BENCH-SCALE TECHNICAL REPORT

TESTS OF THE NANO BUBBLE OZONE TECHNOLOGY (3 HP UNIT)

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ABSTRACT

This technical report presents the bench-scale evaluation of the Nano Bubble Ozone Technology 3-horsepower unit (NBOT) developed by NABAS Group Inc. of Rockville, Maryland. This evaluation was the first to assess NBOT as a potential, in-tank, recirculating ballast water treatment method for the Laurentian Great Lakes.

The evaluation began in March 2019 and ended in June 2019. All analyses occurred at the Lake Superior Research Institute (LSRI) at the University of Wisconsin-Superior (UWS) in Superior, Wisconsin, USA. The treatment technology uses cavitation to create ultrafine microbubbles (nanobubbles) containing ozone (O_3) generated by the system. According to the developer, the resulting ozone and hydroxyl radical biproducts destroy all chemicals containing activated functional groups (aldehydes, ketones, amines, nitrates, etc.), RNA, DNA, peptides, steroids, as well as activated organic compounds (herbicides and pesticides), and microbial toxins.

The ability of NBOT to increase dissolved ozone and oxidation-reduction potential in a 1000-L treatment tank was tested at two water temperatures (~10°C and ~25°C) using both dechlorinated laboratory water and amended dechlorinated laboratory water. Ozone levels observed to be generated by NBOT were lower than anticipated based on observations by Dr. Peter Moeller of the National Oceanic and Atmospheric Administration's (NOAA) National Ocean Service (NOS) who was utilizing a newer model of NBOT. Biological dose effectiveness testing was not completed, per the developer's request, due to below expected levels of ozone.

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1 ACKNOWLEDGEMENTS

LSRI-GWRC would like to thank the American Marine University Research Institute Inc. for their application to our laboratory-based testing program and for providing the NBOT 3 HP ballast water treatment (BWT) testing unit. Brian Domrese of NanoPure Tech provided installation and operational training and technical support prior to the start of and during testing.

This work was supported by the United States Maritime Administration (United States Department of Transportation; Washington, D. C.; Agreement Number DTMA9117H00002) and the United States Environmental Protection Agency's Great Lakes Restoration Initiative.

2 INTRODUCTION

The Lake Superior Research Institute's (LSRI) Great Waters Research Collaborative (GWRC) aims to provide unbiased and independent data to accelerate the development of technologies with potential to prevent the introduction and/or control the spread of non-indigenous organisms within the Laurentian Great Lakes. This document describes the bench-scale evaluation of the Nano Bubble Ozone Technology 3 horsepower unit (NBOT) as developed by NABAS Group Inc. (Rockville, Maryland, USA) and provided by American Marine University Research Institute, Inc. (AMURI).

The NBOT ballast water treatment (BWT) technology produces nanobubbles impregnated with ozone (O_3) that can react with water to generate hydroxyl (OH) radicals. Ozone and hydroxyl radicals are known to have antiseptic properties and can destroy algae, fungi, bacteria, and viruses. The NBOT BWT is a patented technology that has been tested in a laboratory setting by Dr. Peter Moeller of the National Oceanic and Atmospheric Administration's (NOAA) National Ocean Service (NOS) in Charleston, South Carolina. The system has also been applied to commercial field trial treatments of ponds, lakes, and contaminated canals in Florida, Ohio, South Carolina, and Washington D.C. From May to June 2019, the NBOT BWT was evaluated for its applicability to treat Laurentian Great Lakes ballast water as part of GWRC's technology testing program. The NBOT BWT is a proposed in-tank treatment technology, which would treat ballast water on a Great Lakes vessel during the voyage from one port to another. Laboratory, or bench-scale tests, took place at the LSRI of UWS in Superior, Wisconsin, USA. Test objectives included:

1. Determination of the dissolved oxygen concentration, ozone concentration, and oxidation-reduction potential (ORP) in simulated Laurentian Great Lakes ballast water treated using NBOT BWT over time.
2. Determination of the impact of temperature and water quality on the generation of ozone during NBOT treatment.
3. Determination of the aquatic degradation of ozone following NBOT treatment, and the impact of temperature and water quality on the rate of degradation.

