Efficacy testing with AirTree ballast water treatment system

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Contents

Summary

AirTree has developed a Ballast Water Management Systems (BWMS)on the basis of in-tank treatment with ozone that is generated on board. The principle of the treatment is that after uptake an initial dose is given up to 700 mV ORP, followed by a maintenance dose at 600 mV ORP. IMARES has performed two set of tests using a small-scale pilot system, in order to investigate the efficacy of the proposed set-up for ballast water treatment. As realistic filtration with this system was not possible, the test water was sieved using a 50 µm-plankton net and the analyses focussed on organisms <50µm.

In total 5 tests were performed:
In the first two tests, performed in October 2015 with sea water, the in-tank treatment as described above was compared with a single ozone dose, as is applied by BWMS using in-line treatment during uptake. Both treatments received a similar fixed initial dose of 4.8 g/m^3 ozone. After an equilibration period of 60 min, maintenance dosing was started in the in-tank treatment, keeping the ORP between 540 and 600 mV. In the first test, with challenge water complying to marine water conditions, both treatments successfully removed organisms. In the second test, with more challenging conditions due to elevated DOC and TSS values, the AirTree in-tank treatment successfully removed organisms and also inhibited bacterial regrowth, but the single dose treatment failed. This suggest that when a single dose is applied, the capacity of the ozone-generator needs to be much larger compared to a continuous dosing system.

In the following three tests, the efficacy of the AirTree in-tank treatment was evaluated using fresh, brackish and marine water. These tests were executed in January-February 2016, using indoor organism cultures. In all three water types, organisms were successfully removed. In freshwater and marine water a comparable maintenance dose was necessary (5-6 g/m^3 ozone), but for the brackish water test approx. 8 g/m^3 ozone was needed. This water contained most TSS (although less than IMO requirement), very high DOC levels and a relatively high bacteria load. The effectiveness against bacteria was not conclusive, as regrowth still occurred in some tests.

1 Introduction

AirTree Europe GmbH approached IMARES Wageningen UR for support in the development of a Ballast Water Management System (BWMS) that applies ozone as active ingredient to achieve an IMO Type Approval certificate (IMO 2004). Efficacy of an ozone treatment is based on the total amount of organisms and other compounds (mineral and organic particles, dissolved organic carbon) in the ballast water. Depending on the location and specific (natural) circumstances in which the BWMS is being used, systems can be over-efficient or not efficient enough if only a fixed amount of ozone is used. If a BWMS is too efficient, more energy and/or chemicals are being used than what is actually needed, and the treatment is relatively expensive. On the other hand, a BWMS should preferably be reliable under all circumstances that a ship can encounter.

AirTree has recognized that an intelligent BWMS is needed for treatment of ballast water with ozone and developed an in-tank ballast water treatment system that adapts the treatment intensity to the total amount of organic and inorganic material in the water to avoid over and under dosing. Additionally, re-growth in the tank is avoided by using a maintenance dose during the voyage.

The principle of the ballast water treatment procedure by AirTree consists of a four-step approach:

1. filtration at uptake,
2. in-tank ozone treatment at a high level
3. maintenance dose of ozone at a lower level
4. neutralisation with sodium thiosulphate at discharge.
5. At uptake of ballast water a pre-filter (Bollfilter BWT-40; Boll & Kirch) is used followed by in-tank ozone treatment during the voyage with an initial dose to reach an ORP (oxidation-reduction potential) of 750 mV and a maintenance dose keeping the ORP at 600 mV. Maintenance dosing is stopped 48h prior to expected discharge of the ballast water. The ORP value is continuously detected to control the ballast water condition during voyage. An injection system is used to avoid off-gas in the tank and ambient ozone monitors are used for leakage control and crew safety. Potential residual ozone is neutralized with sodium thiosulphate at discharge to reach TRO 0.1 mg/l as Cl_2 equivalents. In Figure 1 an overview of the AirTree BWMS is given.

