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<td>Yang, Zhishan; Jiang, Wenju; Zhang, Yi; Lim, Tuti Mariana</td>
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Inactivation of Dinoflagellate *Scripsiella trochoidea* in Synthetic Ballast Water by Advanced Oxidation Processes

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| Complete List of Authors: | Yang, Zhishan; Sichuan University, College of Architecture and Environment  
Jiang, Wen-ju; College of Architecture and Environment, Sichuan University,  
Zhang, Yi; Fudan University, Department of Environmental Science and Engineering  
Mariana, Lim Tuti; Nanyang Technological University, School of Civil & Environmental Engineering |
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Inactivation of Dinoflagellate Scripsiella trochoidea in Synthetic Ballast Water by Advanced Oxidation Processes

YANG Zhishan\textsuperscript{b}, JIANG Wenju\textsuperscript{b}, ZHANG Yi\textsuperscript{*a} and T.M. LIM\textsuperscript{c}

\textsuperscript{a} Department of Environmental Science and Engineering, Fudan University, 220 Handan Road, Shanghai, P. R. China, 200433.

\textsuperscript{b} College of Architecture and Environment, Sichuan University, Chengdu 610065, P.R. China. Email: yzs126@127.com; jwj@263.net

\textsuperscript{c} School of Civil and Environmental Engineering, Nanyang Technological University, 639798, Singapore.

* Co-corresponding authors:
Tuti Mariana LIM, E-mail: TMLim@ntu.edu.sg, Tel: 65-6790 5269.
ZHANG Yi, Email: zhang-yi@fudan.edu.cn, Tel: 86-21-5566 4354.

Abstract: Ship borne ballast water contributes significantly to the transfer of non-indigenous species across aquatic environments. To reduce the risk of bio-invasion, ballast water should be treated before discharge. In this study, the efficiencies of several conventional and advanced oxidation processes (AOPs) were investigated for potential ballast water treatment, using a marine dinoflagellate species, Scripsiella trochoidea, as the indicator organism. A stable and consistent culture was obtained and treated by ultraviolet light (UV), ozone (O\textsubscript{3}), hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}), and their various combinations. UV apparently inactivated the cells after only 10s of irradiation, but subsequently, photo-reactivation of the cells was observed for all methods involving UV. O\textsubscript{3} exhibited 100\% inactivation efficiency after 5min
treatment, while H$_2$O$_2$ only achieved maximum 80% inactivation in the same duration. Combined methods, e.g. UV/O$_3$ and UV/H$_2$O$_2$ were found to inhibit photo-reactivation and improve treatment efficiency to some degree, indicating the effectiveness of using combined treatment processes. The total residual oxidant levels of the methods were determined, and the results indicated that UV and O$_3$ generated the lowest and highest total residual oxidants (TRO), respectively. The synergic effect of combined processes on TRO generation was found to be not significant, and thus UV/O$_3$ was recommended as a potentially suitable treatment process for ballast water.

**Keywords:** Ballast Water; Water Treatment; Dinoflagellate, Advance Oxidation Processes; *Scrospiella trochoidea*; Inactivation; Photo-reactivation.

1. Introduction

Ballast water is the water stored on board to stabilize ships while sailing (Carlton et al. 1995). It is usually taken when ships are in ports, therefore can contain various fresh and sea water organisms, which can be transported to other places and introduce invasive species into new environments (Tsolaki & Diamadopoulos 2009). For example the zebra mussel *Dreissena polymorpha*, introduced via ballast water into the Great Lakes of North America is estimated to have caused damages of $5 billion between 1985 and 1986, by blocking water intakes and fouling fishing nets, buoys and boat hulls (Ribera 1995). As a consequence, ballast water has been identified as one of the major threats to the global aquatic environment (Bax et al. 2003). To minimize the risk of bio-invasion by ballast water transportation, ships are now required to do ballast water exchange in open sea (IMO 2005). However, deballasting and ballasting during voyage on high seas could expose ships to potential dangers, e.g. physical
stresses on the hull and loss of stability (Bellefontaine et al., 2010). Ships have been recorded to almost capsize during ballast water exchange, (which could severely endanger the ship personnel. Therefore ballast water treatment before discharge is drawing increasing interest as an alternative or supplementary measure to ballast water management and pollution control (Balaji & Yaakob 2011).