3 TEST METHODS

3.1 TEST PLAN AND SOPS

A test plan (Schaefer et al., 2019) and standard operating procedures (SOPs) were used to implement all test activities. These procedures facilitate consistent conformance to technical and quality system requirements and increase data quality in addition to providing unbiased, independent data. The test plan details sample and data collection, sample analysis, sample handling and preservation, and quality assurance/quality control (QAQC) requirements. The test plan was approved by both LSRI-GWRC and the AMURI on May 6, 2019, prior to the start of bench-scale test activities. The procedures followed throughout testing are described in the *Test Methods* section and listed in the *References* section of this report.

3.2 BWT TECHNOLOGY AND EXPERIMENTAL APPARATUS

The NBOT BWT (Model N3), as supplied by the developer, is the three-horsepower unit (Figures 1 and 2) (NABAS, 2019). The stand-alone unit is comprised of a controller, O_3 generator, oxygen generator, a patented motor-mixer, and an intake pump. The system is contained within a metal cabinet which measures approximately 67 cm deep x 141 cm wide x 141 cm tall. The flow rate range is 6-17 m^3/hr. Electrical power to the system is 208 volt (V) single phase with a power consumption of 4.5 kW. The system can operate at temperatures of -29°C to 49°C.

Figure 1. NBOT system with both doors open. The control panel, oxygen generator, and ozone generator are in the left side of the cabinet. The pump and microbubbler are in the right side of the cabinet.

Figure 2. Control panel of NBOT system.

3.3 EXPERIMENTAL WATER PREPARATION

During testing, two water types were used to test the system at two challenge levels. Laboratory water (LW) provides a low water quality challenge to treatment technologies, generally, and amended lab water (LW-TMH) provides a higher challenge. These solutions are defined by a range of chemical parameters including organic carbon, suspended solids, and UV-transmittance. Table 1 outlines the target ranges for each parameter within samples collected prior to the start of each test trial. Treatment processes may alter water quality, therefore, the targets described in Table 1 apply only to test initiation. These water types were prepared as described below:

Laboratory Water (LW): Prior to each test, the 1,000-L control and treatment tank were filled with LW at the approximate test temperature. The LW is municipal water from the City of Superior, Wisconsin, that is passed through an activated carbon column to remove the majority of the chlorine. The remaining residual chlorine is removed through injection of sodium sulfite, resulting in a total residual chlorine concentration of < 5.4 µg/L Cl_2 (LSRI 2019 detection limit for chlorine analysis). Typically, LW has a very low concentration of organic carbon, solids, and a very high UV transmittance.

Amended Laboratory Water (LW-TMH): Prior to each test, the 1,000-L control and treatment tanks were filled with approximately 200 L of LW at the approximate test temperature. While the tank was filling,

LW-TMH was prepared by amending the LW with 12 mg/L pre-sterilized ISO 12103-1, A2 Fine Test Dust (Powder Technology, Inc.; Arden Hills, MN, USA), 12 mg/L pre-sterilized Micromate™ (micronized humate for liquid suspension; Mesa Verde Humates; Bernalillo, NM, USA), and 20 mg/L humic acid (ACROS organics, New Jersey, USA). The amended water was mixed thoroughly in the control and treatment tanks until few visible clumps of Fine Test Dust or Micromate™ remained and a homogenous solution was achieved. Then, both tanks were filled to the 1000-L mark. LW-TMH is used to achieve challenge conditions like those stipulated in the U.S. Environmental Protection Agency (USEPA) Environmental Technology Verification (ETV) Program's *Generic Protocol for the Verification of Ballast Water Treatment Technology*, version 5.1 (USEPA, 2010).

Table 1. Target ranges for LW and LW-TMH water chemistry and water quality parameters.