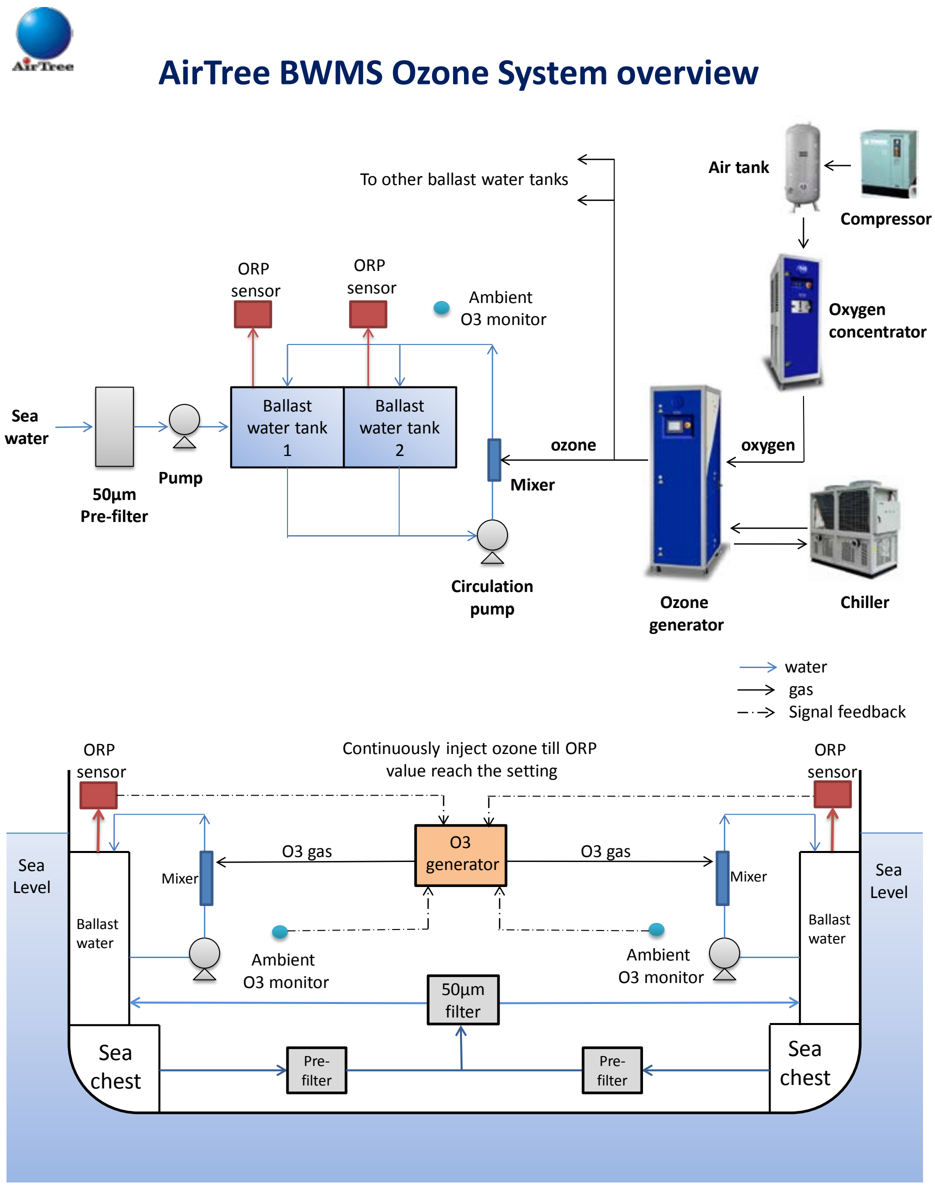

Figure 1 Overview of AirTree BWMS Ozone System to treat ballast water.

There were three relevant assumptions:

1. At low organism densities and (in)organic load, the AirTree BWMS uses less ozone than a BWMS based on a single dose.
2. At high organism densities and/or (in)organic load, the AirTree BWMS is still capable of treating the ballast water efficiently to meet IMO requirements while a BWMS based on a fixed single dose may not.
3. Re-growth is prevented by using a maintenance dose.

To show the differences between a conventional ‘one-dose’ ozone system and AirTree’s in-tank ballast water treatment comparison tests were performed with variable challenging conditions with a pilot BWMS using short term exposures. Based on these results, efficacy tests with the AirTree BWMS were performed with fresh, brackish and marine water. This report describes the test results performed by IMARES using a pilot ozonation system installed by AirTree Europe.

2 Assignment

IMARES was asked to test variable challenging conditions with a pilot treatment system provided by AirTree. The test were short-term (5 days holding period) to show the differences between the conventional ‘one-dose’ ozone treatment and AirTree’s in-tank ballast water treatment (Sneekes & Kaag, 2015). It was assumed that organic load and particles were the most important water quality parameters determining ozone efficacy. Therefore, the tests were conducted at one salinity (sea water) while manipulating TSS and DOC levels. Care was taken to culture sufficient organisms, but the size class ≥50 μm was excluded, as the pilot system did not have its own filter and the test volume (500 l) was too small for a valid assessment of this group of organisms. A final assessment of the efficacy of the AirTree BWMS against the size class ≥50 μm will be done during the land-based tests, where larger volumes are processed and analysed.

Originally, the aim was to determine if at low challenging conditions the AirTree method needs less ozone than a standard system with a fixed single dose. However, in a later stage the client decided that also the AirTree system should start with an initial 2 mg/m^3 ozone treatment, if necessary to be followed by maintenance dosing.

The first two tests aimed at water quality characteristics complying to IMO G8 challenge conditions for sea water and freshwater respectively (IMO MEPC 2008). It was envisaged that both treatment methods would be able to treat this efficiently. The tests would then continue with much more challenging conditions, especially with respect to DOC and organism load.

The first two tests were executed according to the test plan in autumn 2015 and were discussed with the client. Based upon these discussions, the test plan was changed. It was client’s wish to focus on replicated efficacy test with fresh, brackish and marine water using a 5-day holding time, in support of the Basic Approval Dossier. These tests were conducted in the winter of 2016.

3 Materials and Methods

The tests were conducted in a pilot system installed by AirTree at the IMARES laboratory. Here two treatment tanks (640L IBC's) were available enabling parallel testing of both treatments using exactly the same challenge water.

In summary, each test cycle consists of the following activities:

0 Collecting and culturing challenge water
1 Preparing test water with specific quality
2 Filling the 640 L test tanks
3 Sampling both tanks to specify starting conditions
4 Treating the challenge waters
5 In-tank sampling for efficacy
6 Empty and clean containers, pipes and sampling system.