Various ballast water treatment technologies are being developed, which could be classified into three categories: mechanical, including filtration (Parsons & Harkins 2000), hydrocyclone (Waite et al. 2003), and supersaturating gas system (Wright et al. 2003); physical, e.g. heating (Thornton & Chapman 2003), electrical fields (Leffler et al. 2003), ultraviolet radiation (Sassi et al. 2005), and ultrasound (Viitasalo et al. 2005); and chemical, e.g. ozonation (Herwig et al. 2006, Perrins et al. 2006), deoxigenization (McCollin et al. 2007), and various biocides (Raikow et al. 2006, Gregg & Hallegraeff 2007, Wright et al. 2007). A suitable and successful onboard ballast water treatment system should be characterized by its good effect on a wide variety of organisms, high treatment efficiency, low capital and operational cost, safety and ease of use, and no adverse effects on the environment upon discharge (Lloyd’s Register 2010). Cost effective method that meets all the requirements is yet to be found, and research is ongoing for various possible treatment processes.

Conventional oxidation technologies such as ozone (O$_3$) and hydrogen peroxide (H$_2$O$_2$) have been extensively studied in the degradation of various organic substances in fresh water (Glaze et al. 1987, Alvarez et al. 2001). Advanced oxidation processes (AOPs), such as ultraviolet radiation (UV), photocatalysis and combinations of UV, O$_3$, and H$_2$O$_2$ have recently emerged as promising technology for rapid treatment of many organic compounds (Azbar et al. 2004). These processes can produce highly
powerful and non-selective oxidant species like hydroxyl radicals, showing improved efficiency and wide substrate range (Tabrizi & Mehrvar 2004). In recent years, researchers have started to apply oxidation processes in ballast water treatment. O\textsubscript{3} has been tested with bacteria spores, dinoflagellate algae, and organisms in actual seawaters (Perrins et al. 2006), and H\textsubscript{2}O\textsubscript{2} and UV have been used alone or in combination with other oxidation methods to remove various marine species from ballast water (Viitasalo et al. 2005, Smit et al. 2008, Wu et al. 2011). Some of these processes, e.g. ozonation has gone through ship-board trials and are on the way to commercialization (Wright et al. 2010). However, researchers have suggested that, due to the diverse and often varied nature of the microbial composition in natural seawater, a single process might not be sufficient to remove all potentially dangerous species from ballast water (Oemcke & van Leeuwen 2004). Therefore combined advanced oxidation method has potential advantage in ballast water treatment, and their application can benefit from more detailed study.

Up to now the effectiveness of AOPs has not been fully tested in seawater, which has higher salinity and ionic strength compared to fresh water. This study intends to obtain a full comparison of treatment efficiency by various conventional and advanced oxidation methods, namely UV, O\textsubscript{3}, H\textsubscript{2}O\textsubscript{2}, UV/O\textsubscript{3}, and UV/O\textsubscript{3}/H\textsubscript{2}O\textsubscript{2}, using marine alga dinoflagellate as the indicator organism. Dinoflagellates are among the most commonly found toxic organisms in ballast waters and sediments. Due to their prevalence and recalcitrance to treatment processes, they are often used as model species to assess treatment effects (Bolch & Hallegraeff 1993; Montani & Ichimi 1995). In this study, a stable vegetative dinoflagellate culture was maintained and subjected to various treatment procedures. Samples after treatment were obtained and inoculated into fresh medium, and the subsequent regrowth was observed to simulate
the scenario of treatment during deballasting. Dinoflagellate has seldom been tested with AOPs before; therefore this study could further explore the applicability and efficiencies of AOPs in ballast water treatment, especially in a tropical setting.

2. Materials and methods

2.1. Experimental setup

The schematic diagram of the experimental setup is provided in Fig. 1. The reactor was of cylinder shape and made of glass, with a dimension of 6 cm×35 cm (outer diameter×height) and a working volume of 250 mL. A UV lamp was encased in a quartz tube of O.D. 4 cm, and placed at the centre of the reactor to supply the reaction mixture with UV irradiation. Air w/o ozone was pumped into the reactor through Teflon tubing and a glass sinter disk, which was set at the bottom of the reactor vessel. The ozone generator used in this study was supplied by Viresco Singapore Pte. Ltd. The rate of ozone generation as determined by an iodometric method (IOA 1998) was 190 ± 8.3 mg h⁻¹. For treatment involving UV, a 15 W, 253.7 nm lamp (Qidong Fluorescent Lamp Factory, China) was used. The light intensity of the UV lamp was 7.72 ± 0.06 mw cm⁻² as measured at a 1 cm distance by a VECTOR H410 radiometer (SCIENTECH BOULDER CO. Model No, AC5000, USA).

