Effect of Hydroxyl Radicals on Photosynthesis Pigments of Phytoplankton in Ship's Ballast Water of 20t/h

Xiyao Bai, Mindong Bai, Zhitao Zhang, Mindi Bai, Bo Yang

Environmental Engineering Institute, Dalian Maritime University
hjs@dlmu.edu.cn

Abstract— **A pilot-scale system of 20t/h for the treatment of ship's ballast water and the setup of dissolved hydroxyl radical was introduced in this paper. With this experimental system, the kill efficiencies of bacteria, mono-algae, protozoan reach 100% within 2.67s when dissolved OH· concentration is 0.6mg/L. At the same time, the effect of hydroxyl radicals on the photosynthesis pigments of phytoplankton was done. The results indicate that the contents of chl-a, chl-b, chl-c and carotenoid are decreased to 35~64% within 8.0s further to the lowest limit of test after 5 minutes. When dissolved OH· ratio concentration is 0.68mg/L, the attenuation efficiencies of photosynthesis pigment are 100%. Therefore the invasive marine species can be killed in the process of the inputting and discharge ship's ballast water.**

1. INTRODUCTION

The introduction of invasive marine species into new environments by ship's ballast water, attached to ships' hulls and via other vectors has been identified as one of the four greatest threats to the world's oceans by Global Environment Facility (GEF). Vessels of the world are transferring 10 billion tons of ballast water per year. It is estimated that at least 7,000 different species are being carried in ship's ballast tanks around the world. About 110 million plankton specimens are carried in $1m^3$ of ballast water. Until today about 500 different species are known to have been transported with ballast water [1-8].

Near 20 years, main methods for the treatment of ship's ballast water are the mechanical, physical and chemical removal of species [9]. With the open-ocean-exchange in mechanical removal, only 95 % of ballast water was discharged at open ocean injecting 3 times seawater, existing the problems of safety and energy consumption [10]. By the heat treatment in physical removal, higher temperatures would be required to deal with thermophilic (heat loving) organisms or more resistant forms such as bacterial spores. It has to be taken into account that temperature between 30 and 40 would support the growth of bacteria as e.g. Vibrio cholerae [11,12]. It has to be considered that chemical removal is promising method. In 1997, Donald M. Anderson pointed that more than 4,700 effective chemical biocides could be used to kill organisms in oceans and lakes [5]. However Several tonnes were needed to treat the large amounts of ballast water on a bulk carrier calling for a port area without any cargo. In addition, both inorganic and organic biocides would present a range of health and safety problems related to the storage and handling of chemicals, their compatibility with cargoes carried on board ships, as well as those related to the direct and indirect handling of chemicals by crew members [13]. Also the kill duration of biocides needs above 20 minutes, that is impossible to treat over ten thousands ballast water on board. Although a lot of research work was done in the world, Marine Environment Protection Committee (MEPC) and GloBallast think that no any efficient, low cost, no residua method could be used to treat the ship's ballast water [1].

A physics method is studied that the electrons are accelerated and then the gas molecules are aroused using a strong dielectric barrier discharge [14-16]. The strong electric field ($E_d \geq 400Td$, $1Td=10^{-17}Vcm^2$) is formed with the thinner α-Al_2O_3 dielectric layer in the microgap at a high pressure ($P \geq 0.1MPa$ or $n=2.6\times10^{-19}/cm^3$). The electrons achieve the average energy of above 12eV. With this method, the kill of organisms of ship's ballast water was done in laboratory using hydroxyl radicals in 2002 [17,18]. As a result, the mono-algae, protozoan, spore, bacteria were killed 100% with OH· concentration of 0.6mg/L. In this paper, the effect of hydroxyl radicals on the photosynthesis pigments of phytoplankton was studied in the experimental system of 20t/h for the treatment of ship's ballast water.

