Inactivation of dinoflagellate scripsiella trochoidea in synthetic ballast water by advanced oxidation processes

Yang, Zhishan; Jiang, Wenju; Zhang, Yi; Lim, Tuti Mariana

2014

Yang, Z., Jiang, W., Zhang, Y., & Lim, T. (2014). Inactivation of dinoflagellate scripsiella trochoidea in synthetic ballast water by advanced oxidation processes. Environmental technology, 1-10.

<https://hdl.handle.net/10356/102896>

<https://doi.org/10.1080/09593330.2014.960478>

© 2014 Taylor & Francis. This is the author created version of a work that has been peer reviewed and accepted for publication by Environmental Technology, Taylor & Francis. It incorporates referee's comments but changes resulting from the publishing process, such as copyediting, structural formatting, may not be reflected in this document. The published version is available at: [Article DOI: <http://dx.doi.org/10.1080/09593330.2014.960478>].

Downloaded on 26 Apr 2023 11:26:15 SGT

Inactivation of Dinoflagellate *Scropsiella trochoidea* in Synthetic Ballast Water by Advanced Oxidation Processes

Journal:	<i>Environmental Technology</i>
Manuscript ID:	TENT-TENT-2014-0202.R2
Manuscript Type:	Original Article
Date Submitted by the Author:	n/a
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
Keywords:	Ballast Water, Dinoflagellate, Advance Oxidation Processes, Photo-reactivation, <i>Scropsiella trochoidea</i>

SCHOLARONE™
Manuscripts

**Inactivation of Dinoflagellate *Scripsiella trochoidea* in Synthetic Ballast Water by
Advanced Oxidation Processes**

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

^a Department of Environmental Science and Engineering, Fudan University, 220
Handan Road, Shanghai, P. R. China, 200433.

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

^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 (O₃), hydrogen peroxide (H₂O₂), 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. O₃ exhibited 100% inactivation efficiency after 5min

1
2
3 treatment, while H_2O_2 only achieved maximum 80% inactivation in the same duration.
4
5 Combined methods, e.g. UV/ O_3 and UV/ H_2O_2 were found to inhibit photo-reactivation and
6
7 improve treatment efficiency to some degree, indicating the effectiveness of using combined
8
9 treatment processes. The total residual oxidant levels of the methods were determined, and the
10
11 results indicated that UV and O_3 generated the lowest and highest total residual oxidants
12
13 (TRO), respectively. The synergic effect of combined processes on TRO generation was
14
15 found to be not significant, and thus UV/ O_3 was recommended as a potentially suitable
16
17 treatment process for ballast water.
18
19

20
21
22 **Keywords:** Ballast Water; Water Treatment; Dinoflagellate, Advance Oxidation Processes;
23
24 *Scripsiella trochoidea*; Inactivation; Photo-reactivation.
25
26
27

28 29 1. Introduction

30
31 Ballast water is the water stored on board to stabilize ships while sailing (Carlton et
32
33 al. 1995). It is usually taken when ships are in ports, therefore can contain various
34
35 fresh and sea water organisms, which can be transported to other places and introduce
36
37 invasive species into new environments (Tsolaki & Diamadopoulos 2009). For
38
39 example the zebra mussel *Dreissena polymorpha*, introduced via ballast water into the
40
41 Great Lakes of North America is estimated to have caused damages of \$5 billion
42
43 between 1985 and 1986, by blocking water intakes and fouling fishing nets, buoys and
44
45 boat hulls (Ribera 1995). As a consequence, ballast water has been identified as one
46
47 of the major threats to the global aquatic environment (Bax et al. 2003). To minimize
48
49 the risk of bio-invasion by ballast water transportation, ships are now required to do
50
51 ballast water exchange in open sea (IMO 2005). However, deballasting and ballasting
52
53 during voyage on high seas could expose ships to potential dangers, e.g. physical
54
55
56
57
58
59
60

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

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), deoxygenization (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_2O_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_2O_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_3 has been tested with bacteria spores, dinoflagellate algae, and organisms in actual seawaters (Perrins et al. 2006), and H_2O_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_3 , H_2O_2 , UV/ O_3 , and UV/ O_3 / H_2O_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 6cm×35cm (outer diameter×height) and a working volume of 250 mL. A UV lamp was encased in a quartz tube of O.D. 4cm, 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 $190 \pm 8.3 \text{ mg h}^{-1}$. For treatment involving UV, a 15W, 253.7nm lamp (Qidong Fluorescent Lamp Factory, China) was used. The light intensity of the UV lamp was $7.72 \pm 0.06 \text{ mw cm}^{-2}$ as measured at a 1cm 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, $25 \pm 1^\circ\text{C}$; 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 $1.58 \pm 0.03 \text{ mw cm}^{-2}$.