2.2. Vegetative dinoflagellate cultures and culturing conditions

The vegetative culture of a red tide-causing dinoflagellate species, *Scrippsiella trochoidea* was provided by the Tropical Marine Science Institute (TMSI) of Singapore. The cells are approx. 20 µm of size and have typical thecal plate pattern and longitudinal flagellum (Lewis 1991), the morphology of which can be seen in Fig. 2 (a)-(c). The cultures were grown aseptically in 2 L conical flasks containing 500 mL...
synthetic ballast water with f/2 supplementation (Guillard 1975). The incubation conditions were as follows: temperature, 25 ± 1°C; pH, 8.2 ± 0.1; and 12 h D: 12 h L cycles. Lighting was provided by 9 fluorescent light bulbs with the power of 28 W each, and the distance between the culture and the light source was fixed at 60 cm. The light intensity as reached to the flask was measured to be 1.58 ± 0.03 mw cm⁻².

Two kinds of ballast water were used in this study. Natural sea water was obtained from west Singapore coast and storing in sealed 20L polyethylene containers in a dark and cool cupboard for 4 weeks. The turbidity of the original sea water was measured to be 8.13±1.73 NTU, and its salinity approx. 27±3 practical salinity unit (psu). Alternatively, Sea Salt® (Sigma, SG) was added in Milli-Q water to a final concentration of 30 g L⁻¹ and salinity of 35 psu to produce synthetic sea water. The advantage of the synthetic sea water was its constant composition, hence producing consistent and comparable results. However it might not represent the real sea environment adequately, therefore natural sea water was also used to simulate the local tropical marine setting. The two ballast waters were further filter-sterilized through 0.2µm pore size autoclaved membrane filters before use, and the same medium composition and culturing process were maintained for both kinds of waters throughout the study. F/2 medium is a common nutrient combination to stimulate marine organism growth, and its effect was also tested.

2.3 Treatment and regrowth of *Scrippsiella trochoidea* cells

When cell density reached 10⁴ cells mL⁻¹, 250 mL culture was poured into the reactor to be treated by various oxidation processes. In O₃ treatment, ozone generator was engaged and the gas flow rate was set at 6 L min⁻¹. For treatment by UV or H₂O₂, the UV lamp was turned on or liquid H₂O₂ (Sigma Aldrich) was added into the reactor,
while the air flow was maintained without ozone generation. Control samples were bubbled with air only at the same flow rate.

Samples were taken and mortality determined directly after treatment to determine the treatment efficiency. However some researchers discovered that certain organisms have the ability to recover after a period of incubation (Sutherland et al. 2001). Therefore in this study cell viability was indirectly measured by studying the regrowth potential in a grow-out test (Wright et al. 2010), which is not often performed before. 10 mL samples were taken at regular treatment time intervals, and inoculated into 500 mL conical flasks containing 50 mL culturing medium. Cells were allowed to regrow under the same conditions applied in the incubation, and active cells were counted at various intervals subsequently. This could also serve as a simulation to the situation after ballast water discharge into the marine environment. The inactivation efficiency was calculated by the regrowth data after 24h incubation, according to the equation:

\[
\text{inactivation efficiency (\%) = 1} - \frac{C_t}{C_0},
\]

where \( C_0 \) is the cell abundance at treatment time 0 and \( C_t \) is the cell abundance at treatment time \( t \), determined after 24 h grow-out.

2.3. Analytical Methods

Cells were counted using a Sedgewick Rafter counting chamber (50×20×1mm) with a microscope (KEYENCE VH-Z75, Japan) under 100× magnification. Three samples were taken at each sampling point, and three independent sub-cells on the chamber were counted at random for each sample. Moving cells were considered as active, and an assumption was made that the unmoving and missing cells had been inactivated or destroyed by the treatment (Viitasalo et al. 2005). The results were averaged, and the counts were converted to the unit of cell mL\(^{-1}\). The inactivation efficiency was
calculated as the concentration changes of active *S. trochoidea* cells before and after treatment.

The morphologies of *S. trochoidea* cells were observed both with a light and a scanning electron microscope (SEMJSM-5310 LV JEOL, Tokyo, Japan). For SEM observation, the samples were filtered through 0.45µm pore size membranes and fixed with 2% glutaraldehyde for 4 h. The fixed cells were washed by 0.1 mol L⁻¹ sodium cacodylate buffer three times and left for 20 min each time. Cells were subsequently dehydrated in a series of 10 mins washing with 50, 70, 85, 95% ethanol, respectively, and stored in 100% ethanol. The fixed samples were dried with a Critical Point Dryer (E3000; VG Microtech, Eas Grinstead, West Sussex, UK), and then sputter coated with Au-Pd (SPI-Module Sputter coater Structure Probe, West Chester, PA, U.S.A), and finally observed by SEM.

The pH of the solutions was maintained in alkaline range at approx. 8.2±0.1, and was measured with an YSI pH100 portable pH meter. The salinity was determined by a conductivity meter (Hach sensION-5 meter) and turbidity of sea water was measured with a turbidity meter (Hach 2100N turbidimeter). The total residual oxidant (TRO) level was determined as bromine concentration by a HACH DR2400, US method 8016, and a DPD Total Chlorine Reagent Powder Pillow commercially available.