2. Plasma Processes For OH· Radical Formation [19,20]

With the strong dielectric barrier discharge in microgap, H_2O molecules are ionized and excited into OH· radicals as follows.

$$2H_2O + e \rightarrow H_2O^+ + H_2O^* + 2e \quad (1)$$

The dissociation of H_2O^* molecules at excited state:

$$H_2O^* \rightarrow H\cdot + OH\cdot \quad (2)$$

The dissociative ionization of H_2O^+:

$$H_2O^+ + H_2O \rightarrow H_3O^+ + OH\cdot \quad (3)$$

$$H_2O^+ \rightarrow H^+ + OH\cdot \quad (4)$$

The plasma reaction processes of O_2 ionization into OH· are as follows.
Electron-impact ionization:

$$O_2 + e \rightarrow O_2^+ + 2e \quad (5)$$

Electron-impact dissociative ionization:

$$O_2 + e \rightarrow O^+ + O\cdot + 2e \quad (6)$$

Similar dissociative ionization processes to produce N_2^+, N^+ ions. Charge transfer reactions to form additional O_2^+ ions.

$$O_2^+ + H_2O + M \rightarrow O_2^+(H_2O) + M \quad (7)$$

Formation of water cluster ions:

$$O_2^+ + H_2O + M \rightarrow O_2^+(H_2O) + M \quad (8)$$

Dissociative reactions of water cluster ions to form OH·:

$$O_2^+(H_2O) + H_2O \rightarrow H_3O^+ + O_2 + OH\cdot \quad (9)$$

$$O_2^+(H_2O) + H_2O \rightarrow H_3O^+(OH) + O_2 \quad (10)$$

$$H_3O^+(OH) + H_2O \rightarrow H_3O^+ + H_2O + OH\cdot \quad (11)$$

The formation of O_2^+ and H_2O^+ ions is necessary to form OH· radicals in the plasma. Therefore the electron energy has to be larger than the ionization potential of O_2 (12.5eV) and H_2O (12.6eV), respectively. Because the distribution of electron energy conforms to the Maxwell distribution law, a lot of electrons have the energy of ≥12.6eV for the mean electric energy of 12 eV.

With the plasma reactions (1)~(4), per 100eV energy injected into the discharge electric field is possible to produce 2.80 OH· [22]. With the plasma reactions (5)~(10), per 100eV energy injected is possible to make 2.70 water cluster ions to form OH· [22]. Therefore the Strong dielectric barrier discharge is more effective in producing larger numbers of OH· radicals.

The plasma processes of OH· dissolving into water are very complicated chain reactions. OH· is the main product in the system, also having other activated particles such as HO_2^-, $HO_2\cdot$, $HO_3\cdot$, OH^-, O_3OH^+, $O_2^{-}\cdot$, $O_3^{-}\cdot$, O_3, H_2O_2 and so on.

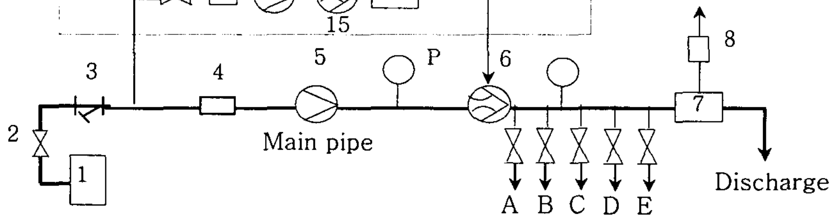

Fig. 1. 20t/h system for the treatment of ship's ballast water

Note: 1. Container of ballast water; 2. Valve; 3. Mechanical filter; 4. Liquid flow-meter; 5. Pump; 6. Liquid / Liquid dissolver; 7. and 16. Gas / liquid separator 8. and 17. Eliminator of residual OH·; 9.Controller; 10. Transformer; 11. Flowmeter; 12. OH· plasma reactor; 13. Electric valve; 14. Check valve; 15. Gas/ liquid dissolver; 18. Dissolved OH· monitor

3. EXPERIMENT METHOD

3.1. Experimental materials

The tested seawater was taken from Dalian port and stored in BC type polyethylene container, from which a part of seawater was taken and put into the glass trough of 1.5m^3. A little of liquid culture medium of 2216E were put into the trough to do the enrichment of algae and bacteria. The enrichment conditions are as follows: the temperature, 23±1°C; pH, 7.2; illumination intensity, 2600Lux. The enriched seawater was poured into the container 2 (in Fig.3), in which the contents of algae, protozoan and bacteria are above 10^4/mL. The phytoplanktons are *Chloreua Pyrenoidesa*, *Chaetocers*, and *Peridinium*; the zooplanktons are *Euplotes*; the germs are *Pseudomonas*, *Flarobacterus*, *Vibrio*, *Acinetobacter*, *Escherichia*, *Alcaligenes*, and *Staphyloccus*.