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 $8.13 \pm 1.73 \text{ NTU}$, 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 $0.2\mu\text{m}$ 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 $10^4 \text{ cells mL}^{-1}$, 250 mL culture was poured into the reactor to be treated by various oxidation processes. In O_3 treatment, ozone generator was engaged and the gas flow rate was set at 6 L min^{-1} . For treatment by UV or H_2O_2 , the UV lamp was turned on or liquid H_2O_2 (Sigma Aldrich) was added into the reactor,

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

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: inactivation efficiency (%) = $1 - C_t/C_0$, 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.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

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. $1.7 \times 10^4 \text{ mL}^{-1}$, 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 O₃ 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 O₃ 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_2O_2 alone

H_2O_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 H_2O_2 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 H_2O_2 concentration. After 5 mins treatment, the inactivation efficiencies reached 57.4%, 67.7% and 87.2% for H_2O_2 concentrations of 10, 40 and 100 mgL^{-1} , respectively. However, the efficiencies under all H_2O_2 dosages exhibited a limit, upon which prolonged treatment time didn't show improvement. Higher H_2O_2 dosages achieved the maximum effect more rapidly, i.e. the inactivation efficiency under 10, 40, and 100 mg L^{-1} H_2O_2 reached the plateau at around 4, 3 and 1 min, respectively.

Previous studies have used H_2O_2 to treat diverse marine organisms, with various results. An H_2O_2 concentration of 10 ppm and contact time of 1 min was enough to inactivate a ctenophore, *Mnemiopsis leidyi*, and 1 ppm H_2O_2 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 H_2O_2 was unable to achieve 100% mortality within 20 mins of treatment (Gavand et al. 2007). In this study, it was found that H_2O_2 was a poor choice for *S. trochoidea* inactivation in comparison to O_3 , as 100% efficiency wasn't achieved even at a high H_2O_2 concentration of 100 ppm and reasonably long treatment duration. Moreover, commercially available H_2O_2 is a toxic liquid and could pose hazard when stored on board. Therefore, for safety reason as well as the economical concern, H_2O_2 alone is not recommended as a suitable method for treating ballast water.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

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 O₃/H₂O₂

To observe if O₃ and H₂O₂ had any synergic effect, 250 mL culture was placed in the reactor, and O₃ was provided together with H₂O₂ dosages of 10, 40 and 100 mgL⁻¹. Fig. 4(d) shows the inactivation efficiencies under various O₃/H₂O₂ combinations. The total efficiency of the combined method appeared to be lower than that of O₃ alone, but showed improvement on those achieved with H₂O₂ of the respective dosages. However, the efficiency plateau was again observed with the two higher dosages, and the H₂O₂ dosage effect was not so pronounced at the presence of O₃. The combination of O₃/10 mg L⁻¹ H₂O₂ 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 ($\cdot\text{OH}$), 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_2O_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_2O_2 and UV/ O_3 // H_2O_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/ $10\text{ mgL}^{-1}\text{ H}_2\text{O}_2$ and UV/ O_3 / $10\text{ mgL}^{-1}\text{ H}_2\text{O}_2$ combinations. Table 2 shows the results, where both methods exhibited 100% inactivation after 5 s of treatment. UV/ $10\text{ mgL}^{-1}\text{ H}_2\text{O}_2$ 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 3min, 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.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

3.3. Photo-reactivation and the advantage of AOPs

3.3.1. Comparison of photo-reactivation of *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 *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 H₂O₂, UV/O₃ and UV/O₃/10 mgL⁻¹ H₂O₂, 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/O₃, 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 10⁴ 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 H₂O₂ or O₃ 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 & Diomadopoulou 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 $\text{mg Br}_2 \text{ L}^{-1}$ 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_2O_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_2O_2 also exhibited a similar trend that longer treatment resulted in slightly lower TRO, which could be due to the decomposition of H_2O_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.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

References

Alvares ABC, Diaper C, Parsons SA (2001) Partial oxidation by ozone to remove recalcitrance from wastewaters - a review. Environ Tech 22: 409-427