3. Results and discussion

3.1. Cultivation of *Scrippsiella trochoidea* cells

In order to study and compare the treatment effects of various oxidation processes, it was necessary to first obtain a stable and consistent culture of the target organism.
Therefore various conditions in the culturing of the *S. trochoidea* cells were first investigated, which included the incubation media, length and inoculation ratio.

Fig. 3(a) compares the growth trends in four types of media, i.e. synthetic saline water or natural sea water, with or without f/2 addition. In both waters, f/2 addition stimulated cell growth and resulted in higher cell abundance in the stationary phase, which could be due to the macronutrients, micronutrients, and vitamins in f/2 providing supplements for microbial growth. Comparing growth trends in the synthetic and natural sea waters, it was observed that w/o f/2 addition, the maximum cell count was much higher in the synthetic saline water than in the natural sea water, likely caused by its higher nutrient content. However, growth in saline water showed a longer lag phase, which might indicate an adaptation process to the synthetic environment. As cultures of 5~10 days age in both types of waters showed similar viable cell counts, the combination of natural sea water (from the same batch) with f/2 addition as growth stimulant was used in the subsequent studies as the medium to obtain stable and active cells.

The inoculation ratio to start a new culture was further investigated as a relevant cultivation parameter, with the aim to determine a suitable ratio that would ensure a healthy and fast-growing sub-culture. Fig. 3(b) shows the growth trends from 5 inoculation ratios, i.e. 5%, 10%, 20%, 30% and 40%, respectively. In 8 days incubation, higher ratio resulted in higher cell abundance, but not necessarily higher growth rate. Therefore, a medium ratio of 20% was chosen for the subsequent studies. A full growth pattern obtained with this ratio is presented in Fig. 3(c), which shows that active growth was sustained until around 2 weeks into the incubation, by which time the viable cell density reached approx. $1.7 \times 10^4$ mL$^{-1}$, a number adequate for
accurate counting (Granéli 2006). Therefore in all subsequent experiments, the cultures were harvested around 1–2 weeks after inoculation, ensuring that each batch used in the treatment study had similar age and properties, and thus yielded comparable data.

3.2. Treatment with various individual and combined oxidation methods

3.2.1. Effect of aeration only on cell activity

As the treatment processes employed aeration as the means of mixing, the effect of aeration alone on *S. trochoidea* cell activity was first studied as the negative control. 250 mL of culture was poured into the reactor, through which air only was bubbled at a flow rate of 6 L min\(^{-1}\). Every minute, a sample of 10 mL was drawn from the reactor and inoculated into 50 mL of medium made of f/2 in natural sea water. After 24h incubation, the abundance of active cells in each culture were counted, and the result shown in Table 1. In 5 min of aeration, no significant change in the number of active cells was observed, therefore the effect of aeration only on *S. trochoidea* cells activity was considered negligible within the test’s time frame, and any deactivation observed in the subsequent tests could be attributed to the oxidation processes employed.

3.2.2. Treatment by ozone in natural and synthetic sea waters

To evaluate the effect of different medium compositions on the activity of the reactive species generated by the oxidation processes, the cells’ relative susceptibilities to a common oxidation method, ozone, were assessed in both media as an indication. Cells cultured in synthetic and natural sea waters (both with f/2 addition) were treated with O\(_3\) of 190 mg h\(^{-1}\) strength (flow rate 6 L min\(^{-1}\)), and the sampling, incubation and enumeration methods were carried out as described in the previous section for the control test. Fig. 4(a) shows that 100% inactivation was achieved by O\(_3\) in both types
of media, and no significant difference was observed thereof. The data suggested that
the reactive species generated reacted with the media and the cells in a similarly
efficient way, and the selection of incubation medium was viable. Furthermore, three
other dosages of O$_3$ were applied to the culture, and their effects are compared in Fig.
4(b). Higher dosage of O$_3$ was observed to inactivate *S. trochoidea* cells with
improved efficiency, i.e. dosages 190 and 228 mg h$^{-1}$ achieved virtually 100%
inactivation within 4 min, while 108 mg h$^{-1}$ of O$_3$ only achieved 85.6% inactivation
after 5 mins treatment. However, higher dosage also entails higher cost, and the
cost-efficiency of the method need to be studied in more detail in future work.