As the pilot system did not include filters, organisms ≥50 µm were removed from the challenge water for all tests by using a zooplankton net. The quality and composition of the challenge water was assessed just before a cycle started by taking samples from the tanks. Then both tanks were treated during which no samples are taken, but ORP was continuously measured. During the treatments, these readings were regularly, but not continuously registered. After 5 days, the tanks were opened and the water thoroughly mixed before taking samples to assess efficacy.

3.1 Comparison tests

For these tests brackish water (IMO G8 specifications; IMO MEPC 2008) were used. The water was cultured in outdoor ponds at the IMARES laboratories in order to provide sufficient organisms densities.

Based upon the different requirements in the IMO G8 guideline for marine and brackish/fresh water (IMO MEPC 2008), two water quality regimes were chosen for the two tests:

WQ1 organisms ≥10-<50µm 10^3/ml; DOC ~1 mg/l, TSS ~1 mg/l
WQ2 organisms ≥10-<50µm 10^3/ml; DOC ~5 mg/l, TSS ~50 mg/l

It was initially assumed that both the single dose treatment and the continuous treatment (AirTree) would be successful treating WQ1 and WQ2, as they are based upon IMO minimum requirements. Further tests with more challenging conditions would then be needed to show a difference in efficacy between the two treatment strategies. In the comparison tests (WQ1 and WQ2), one tank was treated with a single ozone-dose and then left undisturbed for 5 days. The other tank initially received the same dose, was then left undisturbed for 60 minutes, and then received maintenance dosing, keeping the ORP-level between 540 and 600 mV for three days. For practical reasons an initial dose was applied for 30 minutes, resulting in a final dose of 4.8 g/m^3 ozone.

After three days, maintenance dosing was stopped to allow ORP-levels to drop to save levels for 'discharge' at day 5. For these tests only sea water (~32 ‰) was used. The test water was obtained from an outdoor culture at the IMARES laboratories.

In test 1, the test water WQ1 was not extremely challenging and no additional TSS was added.
In test 2, the test water WQ2 was made more challenging. TSS was increased by adding 25 gr Arizona Dust (A2) as mineral matter to achieve 50 mg/l TSS. DOC was increased by adding DOC-rich brackish water, followed by adjustment of the salinity using NaCl. The organism densities (≥10-<50 µm) were more than twice as high compared to Test 1.

3.2 Efficacy tests

In the efficacy tests (WQ3 - WQ5) both treatment tanks were filled simultaneously from a single culture pond and were treated as replicates. The initial dose was applied sequentially to each tank (1500 sec; 25 min). After the initial dosing, the tanks were left undisturbed for 60 minutes before maintenance dosing was started. Tank 2 was treated during this ‘rest-period’ for Tank 1.

The following three water types were tested:

- WQ3 freshwater, with organisms ≥10-<50 µm at IMO challenge levels, supplemented with some organisms <10 µm.
- WQ4 brackish water, with organism densities in both size classes approx. twice as high.
- WQ5 water, in which organisms <10 µm dominated and the size class ≥10-<50 µm did not reach IMO challenge levels.

The test water was obtained from indoor cultures at the IMARES laboratories. Tests were initiated when the densities of organisms in the size class <50 µm was sufficient. The aim was to use mainly organisms in the size class ≥10-<50 µm, but as organisms <10 µm were also present these were also taken into consideration. TSS and DOC were not manipulated, but taken as it was.

3.3 Analyses

3.3.1 Ozone

It was the intention to use an initial dose of 4 g/m^3 (ppm) ozone. The initial ozone dose was not directly measured in the treated water, but calculated on the basis of capacity of the system, flow rate and time running. The system was set to an ozone gas concentration of 4 g/m^3, which was measured by an ozone analyser that sampled the gas flow produced. A gas flow of 20 l/min was used, resulting in an ozone production of 4.8 g/h. The final ozone dose of 4.0 g/m^3 was reached after adding ozone for 1500 sec (25 min) to the tank filled with 500 l test water. Maintenance dosing was characterized by the number of runs (starting at 540 mV and stopping at 600 mV) and the total time (in seconds) that ozone was added during these maintenance runs.

During the tests, the level of ORP in the treatment tanks is continuously measured as mV using the probes that come with the pilot system. As continuous logging of these data is not (yet) possible, the values were registered manually.

As a measure of reactivity, TRO (Total Residual Oxidants) was analysed colorimetric using the DPD-method (WTW) and expressed as Cl_2-equivalents.

3.3.2 Environmental parameters

Basic water quality parameters (temperature, pH, salinity/conductivity, oxygen saturation) were analysed using handheld field equipment.

Turbidity was measured using an optic turbidimeter and expressed as NTU. Total Suspended Solids (TSS) were gravimetrically analysed using pre-weighed GF/F filters.

DOC analyses were performed at the laboratory of NIOZ by combustion using a TOC analyser. Samples were prepared at the laboratory of IMARES following the sample storage procedures from NIOZ.

3.3.3 Organism densities

Organism densities were evaluated with reference to the IMO D-2 standards. Organisms in the size class ≥10-<50 µm are dominated by phytoplankton and sometimes microzooplankton. The viability of these organisms was analysed using vital FDA/CMFDA staining and fluorescence microscopy. During these analysis, visible organisms <10 µm were also registered and enumerated. Separate intake samples were stored on lugol's solution for later analysis of taxonomic composition.