Parameter	Units	Water Type	Acceptable Range for Initiating Bench-Scale Testing
Temperature	°C	LW	22 – 28, 10-15*
		LW-TMH	
pH	NA	LW	6.5 - 9.0
		LW-TMH	
Specific Conductivity	µS/cm	LW	120-170
		LW-TMH	120-170
Salinity	ppt	LW	< 1
		LW-TMH	
Dissolved Oxygen (DO)	mg/L	LW	4 - 12
		LW-TMH	
Total Suspended Solids (TSS)	mg/L	LW	Less than reporting limit
		LW-TMH	11.9 - 30.3
Particulate Organic Matter (POM)	mg/L	LW	Less than reporting limit
		LW-TMH	4.1- 12.1
Dissolved Organic Carbon (DOC)	mg/L	LW	Less than detection - 2
		LW-TMH	4.4 - 6.8
Non-Purgeable Organic Carbon (NPOC)	mg/L	LW	Less than detection - 2
		LW-TMH	5.1 - 13.1
Percent UV Transmittance at 254 nm (%T)	%	LW	93.0 – 100.0 (filtered and unfiltered)
		LW-TMH	25.5 - 35.5 (filtered and unfiltered)

*Tests occurred at two temperatures, ranges are for 25°C and 10°C tests, respectively.

3.4 BWT TECHNOLOGY INSTALLATION AND COMMISSIONING

Prior to testing NBOT, the system was installed in LSRI's Aquatic Toxicology Testing Laboratory by AMURI's Senior Electrical and Mechanical Engineer, Brian Domrese. The UWS campus electrician made the necessary connections between the system and the building's electrical system. LSRI staff members, Olivia Anders, Kimberly Beesley, Christine Polkinghorne, Heidi Saillard, Deanna Regan, and Kelsey Prihoda received hands-on training on the operation of the BWT and were informed of the recommended operating conditions and safety measures required during operation. The NBOT system was operating at an acceptable level upon completion of the installation and details were recorded on GWRC/FORM/22 – *Bench-Scale Ballast Water Management System (BWMS) Installation Acceptance Form* on March 8, 2019.

3.5 BWT TECHNOLOGY TEST DESIGN

LSRI-GWRC tested NBOT's effect on ozone, ORP, and dissolved oxygen (DO) concentrations in a 1000-L tank through "water only" testing in both LW and LW-TMH at two temperatures (10°C and 25°C). Outflow from NBOT was recirculated into the treatment tank throughout the system operation time. During treatment, ozone, ORP, DO, temperature, pH, and specific conductivity were measured prior to the start of system operation and every 15 minutes thereafter until ozone concentrations stopped increasing (or up to three hours). An additional 1000-L tank served as the control and was sampled for ozone and ORP prior to the start of treatment, 15 minutes after initiation, at the mid-point of the run time, and immediately prior to treatment system shut down. The DO, temperature, pH, and specific conductivity were measured on the control tank at the same time points as the treatment tank. Total suspended solids (TSS), particulate organic matter (POM), total non-purgeable organic carbon (NPOC), dissolved organic carbon (DOC), hardness, and alkalinity samples were collected from both the control and treatment tanks prior to starting the treatment system, 15 minutes after initiation, at the mid-point of the run time, and immediately prior to treatment system shut down.

Once ozone concentrations stopped increasing, or after three hours of operation, the system was shut off. Three replicate, one-gallon (3.785 L) bottles of water were collected from both the treatment and control tanks immediately prior to system shut down. The water was held in a dark incubator at the test temperature for a period of 48 hours. The DO, temperature, pH, specific conductivity, ozone and ORP were measured in each bottle at the following time points: 30 minutes, 1 hour, 2 hours, 4 hours, 24 hours, and 48 hours post-treatment. Once ozone measurements in treated water were below the detection limit, no further ozone measurements were collected.

3.6 ANALYTICAL METHODS

Water quality parameters that may have an impact on BWT performance or may impacted by the treatment process were measured during this evaluation. These parameters included TSS, percent transmittance (%T), POM, NPOC, DOC, total alkalinity, total hardness, DO, temperature, specific conductivity, and pH.