O$_3$, which has been used as a disinfectant since the late 1800s, is currently being
studied as a ballast water treatment method for commercial vessels (Perrins et al.
2006). It is a naturally occurring allotrope of oxygen and has a high oxidation
potential. In seawater, O$_3$ can react with bromide to form secondary oxidants,
bromines, which are proposed to be the major oxidizing agents causing motility in
various organisms (Hoigné et al. 1985, Kornmueller 2007). Direct ozone treatment
effect (i.e. by molecular ozone itself) and indirect effect (by bromines) could inactive
cells by disrupting the cell wall and causing the subsequent release of cellular
materials (Schneider et al. 2003), or alter the structures of various cellular
macromolecule, hence resulting in cell inactivation and death. This is also consistent
with our SEM analysis as shown in Fig. 2(d) which displays the rupture of cell surface
and distortion of cell structure of the O$_3$ treated cells.

3.2.3. *Treatment by H$_2$O$_2$ alone*

H$_2$O$_2$ is a relatively strong oxidant with the oxidation potential of 1.76V (Madhavan et
al. 2008), and its effect on *S. trochoidea* was tested in this study. 250 mL culture was
placed in the reactor, and H₂O₂ was added to the final concentrations of 10, 40 and 100 mgL⁻¹, respectively. The results are shown in Fig. 4(c), which indicates that the inactivation efficiency increased with increasing H₂O₂ concentration. After 5 mins treatment, the inactivation efficiencies reached 57.4%, 67.7% and 87.2% for H₂O₂ concentrations of 10, 40 and 100 mgL⁻¹, respectively. However, the efficiencies under all H₂O₂ dosages exhibited a limit, upon which prolonged treatment time didn’t show improvement. Higher H₂O₂ dosages achieved the maximum effect more rapidly, i.e. the inactivation efficiency under 10, 40, and 100 mg L⁻¹ H₂O₂ reached the plateau at around 4, 3 and 1 min, respectively.

Previous studies have used H₂O₂ to treat diverse marine organisms, with various results. An H₂O₂ concentration of 10 ppm and contact time of 1 min was enough to inactivate a ctenophore, Mnemiopsis leidyi, and 1 ppm H₂O₂ could be lethal to plankton of a wide phylogenetic diversity (Kuzirian et al. 2001). On the other hand, Gavand et al. compared the effects of sonication and advanced chemical oxidants on unicellular green algae and cysts, larvae and adults of the brine shrimp, and found 100 ppm H₂O₂ was unable to achieve 100% mortality within 20 mins of treatment (Gavand et al. 2007). In this study, it was found that H₂O₂ was a poor choice for S. trochoidea inactivation in comparison to O₃, as 100% efficiency wasn’t achieved even at a high H₂O₂ concentration of 100 ppm and reasonably long treatment duration. Moreover, commercially available H₂O₂ is a toxic liquid and could pose hazard when stored on board. Therefore, for safety reason as well as the economical concern, H₂O₂ alone is not recommended as a suitable method for treating ballast water.
3.2.4. Treatment by UV alone

250 mL culture was treated by UV under the same air flow rate as used before, and it was discovered that within only 10 seconds of treatment, total apparent inactivation of *S. trochoidea* was achieved, while 2s and 5s UV irradiation resulted in 76% and 93% inactivation, respectively (data not shown). The disinfection ability of UV irradiation lies in the radiation in the UV-C spectral region, which can cause harmful changes in DNA structure. 254nm wavelength UV light can damage DNA and RNA by photo-induced dimerisation of adjacent pyrimidine in the nucleic acid strand. Because of the covalently bound pyrimidine residues, replication of the nucleic acid is hampered or completely blocked, the effect of which could be temporal or lethal depending on the repair mechanisms and the degree of UV resistance (Hess-Erga et al. 2008). In this study it seems that within 24h of regrowth period, the *S. trochoidea* cells could not overcome the negative effect of UV and achieved recovery. However, the inactivation of *S. trochoidea* cells by UV mostly exhibited as the lack of movement of the cells, which might not be equal to cell death. The morphology of UV treated cells can be seen in Fig. 2(e), which indicates that the cell structure was intact.

3.2.5. Treatment by combination of *O*$_3$/H$_2$O$_2$

To observe if *O*$_3$ and H$_2$O$_2$ had any synergic effect, 250 mL culture was placed in the reactor, and *O*$_3$ was provided together with H$_2$O$_2$ dosages of 10, 40 and 100 mgL$^{-1}$. Fig. 4(d) shows the inactivation efficiencies under various *O*$_3$/H$_2$O$_2$ combinations. The total efficiency of the combined method appeared to be lower than that of *O*$_3$ alone, but showed improvement on those achieved with H$_2$O$_2$ of the respective dosages. However, the efficiency plateau was again observed with the two higher dosages, and the H$_2$O$_2$ dosage effect was not so pronounced at the presence of *O*$_3$. The combination of *O*$_3$/10 mg L$^{-1}$ H$_2$O$_2$ did not exhibit efficiency limit, and showed the
highest removal after 5 min treatment. It was postulated that the combined method could generate hydroxyl radical (•OH), a strong and unspecific oxidant, in fresh water (Ormad et al. 1997). It was possible that this effect was hampered by the sea water medium, or the excessive H$_2$O$_2$ acted as scavenger to the reactive species, impairing the killing efficiency on the dinoflagellate *S. trochoidea* cells (Kusic et al. 2006).