Azbar N, Yonar T, Kestioglu K (2004) Comparison of various advanced oxidation processes and chemical treatment methods for COD and color removal from a polyester and acetate fiber dyeing effluent. Chemosphere 55, 35-43

Balaji R, Yaakob OB (2011) Emerging ballast water treatment technologies: a review. J Sustain Sci Manag 6: 126-138

Bax N, Williamson A, Agüero M, Gonzalez E, Geeves W (2003) Marine invasive alien species: a threat to global biodiversity. Mar Policy 27: 313-323

Bellefontaine N, Haag F, Lindén O, Matheickal J (2010) Emerging Ballast Water Management Systems. Proceedings of the IMO-WMU Research and Development Forum. Malmö, Sweden

Bolch CJ, Hallegraeff GM (1993) Chemical and physical treatment options to kill toxic dinoflagellate cysts in ships' ballast water. J Mar Environ Eng 1: 23-29

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

Eker APM, Formenoy L, de Wit LEA (1991) Photoreactivation in the extreme halophilic archaeobacterium *Halobacterium cutirubrum*. Photochem Photobiol 53: 643-651

Gavand MR, McClintock JB, Amsler CD, Peters RW, Angus RA (2007) Effects of sonication and advanced chemical oxidants on the unicellular green alga *Dunaliella tertiolecta* and cysts, larvae and adults of the brine shrimp *Artemia salina*: A prospective treatment to eradicate invasive organisms from ballast water. Mar Pollut Bull 54: 1777-1788

Glaze WH, Kang JW, Chapin DH (1987) The chemistry of water treatment processes involving ozone, hydrogen peroxide, and ultraviolet radiation. Ozone: Sci & Eng: J Int Ozone Assoc 9: 335-352

- Granéli E (2006) Ecology of harmful algae, Springer, New York, pp. 413
- Gregg MD, Hallegraeff GM (2007) Efficacy of three commercially available ballast water biocides against vegetative microalgae, dinoflagellate cysts and bacteria. *Harmful Algae* 6: 567-584
- Guillard RRL (1975) Culture of phytoplankton for feeding marine invertebrates. In: Smith WL, Chanley MH (eds.), *Cultures of Marine Invertebrate Animals*. Plenum Press, New York.
- Herwig RP, Cordell JR, Perrins JC, Dinnel PA, Gensemer RW, Stubblefield WA, Ruiz GM, Kopp JA, House ML, Cooper WJ (2006) Ozone treatment of ballast water on the oil tanker S/T Tonsina: chemistry, biology and toxicity. *Mar Ecol Prog Ser* 324: 37-55
- Hoigné J, Bader H, Haag WR, Staehelin J (1985) Rate constants of reactions of ozone with organic and inorganic compounds in water--III. Inorganic compounds and radicals. *Water Res* 19: 993-1004
- Hess-Erga OK, Attramadal KJK, Vadstein O (2008) Biotic and abiotic particles protect marine heterotrophic bacteria during UV and ozone disinfection. *Aquat Biolog* 4: 147-154
- International Maritime Organization (IMO) (2005) Guidelines for approval of ballast water management systems (G8). International Maritime Organisation Publishing, London
- International Ozone Association (IOA) (1998) Iodometric method for the determination of ozone in a process gas
- Kornmueller A (2007) Review of fundamentals and specific aspects of oxidation technologies in marine waters. *Water Sci Technol* 55: 1-6
- Kumiko O, Hiroyuki K, Shinichiro O (2004) Photoreactivation of *Legionella pneumophila* after inactivation by low or medium pressure ultraviolet lamp. *Water Res* 38: 2757-2763
- Kusic H, Koprivanac N, Bozic AL (2006) Minimization of organic pollutant content in aqueous solution by means of AOPs: UV- and ozone-based technologies. *Chem Eng J* 123: 127-137
- Kuzirian AM, Terry ECS, Bechtel DL, James PL (2001) Hydrogen peroxide: An effective treatment for ballast water. *Biolog Bull* 201: 297-299

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Leffler CE, Rogerson A, Paul W, Germaine G, Elliot M, Antonelli V, Grubs S, Campbel C, Beall G, Salamone A (2003) Electro-sanitization of ballast water. In: 2nd International Ballast Water Treatment R&D Symposium

Lewis J (1991) Cyst-theca relationships in *Scrippsiella* (*Dinophyceae*) and related orthoperidinioid genera. *Botanica Marina* 34: 91-106

Lloyd's register, (2010) Ballast water treatment technology: current status. London EC3M 4BS