3.2. 20t/h system for the treatment of ship's ballast water

20t/h pilot-scale system for the treatment of ship's ballast water using hydroxyl radicals is shown in Fig. 1. The tested seawater was taken from Dalian port and stored in BC type polyethylene container 1. The dissolved hydroxyl radical is injected into liquid/liquid unit 6 for the adequate mixture, which the ratio concentration is 0.68mg/L in main pipeline. The sample points are in three points of A, B, C, D and E respectively, having the duration of 0.0s, 1.33s, 2.67s, 5.33s, 8.00s. The flow velocity of ship's ballast water is 1.5m/s, and the flow rate is 20t/h. The treated ballast water flows into the gas/liquid separator 7, and then is discharged. The residual hydroxyl gas is decomposed into the molecules of H_2O, O_2 in the eliminator 8.

The setup of dissolved hydroxyl radical has the dimension of 0.6m (long) ×0.8m (wide) ×1.5m (high). The self-made power supply with the voltage of 2 kV, frequency of 10kHz and pulse period of 80μs was applied to the discharge electrodes to produce a continuous strong dielectric barrier discharge (DBD). The plasma reactor of stainless steel 12 is rectangular with dimensions 260 mm long, 130 mm wide and 35mm thick. The thinner α-Al_2O_3 dielectric layers, which the dielectric constant is 10 and the thickness is about 0.25mm, are sprayed on the surface of discharge and earthing electrodes by plasma spraying technology. The discharge gap is 0.47mm. According to the theory of gas discharge, the dielectric barrier effectively restrain the enlarging of discharge current as well as the spark or arc discharge in the discharge gap. The properties of dielectric have an important role in DBD plasma. Before applying the DBD discharge, O_2 with the purity of 99.5 % and H_2O gas of 3.5% were introduced into the plasma reactor 12. The high-concentration hydroxyl radicals are injected into the gas/liquid dissolver 15 further dissolved in gas/liquid separator 16. With the mass transfer efficiency of 99.8%, the dissolved $OH^{\cdot}$ ratio concentration reaches 23.4mg/L. The residual hydroxyl gas is decomposed into the molecules of H_2O, O_2 in the eliminator 17.

This pilot scale experiment for 20t/h was done on December, 2002, the temperature of sea water is 8.5°C.

3.3. Test Methods

Bacteria test: The samples of 10mL, 100mL and 150mL were respectively taken from the sampling points, and then were diluted to 10^4 times in the asepsis condition. Three samples are taken in every point for the test. The diluent of 0.1mL is daubed on the ocean 2216E culture plate to do the count of colonies. The detached bacteria are selected from the single colony further to do the second purified separation. The bacterium genera is identified according to its character, cell configuration and test results of biochemistry.

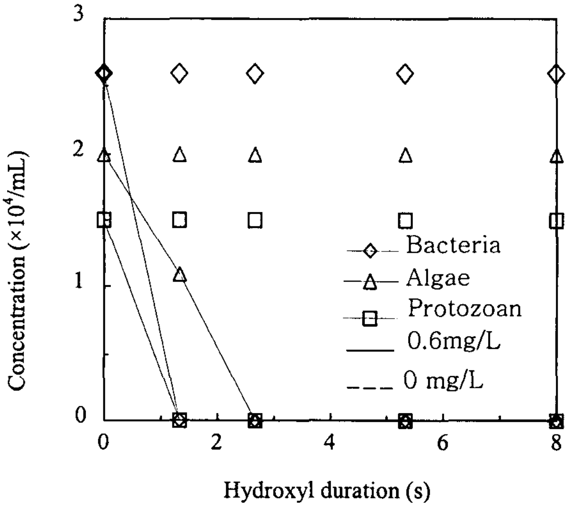

Fig. 3. Hydroxyl duration vs. organism concentration

The bacteria numbers in the sample is difficult to be accurately counted using conventional test method after killed because of the great decrease of bacterium numbers. Therefore the water sample of 150mL is used to inoculate using filter membrane method and to daub on the ocean 2216E plate to do the counts of colony.