3.2.6. Treatment by UV/O$_3$, UV/H$_2$O$_2$ and UV/O$_3$/H$_2$O$_2$ methods

UV alone has shown remarkable effect on *S. trochoidea* cells. To evaluate the effect of UV in combination with other oxidation methods, two batches of treatment tests were further conducted, using UV/O$_3$, UV/10 mgL$^{-1}$ H$_2$O$_2$ and UV/O$_3$/10 mgL$^{-1}$ H$_2$O$_2$ combinations. Table 2 shows the results, where both methods exhibited 100% inactivation after 5 s of treatment. UV/10 mgL$^{-1}$ H$_2$O$_2$ also achieved apparently total inactivation within 10 s, as indicated by 24 h regrowth test shown in Table 3. In comparison, Wu et al. (2011) conducted an inactivation test on a dinoflagellate *Amphidinium* species, and observed low efficiency with UV or ozone alone (15 and 30% after 3 min, respectively), but the combination of the two gave an efficiency of 47%. Because of the fast inactivation by UV alone, the synergic effect of the combined methods was not obvious in this study. However it might be more advantageous to treat real ballast water with combined methods, as they can generate reactive species via multiple mechanisms, suitable for the wider diversity of microorganisms in real sea water.
3.3. Photo-reactivation and the advantage of AOPs

3.3.1. Comparison of photo-reactivation of \textit{S. trochoidea} cells after various UV-related treatments

In the previous section, it was discovered that UV radiation was apparently the most efficient method to inactivate \textit{S. trochoidea} cells. However, numerous studies have reported that microorganisms might resurrect after treatment by UV radiation (Eker et al. 1991, Sancar 2000). This could pose a problem to ballast water treatment if UV is used as the only treatment process during deballasting, as a false high removal efficiency achieved with short UV treatment could lead to subsequent discharge of unsafe ballast water. In order to ascertain if photo-reactivation would occur after UV treatment in this study, samples were obtained and inoculated into fresh medium after 10s treatment by all methods related to UV, i.e. UV, UV/10 mgL$^{-1}$ H$_2$O$_2$, UV/O$_3$ and UV/O$_3$/10 mgL$^{-1}$ H$_2$O$_2$, respectively. Subsequently the number of active cells was counted periodically, and the results are listed in Table 3.

Active cells were observed after 48h of inoculation in all samples except those treated by UV/O$_3$, but the latter also showed photo-reactivation after 72h. The counts of active cells increased significantly with incubation time in all samples after a lag phase of around 9 days, and the highest result after 25 days incubation was observed with the “UV only” method. Therefore, photo-reactivation did occur, and it needs to be taken into consideration in real practices if the ballast water is to be treated by UV-related processes. Combined methods generally showed less significant photo-reactivation than UV alone, but the number of active cells also increased to above $10^4$ mL$^{-1}$ after 25 days. This might be due to the short treatment time used in the test (10s), which might not be long enough for H$_2$O$_2$ or O$_3$ to take effect.
3.3.2 Longer treatment by UV and UV/O₃ method

As 10s proved to be insufficient to prevent photo-reactivation of *S. trochoidea* cells, the treatment time was prolonged to 15, 30, and 60s, respectively, for UV and UV/O₃, and the subsequent photo-reactivation was also observed. Table 4 shows that, for the UV method, longer irradiation time resulted in less photo-reactivation. The effect was most pronounced when the treatment time was prolonged from 15s to 30s, but further increase to 60s was less effective. It was possible that longer UV radiation, e.g. 30s was sufficient for total inactivation of the cells. However, combining O₃ with UV significantly reduced photo-reactivation under shorter treatment duration (15s), suggesting the synergic effect of advanced oxidation might be able to cause more severe cellular damage, thus inhibit photo-reactivation to a greater extent. Therefore AOPs have the potential advantage to achieve shorter retention time and maintain killing effect while used during ballasting or deballasting, hence improve the overall efficiency. Moreover, the effect of UV is directed related to its penetration depth, which is significantly affected by the turbidity of the water (Tsolaki & Diomadopoulos 2010). In real practice the ballast water is likely to be collected from various areas, and the actual turbidity beyond control. The combination of several oxidation processes could help to overcome the limit posed on UV alone by variation in water turbidity.