Madhavan J, Maruthamuthu P, Murugesan S, Anandan S (2008) Kinetic studies on visible light-assisted degradation of acid red 88 in presence of metal-ion coupled oxone reagent. *Appl Catal B-Environ* 83: 8-14

McCollin T, Quilez-Badia G, Josefsen KD, Gill ME, Mesbahi E, Frid CLJ (2007) Ship board testing of a deoxygenation ballast water treatment. *Mar Pollut Bull* 54: 1170-1178

Montani SM, Ichimi K (1995) Chemical and physical treatments for destruction of phytoflagellate cysts. *J Mar Biotechnol* 2: 179-181

Oemcke DJ, van Leeuwen J (2004) Seawater ozonation of the *Bacillus subtilis* spores: implications for the use of ozone in ballast water treatment. *Ozone: Sci & Eng: J Int Ozone Assoc* 26: 389-401

Ormad P, Cortes S, Puig A, Ovelleiro JL (1997) Degradation of organochloride compounds by O₃ and O₃/H₂O₂. *Water Res* 31: 2387-2391

Parsons MG, Harkins RW (2000) The great lakes ballast technology demonstration project filtration mechanical test program. *Mar Technol* 37: 129-140

Perrins JC, Cordell JR, Ferm NC, Grocock JL, Herwig RP (2006) Mesocosm experiments for evaluating the biological efficacy of ozone treatment of marine ballast water. *Mar Pollut Bull* 52: 1756-1767

Raikow DF, Reid DF, Maynard EE, Landrum PE (2006) Sensitivity of aquatic invertebrate resting eggs to SeaKleen (R) (menadione): A test of potential ballast tank treatment options. *Environ Toxicol Chem* 25: 552-559

- Ribera MA (1995) Introduced marine plants, with special reference to macroalgae: mechanisms and impact. *Prog Phycolog Res* 11: 217-268
- Sancar GB (2000) Enzymatic photoreactivation: 50 years and counting. *Mutat Res* 451: 25-37.
- Schneider KR, Pierce RH, Rodrick GE (2003) The degradation of *Karenia brevis* toxins utilizing ozonated seawater. *Harmful Algae* 2: 101-107
- Sutherland TF, Levings CD, Elliot CC, Hesse WW (2001) Effect of a ballast water treatment system on survivorship of natural populations of marine plankton. *Mar Ecol Prog Ser* 210: 139-148
- Thornton GA, Chapman JE (2003) The use of heat for ballast water disinfection-the AquaTherm method. In: Matheickal J, Raaymakeers S (eds.). *International Ballast Water Treatment Symposium*, 2, London, 21-23 July 2003. *Proceedings*. London: Globallast Monograph Series, 15
- Sassi J, Viitasalo S, Rytönen J, Leppäkoski E (2005) Experiments with ultraviolet light, ultrasound and ozone technologies for onboard ballast water treatment. *VTT Tiedotteita-Valtion Teknillinen Tutkimuskeskus* 2313: 1-80
- Smit MGD, Ebbens E, Jak RG, Huijbregts MAJ (2008) Time and concentration dependency in the potentially affected fraction of species: the case of hydrogen peroxide treatment of ballast water. *Environ Technol Chem* 27: 746-753
- Tabrizi GB, Mehrvar M (2004) Integration of advanced oxidation technologies and biological processes: recent developments, trends, and advances. *J Environ Sci Health A39*: 3029-3081
- Tsolaki E, Diamadopoulos E (2010) Technologies for ballast water treatment: a review. *J Chem Technol Biotechnol* 85: 19-32
- Viitasalo S, Sassi J, Rytönen J, Leppäkoski E (2005) Ozone, ultraviolet light, ultrasound and hydrogen peroxide as ballast water treatments - experiments with mesozooplankton in low-saline brackish water. *J Mar Environ Eng* 8: 35-55
- Waite TD, Kazumi J, Lane PVZ, Farmer LL, Smith SG, Smith SL, Hitchcock G, Cap TR (2003) Removal of natural populations of marine plankton by a large-scale ballast water treatment system. *Mar Ecol Prog Ser* 258: 51-63

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Wright DA, Dawson R, Mackey TP, Cutler HG, Cutler SJ (2003) Some shipboard trials of ballast water treatment systems in the United States. In: Matheickal J, Raaymakers S (eds.). International Ballast Water Treatment Symposium, 2, London, 21-23 July 2003. Proceedings... London: Globallast Monograph Series, 15