Algae Test: The samples were taken using 2500mL asepsis glass before and after injecting OH radicals respectively, and then were identified and counted to their living bodies which were done with haemacytometer under microscope.

Photosynthetic pigments test: Following the ocean monitor standard (GB17378.7-1998), UNICO7200 type spectrophotometer is used to test.

OH· concentration: The ratio concentration of OH· is tested using Fluorescence method of benzoic acid and revised by electrochemistry method. The concentration of other activated particles is converted into the OH· concentration according to their oxidation potential.

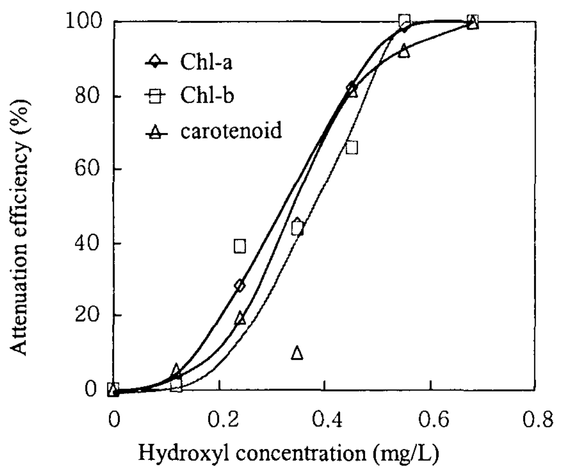

Fig. 4. Hydroxyl concentration vs. attenuation Efficiency of photosynthesis pigment

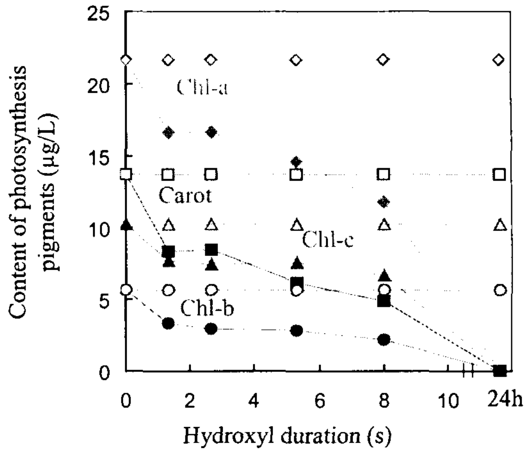

Fig.5. Hydroxyl duration vs. content of photosynthesis pigments

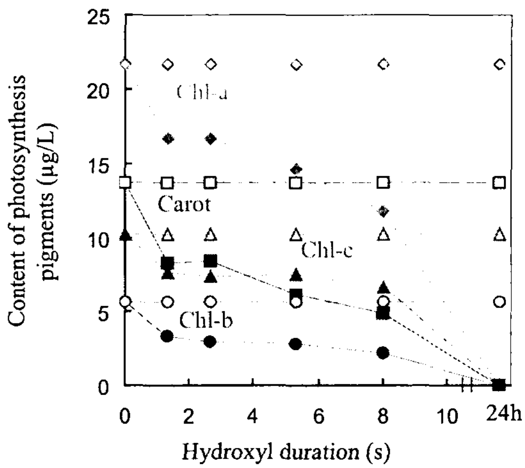

Fig.6. Hydroxyl duration vs. content of photosynthesis pigments

4. RESULTS AND DISCUSSION

4.1. Experiment for the kill of invasive species

The bacteria concentration is 2.6×10^4/mL; the mono-cell algae concentration is 2.0×10^4/mL; the protozoan concentration is 1.5×10^4/mL. After the injection of hydroxyl solution, the samples are taken out in five points of A, B, C, D and E respectively with the duration of 0.0s, 1.33s, 2.67s, 5.33s, 8.00s. The effect of hydroxyl duration on the organism concentration is shown in Fig.3. When the ratio concentration is 0.6mg/L, the duration that the hydroxyl radicals kill all of bacteria and protozoan is only 1.33s, the kill efficiencies reach 100%. When the duration is in the range of 1.33~2.67s, the concentration of algae is decreased into 1.1×10^3/mL and no-test respectively. When the duration is above 2.67s, the kill efficiencies of bacteria, mono-cell algae and protozoan are 100%. The experimental results indicate that all organisms in ballast water could be killed in ship in line.