Bacteria were analysed using the IDEXX procedures for heterotrophic bacteria (HPC), enterococci (Enterolert) and *E.coli* and coliform bacteria (Colilert).

3.3.4 Organism regrowth

During the efficacy tests, the regrowth potential of phytoplankton was assessed by adding nutrients to a 1 litre sample taken at day 5 and incubating this with continuous illumination at 15°C. Primarily chlorophyll-a was measured and when this indicated growth, the number of cells was counted using vital staining as described above.

4 Results

4.1 Comparison tests

During tests WQ1 and WQ2, a 30 min (1800 sec) dosing period was applied, resulting in an initial dose of 4.8 g ozone /m^3. Some dosing characteristics are presented in Table 1.

Table 1 Dosing characteristics WQ1 and WQ2. ORP in mV

Dosing	**WQ1**	**23 Oct '15**	**WQ2**	**29 Oct'15**
	Single dose	**In-tank**	**Single dose**	**In-tank**
ORP baseline	188	255	228	277
ORP after 30 min dosing	716	769	364	394
ORP 5 days	450	611	192	323
start-up runs	1	1	1	1
maintenance runs	0	1	0	21
maintenance seconds	0	89	0	3749
secs/run	-	89	-	178.5
First dose (g/m^3 ozone)	4.8	4.8	4.8	4.8
Maintenance dose (g/m^3)	-	0.24	-	10.0
TRO (mg/l Cl_2) Day 0	0.01	0.01	0.03	0.01
TRO (mg/l Cl_2) Day 5	0.06	0.08	0.02	<0.01

Table 2 Water quality measurements and organisms counts (±sd) in challenge water and after 5 holding during Test 1

WQ1	**IMO Marine water**	**Challenge**	**23 Oct '15**	**Day 5**	**28 Oct'15**
		Single dose	**In-tank**	**Single dose**	**In-tank**
Salinity (‰)	>32	32.6±0.1	32.7±0.3	32.7±0.1	32.6±0.1
Temperature (°C)		21.1±0.8	21.4±1.3	16.1	16.0
pH		8.34±0.01	8.38±0.00	8.28±0.04	8.30±0.01
O_2 (%)		101.8±0.7	103.6±0.4	104.1±0.4	102.1±0.2
Turbidity (ntu)	-	0.60±0.06	0.64±0.07	0.14±0.03	0.18±0.02
TSS (mg/l)	>1	1.3	2.4	0.4	0.1
DOC (mg/l)	>1	4.06±0.08	3.94±0.10	4.63±0.07	3.37±0.06
Total chl-a (µg/l)	-	9.67±0.38	8.73±2.25	<3	<3
Diatoms (µg/l)	-	8.22±0.31	7.38±1.75	<3	<3
Total chl-a activity (%)	-	24.88±0.74	26.74±1.39	-	-
Counts					
Org ≥10-<50 µm (n/ml)	>1000	2105±0.0	2550±21	0	0
Org <10 µm (n/ml)	-	0	0	0	0
Enterococci (cfu/100ml)	-	53	10	<10	<10
Total coliforms (cfu/100ml)	-	53	10	<10	<10
E. coli (cfu/100ml)	-	<10	<10	<10	<10
HPC (cfu/100ml)	-	478	531	20	20

The challenge water in Test WQ1 complied to IMO requirements for challenge conditions in marine water (Table 2). Both treatments reached an ORP of more than 700 mV after dosing 4.8 g/m^3 ozone to the system (during a 30 min treatment). In the single dose treatment the ORP dropped to 450 mV after 5 days holding in a treatment tank. The in-tank treatment only needed one additional dose of 89 seconds, providing 0.24 g/m^3 ozone to maintain the ORP at the required maintenance level. In this tank the final ORP after 5 days was 611 mV (Figure 2, Table 1). TRO (expressed as mg/l Cl_2) was close to background levels after 5 days. Both treatments successfully removed the living organisms from the challenge water (Table 2).

For Test WQ2, much more challenging water was provided, although the TSS-level was lower than expected, as it settled rather quickly (Table 3). After dosing 4.8 g ozone/m^3 into the systems, neither system reached an ORP of 400 mV. Consequently, the maintenance dosing immediately proceeded when tank 2 was restarted after a 60 min pause. At that point ORP had dropped to 254 mV. During the next 3 days, 21 maintenance runs, with a total of 3749 seconds dosing, were needed, adding 10 g/m^3 ozone to the test water. At day 5, the final ORP after 2 days detoxification was 323 mV. In the single dose treatment the final ORP had dropped to 192 mV, which is lower than the start values (average 250 mV) (Figure 3, Table 1). In both treatments, the TRO was at baseline levels after 5 days.