3.4. Total residual oxidant of the various methods

The total residual oxidant (TRO) level is an important parameter in evaluating ballast water quality and treatment efficiency. In the case of treatment during ballasting, a proper TRO concentration can ensure a residual killing effect on the organisms in the ballast water storage tanks, but too high a level could lead to tank corrosion. If treatment occurs during deballasting, the residual oxidant level in the discharged
water has to be controlled so it doesn’t cause toxic effect on the marine environment.

In this study, the TRO level in natural sea water was measured as mg Br$_2$ L$^{-1}$ after 10 and 300s treatment, respectively, by various oxidation processes (Table 5). Among the single methods, UV gave the lowest TRO and O$_3$ the highest, and both methods exhibited higher TRO with longer treatment time. H$_2$O$_2$, on the other hand, showed a medium level TRO after 10s, which slightly decreased after 5 min. For the AOPs, any methods associated with H$_2$O$_2$ also exhibited a similar trend that longer treatment resulted in slightly lower TRO, which could be due to the decomposition of H$_2$O$_2$ with aeration.

In terms of synergic effect, the combination of UV/O$_3$ had a similar TRO level as the addition of the two single methods. This was consistent with the results of S. trochoidea inactivation, where the combined method didn’t exhibit improved efficiency over UV alone. The TRO level generated by O$_3$ in 10s was very low, which might not have made significant contribution in the combined AOP. Wu et al. (2011) studied the inactivation of Amphidinium sp. by photocatalytic process/AOPs and observed a similar result that UV alone did not generate additional TRO and the synergic effect was not significant. However it might not be a disadvantage, as a lower TRO level might be preferred if the water is discharged immediately after treatment. In this sense, the method UV/O$_3$ is recommended with 15~30 s treatment duration. This combined UV/O$_3$ treatment is promising as it gives high treatment efficiency and fewer tendencies for the organisms to revive after discharge. In addition, it also results in lower level of residual toxicity to the marine environment.
4. Conclusion

- Several conventional and advanced oxidation processes were tested as potential treatments for ballast water, using a dinoflagellate species, *Scripsiella trochoidea* as the indicator species.
- UV radiation was found to be a quick and effective technology for apparent cellular inactivation.
- O₃ had good inactivation efficiency and the cells showed cellular damage.
- H₂O₂ alone was not efficient in ballast water treatment, due to its high cost and requirement of storage space onboard.
- Photo-reactivation was observed with treatments associated with UV.
- UV/O₃ and UV/O₃/H₂O₂ inhibited cell regrowth to some degree, especially under short treatment duration.
- UV generated the lowest level of total residual oxidant, and O₃ the highest. H₂O₂ might decompose with longer aeration time, causing TRO to drop.
- UV/O₃ of short duration could be a good combination for ballast water treatment, as it provided good effect, high efficiency and low TRO upon discharge.

Acknowledgement

The authors would like to express their deep appreciation to the TMSI (Tropical Marine Science Institute) of Singapore, for the supply of the dinoflagellate species *Scripsiella trochoidea* and advice on its cultivation.
References


Carlton JT, Reid DM, van Leeuwen H (1995). The role of shipping in the introduction of nonindigenous aquatic organisms to the coastal waters of the United States (other than the Great Lakes) and an analysis of control options. Report No. CG-D-11-95 National Technical Information Service Springfield, Virginia


URL: http://mc.manuscriptcentral.com/tent


International Ozone Association (IOA) (1998) Iodometric method for the determination of ozone in a process gas


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Figure captions

Figure 1. Schematic diagram of the reactor system.

Figure 2. Images of *S. trochoidea* cells at various treatment stages: (a) original cells under light microscope, 750×; (b) before treatment under SEM, 750×; (c) before treatment under SEM, 5000×; (d) after 4 mins treatment by O₃; (e) after 1 min treatment by UV; (f) after 1 min treatment by UV/O₃.

Figure 3. Comparison of growth trends under various conditions. (a) Growth trends with and without f/2 medium addition in synthetic saline water and natural sea water. (▲) natural sea water with f/2 medium; (■) natural sea water without f/2 medium; (◇) synthetic saline water with f/2 medium; (□) synthetic saline water without f/2 medium; (b) Growth trends at various inoculation ratios in natural sea water with f/2 addition. (◆) 5%; (■) 10%; (▲) 20%; (●) 30%; (△) 40%; (c) Full growth pattern under 20% inoculation ratio in natural sea water with f/2 addition.