4.2. Effect of hydroxyl radicals on the attenuation efficiency

Hydroxyl radical has strong oxidized and decolored effects on phytoplankton (mono-cell algae). The samples were taken at the point C and tested after 5 minters. The effect of OH· ratio concentration on the attenuation efficiencies of photosynthesis pigment is shown in Fig.4. When OH· ratio concentration is in the range of 0.1~0.5mg/L, the hydroxyl radicals greatly restrain the increases of Chl-a, Chl-b and carotenoid, having a sharp attenuation efficiency which increases with the increase of hydroxyl ratio concentration and their curve inflexions are 0.55mg/L.

Chl-b, Chl-a and carotenoid have the similar curves. When OH· ratio concentration is 0.55mg/L, Chl-a concentration decreases from 15.39μg/L to no-test value with the attenuation efficiency of 100%, considering chl-a to be decomposed completely. The chl-b concentration decreases from 17.5μg/L to no-test value with the attenuation efficiency of 100%, also considering chl-b to be decomposed completely. The concentration of carotenoid decreases from 13.70μg/L to 1.06μg/L, the attenuation efficiency is 92.3%. When OH· ratio concentration is 0.68mg/L, the attenuation efficiency of carotenoid is 100%. Therefore, the hydroxyl radicals have much stronger effects on chl-a, chl-b and carotenoid.

4.3. Effect of hydroxyl radicals on the photosynthesis pigments

The effect of hydroxyl duration on the photosynthesis pigments is shown in Fig. 5. The original contents of chl-a, chl-b, chl-c and carotenoid are 21.69μg/L, 5.62μg/L, 10.32μg/L and 13.74μg/L respectively. When the dissolved OH· ratio concentration was 0.6mg/L in the main pipe of ballast water, the samples were taken at the five point of A, B, C, D and E respectively and the experiments of photosynthesis pigment were done. The curves of chl-a, chl-b, chl-c andcarotenoid are very similar, and the pigment contents decrease to 11.81μg/L, 2.16μg/L, 6.71μg/L and 4.89μg/L respectively after 8 seconds. The all pigment contents were no test after 24 hours.

All of the experimental data indicate that the hydroxyl radicals make phytoplankton lose its activity finally resulting in all mono-cell algae to be killed.

4.4. Effect of hydroxyl radicals on the phaeophytin

The effect of hydroxyl radical on the phaeophytin of phytoplankton is shown in Fig. 6. The original content of mono-cell phaeophytin in the ballast water is 4.78μg/L. With the hydroxyl concentration of 0.6mg/L after 8 seconds, the content of phaeophytin is increased to 47.21μg/L, the content increases about 10 times. Therefore, hydroxyl radicals have very strong effect on the photosynthesis pigment of phytoplankton.

5. CONCLUSIONS

The treatment of ship's ballast water using OH· radicals is a kind of advanced oxidation method, which realizes Atom Economy, Zero Emission and Zero Pollution in the process of the production of OH· radicals and the kill of organisms of ship's ballast water. Invasive marine species can be killed in ship in the process of the discharge or inputting ballast water.

(1) OH· radicals are dominantly produced from the positive ions O_2^+ reacting with H_2O to form the water cluster ions.
(2) With the 20t/h pilot-scale system for the treatment of ship's ballast water, the kill efficiencies of bacteria, mono-algae, protozoan reach 100% within 2.67s when dissolved OH· concentration is 0.6mg/L.
(3) The contents of chl-a, chl-b, chl-c and carotenoid are decreased to 35%-64% within 8.0s further to the lowest limit of test after 5 minutes. The content of phaeophytin is increased to ten times. Hydroxyl radicals make phytoplankton lose its activity finally resulting in all mono-cell algae to be killed.
(4) The equipment of dissolved hydroxyl radicals has some advantages such as small volume, simple operation and low running cost which is only 1/30 in comparison with the open-ocean-exchange of ship's ballast water.