3.6.1 TOTAL SUSPENDED SOLIDS, PARTICULATE ORGANIC MATTER, AND MINERAL MATTER

TSS analysis was conducted according to LSRI/SOP/SA/66, v.1 – *Analyzing Total Suspended Solids (TSS), Particulate Organic Matter (POM), and Mineral Matter (MM)* (LSRI, 2017). Accurately measured sample volumes (±1%) were vacuum filtered through pre-washed, dried, and pre-weighed glass fiber filters (Whatman 934-AH, 1.5 µm pore diameter). After each sample was filtered, it was dried in an oven and brought to constant weight. TSS values were determined based on the weight of particulates collected on the filter and the volume of water filtered. To determine POM, the residue from the TSS analysis was ignited to a constant weight at 550°C in a muffle furnace. The concentration of POM was determined by the difference of the dry weight of the particulates on the filter before and after ignition (the mass lost to combustion). Mineral matter was defined, and calculated, as the difference between TSS and POM.

3.6.2 PERCENT TRANSMITTANCE AT 254 NM

Percent transmittance analysis was conducted according to LSRI/SOP/SA/69 – *Laboratory Determination of Percent Transmittance (%T) of Light in Water at 254 nm* (LSRI, 2018b). The %T was measured on both unfiltered and filtered aliquots of each sample collected using a Perkin Elmer Lambda 35 UV-Vis spectrophotometer. For analysis of the filtered aliquot, an appropriate volume of sample was filtered through a glass fiber filter (Whatman 934-AH, 1.5 µm pore diameter). Deionized water was used as a reference to adjust the spectrophotometer to 100%T, and then each aliquot was measured in a pre-rinsed sample cuvette with a 1-cm path length.

3.6.3 ORGANIC CARBON ANALYSIS

NPOC/DOC analysis was conducted according to LSRI/SOP/SA/47, v.1 – *Measuring Organic Carbon in Aqueous Samples* (LSRI, 2006) and using a Shimadzu Model TOC-L Total Organic Carbon Analyzer. After collection, DOC samples were filtered through a glass fiber filter (Whatman GF/F, 0.7 µm effective pore size). Before analysis, the samples were acidified to a pH<2 with concentrated hydrochloric acid (HCl; ~ 0.2% v/v). Samples were then purged with high-purity air to remove the inorganic carbon and purgeable organic carbon and injected into the analyzer. Amended samples (LW-TMH) were sonicated for a minimum of 30 minutes and were stirred continuously, using a stir bar and stir plate, while being manually injected into the instrument. A 1000 mg/L organic carbon stock solution was used to prepare a working standard of 50 mg/L carbon, which was also acidified to a pH <2 with concentrated HCl. The standard was used to generate a calibration curve from which the organic carbon concentration of the samples was determined.

3.6.4 HARDNESS AND ALKALINITY

Total hardness was analyzed using the ethylenediaminetetraacetic acid (EDTA) titrimetric method through manual titration according to the method described in LSRI/SOP/GLM/02, v.3 – *Procedure for Measuring Total Hardness* (LSRI, 1991b). Total hardness is reported as mg/L $CaCO_3$. Analysis of total alkalinity was conducted using the sulfuric acid titrimetric method through manual titration and

according to the method described in LSRI/SOP/GLM/01, v.3 – *Procedure for Measuring Alkalinity* (LSRI, 1991a). Total alkalinity is reported as mg/L $CaCO_3$.

3.6.5 DISSOLVED OXYGEN ANALYSIS

Analysis of DO was conducted using a YSI ProSolo handheld meter and optical dissolved oxygen/temperature probe, which was calibrated daily following LSRI/SOP/GLM/34 – *Calibrating, Maintaining and Using the YSI ProSolo Handheld Meter and Optical Dissolved Oxygen/Temperature Probe* (LSRI, 2019).

3.6.6 TEMPERATURE, CONDUCTIVITY, AND pH

Temperature was measured using a Fisher digital thermometer that was verified quarterly following LSRI/SOP/GLM/17, v.2 – *Procedure for Thermometer Verification and Calibration* (LSRI, 1995). Specific conductivity was measured using an Oakton Model CON 110 Conductivity/TDS/Temperature Meter that was calibrated on a monthly basis following LSRI/SOP/GLM/26, v.2 - *Procedures for Calibrating and Using the Oakton CON 110 Conductivity/TDS/Temperature Meter* (LSRI, 2011a). Its accuracy was verified daily prior to sample analysis using a potassium chloride check standard. pH analysis was conducted using an Orion 3 Star meter and Orion 8157BNUMD pH probe. Both instruments were calibrated daily following LSRI/SOP/GLM/05, v.6 – *Procedure for Calibration and Operation of pH Meters Utilizing Automatic Temperature Compensation (ATC)* (LSRI, 1992). A check buffer of 8.00 was measured after calibration to verify the accuracy of the calibration.

