. The treatment reduced the diversity of bacterial genera compared with untreated water. However, there were still 22 genera in the treated T0, including a variety of pathogenic genera, such as Vibrio (2.16%–2.24%) and Pseudomonas (0.5%–0.9%).

3.4. Inference of functional of bacterial community

Metabolic functional prediction of the bacterial community was carried out using PICRUSt1. Predictive inference of functions of the bacterial community revealed that most of the core functions of the bacterial community were expressed to a lower degree in treated water than in untreated water. However, during the process, the expression of a few specific core functions was increased, and mainly included amino acid transport and metabolism, nucleotide transport and metabolism, carbohydrate transport and metabolism, and transcription (Figure 4). An increase in specific bacterial clades could be related to the core metabolic functions expressed by bacteria inside the BW.
4. DISCUSSION

Important observations from this study of treatment effects on BW are that (a) the AEOP treatment can cause a reduction of the bacterial abundance and activity, and that the density of indicator bacteria met the D-2 Standard; (b) the bacterial diversity in the treated water differed from the original diversity with \( \alpha\)-Proteobacteria as the major class of original water, and the proportion of Bacteroidetes, \( \alpha\)-Proteobacteria, and Actinobacteria increased in treated water; (c) although the treatment is effective in reducing bacterial diversity, potential pathogens Vibrio and Pseudomonas, which can still thrive in treated water, also include bacteria that have developed adaptive mechanisms; and (d) the increased expression of a few core metabolic functions in treated samples may be related to the increase of specific bacterial clades.

In our study, most of the bacteria were eliminated after treatment without holding time, such as Tenericutes and Cyanobacteria on class level (Figure 3(b)). Previous studies have shown that some bacteria in BW are susceptible to a variety of environmental factors (Khandeparker et al. 2020). For example, Cyanobacteria photosynthetic autotrophs are commonly found in BW, but their numbers decline rapidly during the voyage. Most studies have shown that phototrophs, a group of organisms unable to cope with the dark conditions inside the ballast tanks (Tomaru et al. 2014; Ng et al. 2015), and non-spore-forming bacteria will gradually die out as the environment changes. In contrast, we should pay more attention to those species that can survive during stress conditions.

In relation to the other major taxa, \( \alpha\)-Proteobacteria (mostly \( \alpha\) and \( \gamma\)) dominated untreated water, and previous research from in 2010 showed that Proteobacteria are among the most successful bacteria in the sea (Hess-Erga et al. 2010), and have an advantage in quantity (Li et al. 1999; Utchicke & McGuire 2007; Zhang et al. 2007; Toes et al. 2008) (Figure 3(a)). \( \gamma\)-Proteobacteria reduced fastest in the treated T0 samples, which supports that \( \gamma\)-Proteobacteria can respond quickly to environmental changes and are easily affected by the environment (Eilers et al. 2000). Previous studies also identified \( \gamma\)-Proteobacteria was usually the most abundant class of bacteria and accounted for more than 50% of the total microbial community in the marine environment (Kerfahi et al. 2014). In addition, a variety of pathogenic bacteria has been found in \( \gamma\)-Proteobacteria (Zhou et al. 1989; Holt 2013).

Our results show that \( \alpha\)-Proteobacteria seem to be more resistant to stress, and it was most abundant in treated T0 samples. A possible explanation for this increase in numbers and stabilization is a succession where resource limitation and competition are beneficial to the survival of this kind of bacteria. \( \alpha\)-Proteobacteria is one of the most dominant bacterial groups, especially in coastal ecosystems (Gonzalez & Moran 1997), and plays a significant role in biogeochemical processes (Pujalte et al. 2014). Members of the family Rhodobacteraceae are also known to grow under phosphate limiting conditions by employing multiple strategies to overcome the stress (Smith et al. 2018). Rhodobacteraceae, due to its innate ability to grow under low nutrient conditions, flourished and dominated the bacterial community in the treated water (Beech et al. 2005). Naturally, they could have a major economic impact due to their strong ability to survive if translocated into a new environment.

Actinobacteria and Bacteroidetes were also detected in treated samples. Previous studies showed that Actinobacteria in wastewater have good capabilities for the production of the tested enzymes, such as lignocellulose, keratin, and chitin, which play a key role in the hydrolysis and biotransformation of organics (Hozzein & Wadaan 2013). Moreover, Bacteroidetes were also detected in treated T0 samples. Bacteroidetes were often found in saline sediments (Newton et al. 2013) and they could degrade various complex organics through excreting extracellular enzymes (Williams et al. 2013; Liu et al. 2015). The hydrolytic capability was helpful for Bacteroidetes when the labile carbon source was deficient (Carr et al. 2013). In consequence, the oligotrophic conditions might contribute to the presence of Actinobacteria and Bacteroidetes in BW.

From the marine bioinvasion perspective, the treatment could not completely eliminate the pathogenic bacteria. The introduction of bacterial communities, which have mechanisms to cope with the competition, into non-native port environments, will result in the bacterial population re-establishing if the appropriate nutritional conditions were present, which would be of concern to the prevailing microbiome and could also cause economic loss to the aquaculture industry (Hess-Erga et al. 2019; Petersen et al. 2019). Therefore, manufacturers need to pay more attention to the bacterial diversity of ballast water, and the system should be improved to remove bacteria efficiently to reduce the potential invasion of bacteria such as Proteobacteria.

5. CONCLUSION

AEOP is an effective contingency reception technique for removing bacteria from ballast water. Treatment caused the inactivation rate of total viable bacteria to be as high as 99% and the density of indicator bacteria met the D-2 Standard.
Additionally, the ability of a bacterial community to invade a new environment should take into account the ability of the microorganisms to display mechanisms—bacteria that develop adaptive mechanisms have a greater potential for invasion. For monitoring the efficiency of ballast water treatment, bacterial diversity appears to be a more important indicator than densities of indicator bacteria.

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DECLARATIONS CONFLICTS OF INTEREST

The authors (Yulin Xu; Qiong Wang; Huixian Wu) declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. No conflict of interest exists in the submission of this manuscript, and the manuscript is approved by all authors for publication. All the authors listed have approved the manuscript.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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