REFERENCES

[1] The problem, Global Ballast Water Management Program. html

[2] Gregory M Ruiz etc, "Global spread of microorganisms by ships", *Nature*, 2000, 408:49~50

[3] Mackenzie etc, "Alien invaders", *New Scientist*, 1999, 162:18~19

[4] Geoff Rigby, "From ballast to bouillabaisse", *Science*, 2000, 289:241

[5] Donald M Anderson, "Turning back the harmful red tide", *Nature*, 1997, 388:513~514

[6] Hallegraeff G M, Bolch C J, "Transport of Diatom and Dinoflagellate Resting Spores in Ships' Ballast Water: Implications for Plankton Biogeography and Aquaculture", *J. Plankton Res*, 1992, 14(8): 1067~1084

[7] Carlton J T., "Biological Invasions and Biodiversity in the Sea: The Ecological and Human Impacts of Non-indigenous Marine and Estuarine Organisms", *Proc. Conf. Wkshop*. NOAA, 1994, 5~11

[8] Holmes J M C, Minchin D., "Two Exotic Copepods Imported into Ireland with the Pacific Oyster *Crassostrea gigas* (Thunberg)", *Ir. Nat. J.*, 1995, 25:17~20

[9] Bai Xiyao, Bai Minong, Yang Bo, "Studies on the Disaster and Treatment of Alien Invaders", *J. Nature (China)*, 2002, 24(4): 223~226

[10] Rigby, G. and Hallegraeff, G. M., "Ballast Water Exchange Trials and Marine Plankton Distribution on the MV "Iron Whyalla"", *Australian Government Publishing Service*, Canberra, 1993, Vol. 2, p.123

[11] Yount, J. D., "Ecology and Management of the Zebra Mussel and other Introduced Aquatic Nuisance Species", *EPA Workshop on Zebra Mussels and other Introduced Aquatic Nuisance Species*, Saginaw Valley State University, USA, 1990, pp. 1-45

[12] Armstrong, G., "Ballast System Design for a Flow-through Exchange of Ballast Water", *Prepared for a technical meeting at the Institute of Marine Engineers*, London, 25 March, 1997, p. 11

[13] Müller, K.:, "Disinfection of Ballast Water. A Review of Potential Options", *Lloyd's Register*, 1995, p. 29+ appendix

[14] Bai Mindong, Zhang Zhitao, Bai Xiyao et al, "Plasma Synthesis of Ammonia with a Microgap Dielectric Barrier Discharge at Ambient Pressure," *IEEE Trans. Plasma Sci.*, 2003, 31 (6)

[15] Bai Xiyao, Zhang Zhitao, Han Hui et al, "Research Situation and Progress of Non-equilibrium Plasma Chemistry," *Chinese Science Bulletin (China)*, 2002, 47(7): 529-530

[16] Zhang Zhitao, Xian Yuze, Bai Mindong, "Studies of Strong Ionization Discharge", *Journal of Northeastern University*, 2002, 23(5):207~510

[17] Bai Mindong, zhang Zhitao, Bai Xiyao et al, "The killing of organisms in ship's ballast water using hydroxyl radicals", *Oceanologia et Limnologia Sinica (china)*, 2003, 34(5): 484~489

[18] Zhou Xiaojian, Bai Mindong, Deng Shufang, "Study on Killing Monads in Ballast Water by Hydroxyl Radical Pharmaceuticals", *Environmental Protection in Transportation (China)*, 2002, 23(5):1~3

[19] Bernie M Penetrante, J Norman Bardsley, Mark C Hsiao, "Kinetic Analysis of Non-Thermal Plasmas Used for Pollution Control," *Jap. J. Appl. Phys*, 1997, 36(7B): 5007-5017

[20] Sun Chunpu, Zhang Jianzhong, Duan Shaojin, "Free Radical Biology Introduction," *China Science and Technology University Publication*, Hefei, China, 1999.