Wright DA, Dawson R, Cutler SJ, Cutler HG, Orano-Dawson CE, Graneli E (2007) Naphthoquinones as broad spectrum biocides for treatment of ship's ballast water: Toxicity to phytoplankton and bacteria. Water Res 41: 1294-1302

Wright DA, Gensemer RA, Mitchelmore CL, Stubblefield WA, van Genderen E, Dawson R, Orano-Dawson CE, Bearn JS, Mueller RA, Cooper WJ (2010) Shipboard trials of an ozone-based ballast water treatment system. Mar Pollut Bull 60: 1571-1583

Wu DH, You H, Zhang R, Chen C, Lee D-J (2011) Inactivation of Amphidinium sp. In ballast waters using UV/Ag-TiO₂+O₃ advanced oxidation treatment. Bioresour Technol 102: 9838-9842

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 $\times$; (b) before treatment under SEM, 750 $\times$; (c) before treatment under SEM, 5000 $\times$; (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 $\times$; (b) before treatment under SEM, 750 $\times$; (c) before treatment under SEM, 5000 $\times$; (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_2O_2 of various dosages, (◆) 10 $mg\ L^{-1}$ (■) 40 $mg\ L^{-1}$ (▲) 100 $mg\ L^{-1}$; (d) by O_3/H_2O_2 of several combinations, (◆) $O_3/10\ mg\ L^{-1}$ (■) $O_3/40\ mg\ L^{-1}$ (▲) $O_3/100\ mg\ L^{-1}$.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Table 1. Effect of aeration on *S. trochoidea* cells activity.

Aeration time	Number of active cells (mL ⁻¹)
(min)	average ± standard deviation
0	4667 ± 1118
1	4889 ± 1269
2	4556 ± 1014
3	4556 ± 1130
4	4778 ± 667
5	4778 ± 667

Table 2. Inactivation efficiency of *S. trochoidea* cells by UV/O₃ and UV/O₃/10 mg L⁻¹ H₂O₂ methods.

Time	UV/O ₃		UV/O ₃ /10 mg L ⁻¹ H ₂ O ₂	
(s)	No. of active cells	1-C/C ₀	No. of active cells	1-C/C ₀ (%)
	(mL ⁻¹)	(%)	(mL ⁻¹)	
0	4889	0	2000	0
5	5	99.9	2	99.9
10	0	100	0	100
15	0	100	0	100

Table 3. Comparison of photo-reactivation after 10s treatment by various methods.

Incubation time (day)	UV	UV/10 mg L ⁻¹		UV/O ₃ /10 mg L ⁻¹	
		H ₂ O ₂		H ₂ O ₂	
		Number of active cells (mL ⁻¹)			
0	0	0	0	0	
1	0	0	0	0	
2	3	5	0	3	
3	20	17	20	19	
4	30	30	31	35	
5	58	70	63	80	
6	107	146	154	203	
7	207	132	216	211	
8	218	312	300	268	
9	265	335	265	409	
11	531	650	568	920	
13	1750	2000	1750	2000	
15	4000	4250	3250	4500	
17	5000	5000	5500	8000	
25	28700	13000	16500	20000	

Table 4. Comparison of photo-reactivation after longer treatment by UV and UV/O₃ methods.

Incubation		UV			UV/O ₃		
time (day)	15s	30s	60s	15s	30s	60s	
0	0	0	0	0	0	0	
1	0	0	0	0	0	0	
2	4	1	0	2	0	0	
3	14	1	0	9	0	0	
4	41	0	0	7	3	0	
5	26	2	0	26	0	0	
6	29	2	1	32	5	1	
8	54	1	1	42	0	1	
9	91	0	1	98	0	0	
10	167	2	1	88	1	1	
11	211	0	2	92	1	0	
12	213	2	1	117	2	1	
13	252	2	1	154	0	2	
14	826	1	0	175	0	0	
15	1500	0	0	750	1	0	

Table 5. Total residue oxidant levels after treatment by various oxidation processes.

Treatment length	10s	300s
Oxidation process	TRO concentration (mg Br ₂ L ⁻¹)	
UV	0.04	0.10
O ₃	0.08	7.77
H ₂ O ₂	2.55	1.87
UV/10 mg L ⁻¹ H ₂ O ₂	2.61	1.74
O ₃ /10 mg L ⁻¹ H ₂ O ₂	2.50	1.50
UV/O ₃	0.04	3.01
UV/O ₃ /10 mg L ⁻¹ H ₂ O ₂	2.27	1.04