3.6.7 OXIDATION REDUCTION POTENTIAL

The ORP was measured following *LSRI/SOP/SA/54, v.2 - Determination of Oxidation-Reduction Potential (ORP)* (LSRI, 2011b). ORP was measured using a Thermo Scientific Orion Epoxy Refillable ORP/ATC Triode, with a platinum indicator electrode and a silver/silver chloride reference electrode. Calibration was performed daily with an ORP standard (Thermo Scientific, Orion #967901). Accuracy was verified daily using an externally-sourced reference standard (600 or 200 mV vs Ag/AgCl; RICCA Chemical Company).

3.6.8 OZONE

Ozone concentration was measured according to the method in *LSRI/SOP/SA/73 - Analyzing Ozone Concentrations in Water* (LSRI, 2019). Test water was reacted immediately with an Indigo Reagent. Ozone reacts quickly with the reagent so a decrease in absorbance at 600 nm can be related to ozone concentration. The detection range of this method is 0.05-0.5 mg/L. To measure ozone at higher concentrations, samples were diluted so ozone concentrations were within the measurable range. It should be noted that according to Baird et al. (2017) this method measures residual ozone in aqueous solutions. It is unknown if this method is able to measure ozone confined within nanobubbles. Due to this, it is possible ozone concentrations were underestimated by this method.

3.7 DEVIATIONS

During the course of NBOT testing, two deviations from the TQAP and SOPs occurred. These deviations are listed in Table 2 along with corrective actions that were taken as a response to the deviations and the perceived impact of the deviations on the test results.

Table 2. Deviations encountered during NBOT bench-scale testing, potential impact, and corrective actions.

Test Date(s)	Description and Root Cause of Deviation or Quality Control Failure	Description of Corrective Action(s)	Describe the Impact on the Project/Test	Do the Data Need to be Qualified? (Y/N)	Analyst Name
26 June 2019	POM values in the Treatment LW-TMH Stock were outside the acceptable ranges for initiation bench-scale testing that were listed in the test plan.	Add TMH to the tanks when they have 200 liters of lab water in them so the TMH is allowed to mix as the tank is filling the rest of the way. Be sure samples are collected beneath the surface of the water in the Control and Treatment tanks and that they are collected from the same spot within the tank each time.	Minimal, all other Control and Treatment LW-TMH Stock parameters were within the acceptable ranges for initiating bench-scale testing.	N	Kimberly Beesley
4 June 2019, 5 June 2019, 26 June 2019, 27 June 2019	No POM reference standards were analyzed over the course of the project	LSRI SOP SA/66-Analyzing Total Suspended Solids (TSS), Particulate Organic Matter (POM) and Mineral Matter (MM) was updated to clarify when the POM reference standard should be analyzed and when the combined TSS/POM reference standard should be analyzed.	Minimal, all water quality parameters, excepting a single test's POM (see above), were within the acceptable range for test initiation for all tests. Additionally, all TSS reference standards were within the acceptable range. These suggest a POM reference standard would likely also have been with the acceptable range because TSS samples undergo most of the same handling as POM samples. Despite this, the POM data should be qualified as were not verified as accurate on a daily basis.	Y	Kimberly Beesley

4 TEST RESULTS

4.1 NBOT BWT OPERATIONAL PERFORMANCE

Prior to initiating testing, the UWS Environmental Health and Safety Director measured ozone levels in the laboratory air while the NBOT system was running. Ozone concentrations were found to be below

levels of concern for LSRI staff. During an initial testing attempt, the NBOT system was not pumping water at a consistent rate, and the pressure gauge was fluctuating. Discussions with Brian Domrese determined that there was a leak in the system. Mr. Domrese traveled to LSRI and replaced hoses and connections to repair the system.