Figure 4. Comparison of inactivation efficiency by various methods. (a) by O₃ of 190 mg h⁻¹ strength (▲) in natural sea water (■) in synthetic saline water; (b) by O₃ of various strengths, (■) 108 mg h⁻¹ (◆) 118 mg h⁻¹ (▲) 190 mg h⁻¹ (×) 228 mg h⁻¹; (c) by H₂O₂ of various dosages, (◆) 10 mg L⁻¹ (■) 40 mg L⁻¹ (▲) 100 mg L⁻¹; (d) by O₃/H₂O₂ of several combinations, (◆) O₃/10 mg L⁻¹ (■) O₃/40 mg L⁻¹ (▲) O₃/100 mg L⁻¹.
Figure 1. Schematic diagram of the reactor system: 1, air inlet; 2, ozone generator; 3, gas inlet; 4, draining port; 5, sampling port; 6, UV lamp; 7, fume extractor; 8, ozone scrubber; 9, exhaust gas; 10, quartz tube; 11, reactor chamber; 12, porous plate.
Figure 2. Images of *S. trochoidea* cells at various treatment stages: (a) original cells under light microscope, 750×; (b) before treatment under SEM, 750×; (c) before treatment under SEM, 5000×; (d) after 4 mins treatment by O$_3$; (e) after 1 min treatment by UV; (f) after 1 min treatment by UV/O$_3$. 
Figure 3. Comparison of growth trends under various conditions. (a) Growth trends with and without f/2 medium addition in synthetic saline water and natural sea water. (▲) natural sea water with f/2 medium; (■) natural sea water without f/2 medium; (◇) synthetic saline water with f/2 medium; (□) synthetic saline water without f/2 medium; (b) Growth trends at various inoculation ratios in natural sea water with f/2 addition. (●) 5%; (■) 10%; (▲) 20%; (●) 30%; (△) 40%; (c) Full growth pattern under 20% inoculation ratio in natural sea water with f/2 addition.
Figure 4. Comparison of inactivation efficiency by various methods. (a) by O$_3$ of 190 mg h$^{-1}$ strength (▲) in natural sea water (■) in synthetic saline water; (b) by O$_3$ of various strengths, (■) 108 mg h$^{-1}$ (◆) 118 mg h$^{-1}$ (▲) 190 mg h$^{-1}$ (×) 228 mg h$^{-1}$; (c) by H$_2$O$_2$ of various dosages, (◆) 10 mg L$^{-1}$ (■) 40 mg L$^{-1}$ (▲) 100 mg L$^{-1}$; (d) by O$_3$/H$_2$O$_2$ of several combinations, (◆) O$_3$/10 mg L$^{-1}$ (■) O$_3$/40 mg L$^{-1}$ (▲) O$_3$/100 mg L$^{-1}$.
Table 1. Effect of aeration on *S. trochoidea* cells activity.

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<th>Aeration time (min)</th>
<th>Number of active cells (mL⁻¹) average ± standard deviation</th>
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<tr>
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<tr>
<td>1</td>
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<tr>
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<tr>
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<td>4778 ± 667</td>
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<tr>
<td>5</td>
<td>4778 ± 667</td>
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Table 2. Inactivation efficiency of *S. trochoidea* cells by UV/O₃ and UV/O₃/10 mg L⁻¹ H₂O₂ methods.

<table>
<thead>
<tr>
<th>Time (s)</th>
<th>UV/O₃</th>
<th>UV/O₃/10 mg L⁻¹ H₂O₂</th>
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Table 3. Comparison of photo-reactivation after 10s treatment by various methods.

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<th>Incubation time (day)</th>
<th>UV/10 mg L(^{-1})</th>
<th>UV/O(_3)/10 mg L(^{-1})</th>
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<tr>
<td></td>
<td>UV/10 mg L(^{-1})</td>
<td>UV/O(_3)/10 mg L(^{-1})</td>
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<tr>
<td></td>
<td>H(_2)O(_2)</td>
<td>H(_2)O(_2)</td>
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<td>Number of active cells (mL(^{-1}))</td>
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Table 4. Comparison of photo-reactivation after longer treatment by UV and UV/O₃ methods.

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<th>UV 30s</th>
<th>UV 60s</th>
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Table 5. Total residue oxidant levels after treatment by various oxidation processes.

<table>
<thead>
<tr>
<th>Treatment length</th>
<th>10s</th>
<th>300s</th>
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<tr>
<td>Oxidation process</td>
<td>TRO concentration (mg Br₂ L⁻¹)</td>
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<tr>
<td>UV</td>
<td>0.04</td>
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<tr>
<td>O₃</td>
<td>0.08</td>
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<td>H₂O₂</td>
<td>2.55</td>
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<tr>
<td>UV/10 mg L⁻¹ H₂O₂</td>
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<td>1.74</td>
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<tr>
<td>O₃/10 mg L⁻¹ H₂O₂</td>
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<td>UV/O₃/10 mg L⁻¹ H₂O₂</td>
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