Table 3 Water quality measurements and organisms counts (±sd) in challenge water and after 5 holding during Test 2

WQ2	**IMO fresh/brackish water**	**Challenge 29 Oct '15 Single dose**	**Challenge 29 Oct '15 In-tank**	**Day 5 3 Nov '15 Single dose**	**Day 5 3 Nov '15 In-tank**
Salinity (‰)	<32	31.9±0.1	31.8±0.0	31.9±0.1	32.2±0.1
Temperature (°C)		14.2	14.1	16.1±0.1	15.9±0.0
pH		8.39±0.01	8.41±0.03	8.34±0.00	8.36±0.00
O_2 (%)		99.0±0.6	99.8±1.3	82.8±0.9	102.5±0.6
turbidity (ntu)	-	9.26±0.35	8.66±0.25	14.52±0.42	12.69±0.39
TSS (mg/l)	>50	19.9	18.6	31.1	22.7
DOC (mg/l)	>5	9.35±0.09	8.80±0.07	9.80±0.13	10.96±0.08
Total chl-a (µg/l)	-	21.11±0.33	21.46±0.24	<3	<3
Diatoms (µg/l)	-	16.52±0.49	16.90±0.08	<3	<3
Total chl-a activity (%)	-	23.69±0.44	20.43±0.79	-	-
Counts					
Org ≥10-<50 µm (n/ml)	>1000	3930±99	3675±233	345±16.5	12.0±1.4
Org <10 µm (n/ml)	-	1580±255	1780±0.0	170±0.0	4.0±1.4
enterococci (cfu/100ml)	-	<10	<10	<10	<10
total coliforms (cfu/100ml)	-	384	306	10112	<10
E. coli (cfu/100ml)	-	<10	<10	<10	<10
HPC (cfu/100ml)	-	>2001	>2001	>24196	52

The single-dose treatment did reduce the number of algae in the test water, but not sufficient. Coliform bacteria and heterotrophic plate count even increased during the holding time (for HPC this could not be substantiated, as the limit of detection was too low for the challenge conditions).

The efficacy of the in-tank treatment was significantly better. Bacteria and algae were nearly completely eliminated, the level of organisms ≥10-<50 µm remaining just above the D-2 standard (Table 3).

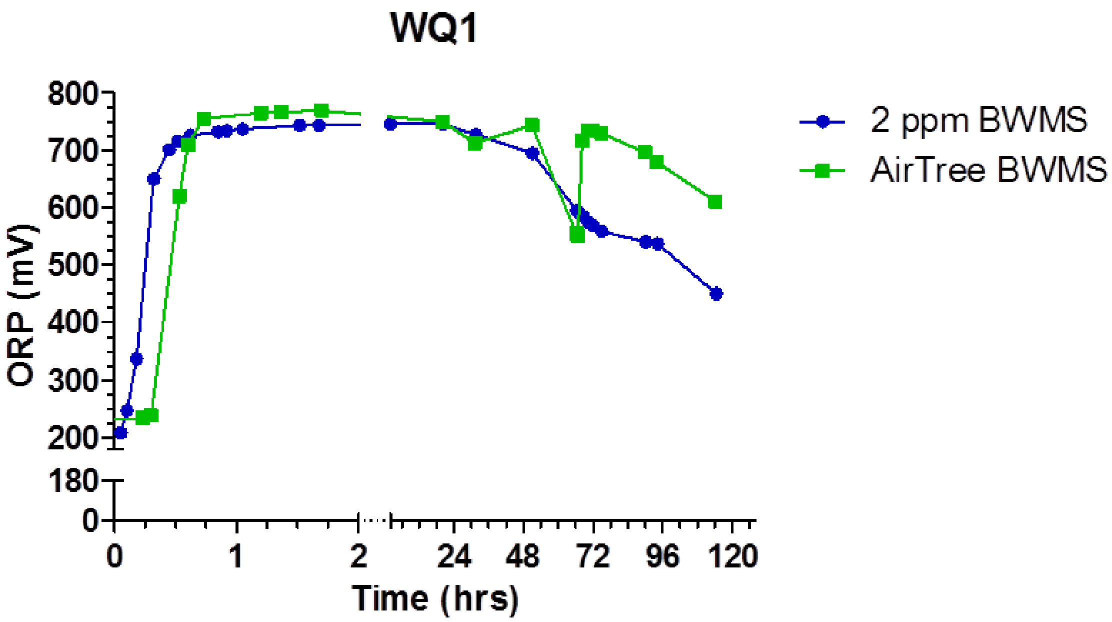

Figure 2 *ORP measurements during the WQ1 test, showing the effect of the maintenance dose just before termination of dosing after 72h.*