During the first test run in LW at 25°C, the system was stopped due to the ammeter dropping below 1 A. Data from that test is not included in this report. In the following tests, the ammeter was monitored and if the ammeter dropped below 1 A, the ozone adjuster was increased until the ammeter read > 1 A. No other operational issues were observed by LSRI staff during the remaining water-only tests.

Due to lower than desired ozone concentrations achieved during all testing with the 3 HP system, the developer suggested a newer version of the NBOT system (2.5-horsepower model) be employed for further efficacy testing of the BWT.

4.2 WATER QUALITY

Water quality parameters were within target ranges for all tests except the LW 25°C test (Table 3). In the LW 25°C test, POM concentrations were greater than the target range. The effects of this deviation are discussed in the *Deviations* section (Table 2).

During the LW tests, TSS, POM, MM, NPOC, DOC, and %T measurements were similar between the control and treatment tanks. During LW-TMH tests, in both the treatment and control tanks, TSS, POM, and MM decreased with experiment time. These results suggest that this was not an effect of the treatment process but was due to incomplete suspension at the initiation of the experiment. This may have resulted in settling out of larger particle aggregates since decreases occurred in both tanks, although the control tank was stirred prior to each sample point and the treatment tank was significantly agitated by the NBOT system. It is unknown if the incomplete suspension affected the results of these tests. TSS, POM, and MM initial concentrations in the control and treatment tanks at initiation of the LW-TMH 25°C test were within the target ranges (Table 1) but were not similar to one another (Table 3). Some variability observed in these measurements may have arisen due to uneven distribution of the Micromate™ in the LW-TMH coupled with inconsistent sampling methods. To avoid this in future tests, LSRI staff developed guidelines for preparation of LW-TMH and consistent sampling (LSRI, 2020).

In the treatment tank, NPOC and DOC both decreased slightly in all LW-TMH tests. In the control tanks, NPOC decreased slightly and DOC increased. NPOC decreases in the treatment and control tank were comparable, suggesting the NPOC decrease observed in the treatment tank may not be driven by treatment.

During treatment of LW-TMH water, large increases in filtered %T (23.2% at 25°C and 26.3% at 10°C) were observed in the treatment tank, but not the control tanks. This result suggests that NBOT is somewhat effective at removing UV-absorbing dissolved organic matter, as UV transmittance increased with treatment. Additionally, the LW-TMH water became visibly lighter in color during the treatment

period, indicating that colored dissolved organic matter was also being removed during treatment (Figure 3).