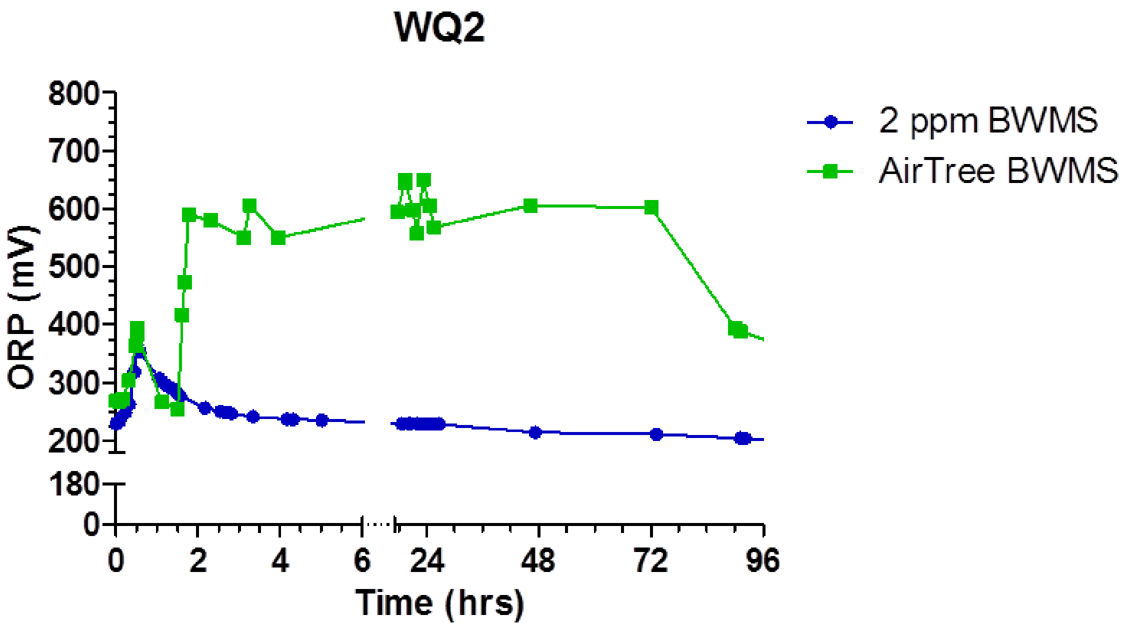

Figure 3 *ORP measurements during the WQ2 test clearly show the difference between the two treatments.*

4.2 Efficacy tests

The intention was to use an initial dose of 4 g/m^3 ozone during the efficacy tests, equivalent to a 25 min initial exposure period. Due to start-up problems this was stretched to 32 minutes in the freshwater test WQ3. Some dosing characteristics are presented in Table 4.

Table 4 Dosing characteristics WQ3, WQ4 and WQ5. ORP in mV

Dosing	**WQ3 29 Jan '16**		**WQ4 5 Feb '16**		**WQ5 18 Feb '16**	
	Tank 1	**Tank 2**	**Tank 1**	**Tank 2**	**Tank 1**	**Tank 2**
ORP baseline	229	233	208	216	200	211
ORP after 30 min dosing	668	871	372	403	509	535
ORP 5 days	276	290	305	288	352	360
start-up runs	3	2	1	1	1	1
maintenance runs	55	43	53	60	22	26
maintenance seconds	2152	1380	3325	2906	2466	2247
secs/run	39	32	63	48	112	86
First dose (g/m^3 ozone)	5.12	5.12	4.00	4.00	4.00	4.00
Maintenance dose (g/m^3)	5.74	3.68	8.87	7.75	6.58	5.99
TRO (mg/l Cl_2) Day 0	0.08	0.10	0.52	0.11	1.36	0.96
TRO (mg/l Cl_2) Day 5	0.05	0.10	0.34	0.37	1.56	1.22

4.2.1 Freshwater test

The salinity of the challenge water that was used for test WQ3 was 0.3 ‰. The TSS was low (<1 mg/l), but no additional solids were added. With 24.5±4.9 mg/l, the DOC was very high. The number of organisms in the size class ≥10-<50 µm was approx. 900/ml, while also some 400 organisms <10 µm were present. Bacteria load was low (Table 5). There were no significant differences between the replicate treatment tanks.

Due to leaking connections, it took some effort to get started, especially for treatment tank 1. In the end the initial dose added up to >5 g/m^3, which resulted in an initial ORP value of only 668 mV for Tank 1, compared to 871 mV for Tank 2. Consequently, Tank 1 needed a higher maintenance dose of in total 5.7 g/m^3, compared to 3.7 g/m^3 for Tank 2. Overall, the average duration of the maintenance runs became comparable (39 vs 32 sec), but the first maintenance doses in Tank 1 lasted 65 sec, whereas the first in Tank 2 lasted only 40 sec. The final ORP at discharge was 276 and 290 mV respectively (Table 4). The TRO levels were at baseline levels after 5 days.

After a 5 day holding period, hardly any living algal cells were found in the test water. The number of heterotrophic bacteria remained stable (tank 2), or even showed some increase (Tank 1) as can be seen in Table 5. No regrowth was observed in 1 litre test water incubated with algal nutrients in the light for 14 days.

Table 5 *Water quality measurements and organisms counts (±sd) in challenge water and after 5 holding during Test WQ3*

WQ3	IMO fresh water	Challenge Tank 1	29 Jan '16 Tank 2	Day 5 Tank 1	3 Feb '16 Tank 2
Salinity (‰)	<3	0.3±0.01	0.3±0.00	0.3	0.3
Temperature (°C)		15.7	15.5	21.2*	21.0*
pH		8.57	8.54	7.93	7.98
O_2 (%)		103.2±0.3	102.9±0.1	109.9	107.9
Turbidity (ntu)	-	0.63±0.09	0.52±0.04	0.20±0.05	0.08±0.03
TSS (mg/l)	>50	0.8	0.3	0.2	0.2
DOC (mg/l)	>5	21.08	27.99	5.04	3.71
Total chl-a (µg/l)	-	4.13±0.07	4.05±0.18	<3	<3
Total chl-a activity (%)	-	39.56±2.98	47.34±3.61	-	-
Counts					
Org ≥10-<50 µm (n/ml)	>1000	872±148	920±56	2.0±2.1	0±0.0
Org <10 µm (n/ml)	-	390±71	412±28	0±0.0	0±0.0
Enterococci (cfu/100ml)	-	<1	<1	<1	<1
Total coliforms (cfu/100ml)	-	1	<1	<1	<1
E. coli (cfu/100ml)	-	<1	<1	<1	<1
HPC (cfu/100ml)	-	39.3	41.1	95.6	35.8

* Sample stored at ambient temp

4.2.2 Brackish water test

The salinity of the water used for test WQ4 was 11.5‰. TSS level was low, but was not artificially augmented. The DOC level was high. Although both tanks were simultaneously filled, the challenge water in tank 1 appeared to have higher values of TSS and DOC than the water in tank 2. The difference in TSS levels was not supported by a different turbidity. The organism levels were comparable in both tanks. Both tanks contained approx. 2000 org/ml in the size class ≥10-<50 µm and ca. 400 org/ml in the size class <10 µm. The number of bacteria was high (Table 6).

The initial dose of 4 g/m^3 ozone was delivered in one run, resulting in ORP values of 372 and 403 mV for Tank 1 and Tank 2 respectively. Tank 1 needed less maintenance doses, but these lasted much longer than those for Tank 2. As a result, Tank 1 received a maintenance dose of 8.87 g/m^3 ozone compared to 7.75 g/m^3 ozone for Tank 2. The final ORP at discharge was 305 and 288 mV respectively (Table 4). The baseline TRO levels were higher and more variable compared to the freshwater tests, but were not raised after 5 days.

The treatment successfully reduced the number of organisms in both treatment tanks. Although still some chlorophyll-a was measured, organisms in the size class ≥10-<50 µm (only 1 live cell in 6 ml assessed) and <10 µm were completely absent, enterococci were halved, coliforms removed, and heterotrophic bacteria strongly reduced (Table 6). In the 1 litre regrowth samples, the level of chlorophyll-a was further reduced after a 10-d incubation period, but not below detection. Also a few living cells in the size class ≥10-<50 µm were found (4 resp. 20 per ml), as well as ciliates and other heterotrophic organisms <10 µm.

Table 6 *Water quality measurements and organisms counts (±sd) in challenge water and after 5 holding during Test WQ4*

WQ4	**IMO brackish water**	**Challenge Tank 1**	**5 Feb '16 Tank 2**	**Day 5 Tank 1**	**10 Feb '16 Tank 2**
Salinity (‰)	3-32	11.43	11.54	11.62	11.61
Temperature (°C)		17.2	16.9	15.1	14.9
pH		8.38	8.39	8.13	8.18
O_2 (%)		95.1	95.1	96.2	95.8
Turbidity (ntu)	-	2.17±0.01	2.18±0.02	0.51±0.01	0.51±0.02
TSS (mg/l)	>50	16.5	6.3	3.6	3.7
DOC (mg/l)	>5	42.99	29.09	11.56	9.95
Total chl-a (µg/l)	-	46.71	46.22	8.27	9.49
Green algae (µg/l)	-	40.63	40.23	8.05	9.24
Total chl-a activity (%)	-	50.45	50.06	33.51	30.34
Counts					
Org ≥10-<50 µm (n/ml)	>1000	1823±42	2057±55	0±0.0	0.3±0.6
Org <10 µm (n/ml)	-	390±71	412±28	0±0.0	0±0.0
Enterococci (cfu/100ml)	-	78.9	124.6	35.5	53.8
Total coliforms (cfu/100ml)	-	816.4	689.3	<1	3.1
E. coli (cfu/100ml)	-	<1	<1	<1	<1
HPC (cfu/100ml)	-	>2419.6	>2419.6	105	96

4.2.3 Marine water test

The salinity of the water used for test WQ5 was 34.9‰. The TSS was approx. 8 mg/l in both tanks, DOC around 5 mg/l. Only around 600 organisms/ml in the size class ≥10-<50 µm were present, but this was compensated by the number of organisms <10 µm (ca. 2500/ml). The IDEXX assessments only revealed some 800-1000 cfu/ml heterotrophic bacteria (Table 7).

The initial dose of 4 g/m^{3}ozone was supplied in one dose, resulting in an ORP of 509 and 535 mV in tank 1 and tank 2 respectively. Both tanks needed a similar number of maintenance doses (22 vs 26), but in tank 1 the average maintenance dose lasted 112 sec, versus 86 seconds in tank 2. The total maintenance dose in tank 1 was, therefore, higher than in tank 2. The final ORP at discharge was 352 and 360 mV respectively (Table 4). The baseline TRO-levels in WQ5 were clearly higher than in the tests with freshwater (WQ3) and brackish water (WQ4), and also compared to test WQ1 and WQ2. After 5 days the TRO levels were slightly higher than at the start, but it is not clear if this is treatment related.

The treatment successfully removed organisms ≥10-<50 µm and nearly all <10 µm. The latter group showed some regrowth after 8d incubation of a 1 litre discharge sample. Heterotrophic bacteria were slightly reduced in tank 1, but showed a strong increase in tank 2 (Table 7).

Table 7 *Water quality measurements and organisms counts in challenge water and after 5 holding during Test 5*

WQ5	IMO marine water	Challenge Tank 1	18 Feb '16 Tank 2	Day 5 Tank 1	23 Feb '16 Tank 2
Salinity (‰)	>32	34.9	34.9	34.7	34.9
Temperature (°C)		15.6	15.6	14.3	14.3
pH		8.41	8.47	8.12	8.16
O_2 (%)		94.1	94.1	96.4	96.4
Turbidity (ntu)	-	2.34±0.21	2.53±0.20	0.66±0.01	1.00±0.08
TSS (mg/l)	>1	8.7	7.4	9.4	5.0
DOC (mg/l)	>1	7.14	4.36	4.93	4.64
Total chl-a (µg/l)	-	3.84	3.97	<3	<3
Diatoms (µg/l)	-	<3	<3	<3	<3
Total chl-a activity (%)	-	46.1	48.8	-	-
Counts					
Org ≥10-<50 µm (n/ml)	>1000	602±40	577±103	0±0.0	0±0.0
Org <10 µm (n/ml)	-	2525±118	2457±268	0±0.0	0.3±0.6
Enterococci (cfu/100ml)	-	<10	<10	<10	<10
Total coliforms (cfu/100ml)	-	<10	<10	<10	<10
E. coli (cfu/100ml)	-	<10	<10	<10	<10
HPC (cfu/100ml)	-	829	1056	609	6488

5 Conclusions

5.1 Comparison tests

The aim of these experiments was to test three relevant assumptions:

1. At low organism densities and/or (in)organic loads the AirTree BWMS uses less ozone than a BWMS using a single dose
2. At high organism densities and/or (in)organic loads the AirTree BWMS is still capable of treating the ballast water efficiently to meet IMO requirements, while a BWMS based on a fixed single dose may not.
3. Re-growth is prevented by using a maintenance dose.

In a later stage, the client decided to use a fixed initial dose instead of aiming at a minimum ORP level. Therefore, the first assumption could not be evaluated.

The second assumption was very clearly demonstrated and even quicker than originally envisaged. Originally, WQ1 and WQ2 were set-up as minimum conditions according to IMO G8 and it was expected that a single dose would be sufficient to treat the water in both tests, as ozone systems following this principle have already been type approved. It was, therefore, a complete surprise to find that the single dose treatment already failed at WQ2, which was intended to be close to IMO G8 requirements for fresh and brackish water. Admittedly, DOC and the number of organisms were well over the minimum requirements, but not extreme and TSS was lower.

Bacteria levels were significantly reduced indicating there was no regrowth of bacteria in these tests.

5.2 Efficacy tests

In the efficacy tests, TSS-levels were below IMO challenge conditions in the freshwater (WQ3) and brackish water (WQ4) tests. Augmenting TSS with Arizona Dust was not considered relevant due to the rapid settling out observed in the previous tests. The DOC-levels on the other hand were much higher than required.

The efficacy tests were initiated when the number of organisms (≥10-<50 µm) was sufficient in the cultures, except for the marine test (WQ5) where compensation by the high number of organisms <10 µm was considered acceptable.

During start-up of the WQ3 freshwater test, the treatment tanks were leaking at the connections of the tubing. Especially for tank 1 it took some time to secure all connections, resulting in a lower ORP start level and more maintenance runs compared to tank 2. During the brackish (WQ4) and marine (WQ5) tests, no leaking was observed anymore and the primary dose could be delivered in one run. Still in these two tests also, tank 1 received a higher total maintenance dose than tank 2. Although the total number of maintenance runs was lower in tank 1, the maintenance runs lasted longer. Overall, the highest maintenance dose was needed in the test with brackish water, whereas the total dose in freshwater and marine water was comparable.

Notwithstanding these differences, in both tanks and all three water types the number of organisms in the size class ≥10-<50 µm was successfully reduced to levels below the D-2 standard. Also the organisms <10 µm were nearly completely removed from the test water. Although these are not regulated in the D-2 standard, the treatment can be considered effective for this group too. In the brackish test, high concentrations of chlorophyll-a were still present in the test water after 5 days, although living algal cells could hardly be found using vital staining. Exposing a water sample to continuous light and some aeration to promote regrowth showed a further decline of chlorophyll-a after 10 days culturing and only a limited regrowth of organisms. Apparently the treatment did not completely destruct the chlorophyll-a in dead cells. In the marine test, the regrowth test (8 days) showed recovery of organisms <10 µm, accompanied by some increase in chlorophyll-a.

In contrast to the first tests (WQ1 and WQ2), the effectiveness against bacteria is not clear. The effect on heterotrophic bacteria, as measured with the IDEXX HPC method, was variable, sometimes even showing significant regrowth. It is possible that these micro-organisms rapidly develop on the available dead organic material during the last two days when dosing is stopped prior to discharge (Perrins *et al.*, 2006).

5.3 Recommendations

In conclusion, in-tank treatment with ozone is effective against organisms in the size class ≥10-<50 µm and also to smaller organisms. The treatment is also effective at more challenging conditions where a single treatment would fail, due to the fact that ozone is delivered into the system as long as it is consumed by organic material and other sinks. However, the water is not completely sterilized and low numbers of organisms survive, even in the confined space of the test containers. The regrowth potential of bacteria needs attention.

The following steps are recommended to further develop the system:

1. Analysis of DBP-formation and residual toxicity during long-term exposure as is already under discussion.
2. Investigate bacterial regrowth during the last two non-exposure days.
3. The leaking of ozone during test WQ3 did not seem to have had consequences for the efficacy. Leakage will be unavoidable in large-scale treatments. When tests are scaled up to relevant volumes, the larger treatment volume may compensate for leakage as less ozone will be vented off.

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Justification

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The scientific quality of this report has been peer reviewed by a colleague scientist and a member of the Management Team of IMARES.

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