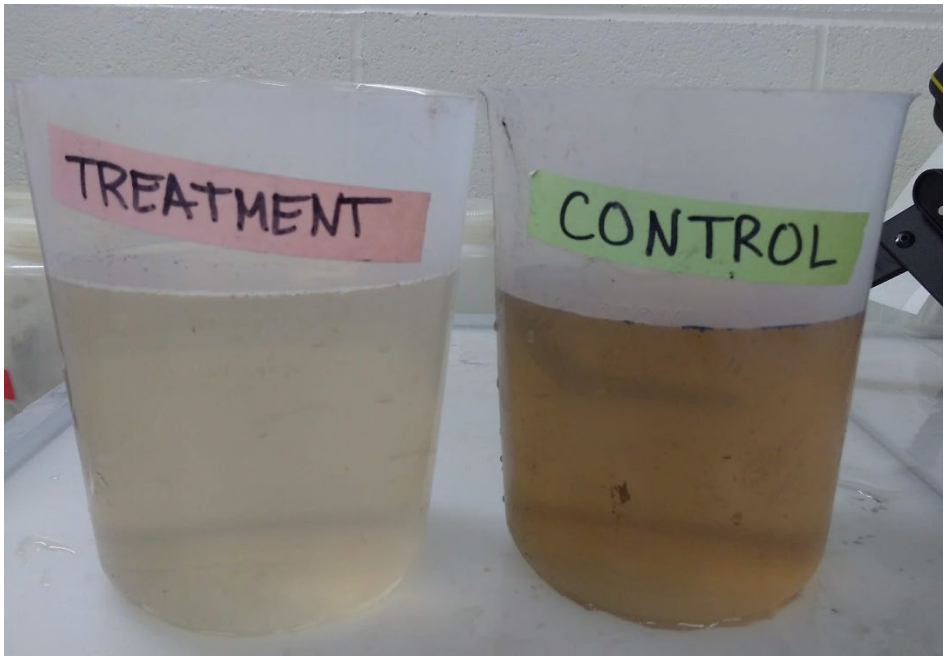

Figure 3. Post testing treatment and control water from LW-TMH 25°C test.

Table 3. Water quality parameters measured in treatment and control tanks during NBOT trials.

		TSS (mg/L)		Percent Transmittance Filtered/Unfiltered (%)		NPOC (mg/L)		DOC (mg/L)		POM (mg/L)		MM (mg/L)	
Water Type and Target Temp.	Duration (min.)	Treatment	Control	Treatment	Control	Treatment	Control	Treatment	Control	Treatment	Control	Treatment	Control
LW 25°C	0	<1.25	<1.25	96.3/96.8	96.9/96.6	1.0[J]	1.5[J]	1.2[J]	1.3[J]	<1.25	<1.25	<1.25	<1.25
	15	<1.25	<1.25	98.0/97.9	96.7/96.9	1.0[J]	1.1[J]	1.2[J]	1.2[J]	<1.25	<1.25	<1.25	<1.25
	90	<1.25	<1.25	98.7/98.6	96.2/96.6	1.2[J]	2.4	1.4[J]	2.3[J]	<1.25	<1.25	<1.25	<1.25
	180	<1.25	<1.25	98.9/99.0	96.7/97.0	0.79[J]	1.4[J]	1.1[J]	1.6[J]	<1.25	<1.25	<1.25	<1.25
LW 10°C	0	<1.25	<1.25	96.3/96.4	96.2/96.3	1.2[J]	1.3[J]	1.3[J]	1.4[J]	<1.25	<1.25	<1.25	<1.25
	15	<1.25	<1.25	97.6/97.1	96.4/96.5	1.4[J]	1.1[J]	1.3[J]	1.2[J]	<1.25	<1.25	<1.25	<1.25
	90	<1.25	<1.25	98.5/98.3	96.5/96.4	0.90[J]	1.5[J]	0.95[J]	1.4[J]	<1.25	<1.25	<1.25	<1.25
	180	<1.25	<1.25	99.0/98.6	96.1/96.2	<0.70	1.2[J]	0.83[J]	1.1[J]	<1.25	<1.25	<1.25	<1.25
LWTMH 25°C	0	27.5	19.6	29.2/26.5	28.6/26.2	9.2	9.5	6.5	5.9	14.2	8.1	13.3	11.5
	15	18.2	13.6	31.6/28.7	28.5/26.3	9.0	8.9	6.4	6.7	8.1	5.8	10.1	7.8
	90	15.8	17.5	40.2/34.3	28.5/26.1	8.4	10.1	5.4	6.9	7.7	9.3	8.1	8.2
	180	11.5	10.2	52.4/47.8	28.5/26.3	8.2	9.0	5.3	7.0	4.9	4.5	6.6	5.7
LWTMH 10°C	0	15.8	15.2	28.6/26.4	27.5/25.6	8.4	9.1	6.6	6.0	7.4	6.3	8.4	8.9
	15	16.3	12.0	31.4/28.8	27.8/25.6	8.7	10.4	6.3	7.0	6.8	4.9	9.5	7.1
	90	13.7	7.80	42.2/39.1	27.7/25.6	8.0	8.0	5.5	6.8	5.7	3.1	8.0	4.7
	180	13.0	7.10	54.9/49.2	27.6/25.7	7.8	8.0	5.4	6.5	5.9	4.1	7.1	3.0

[J] indicates values above the detection limit but below the limit of quantification of the analysis method.

4.3 MEASUREMENTS DURING TREATMENT

During treatment, ozone, ORP, pH, DO, conductivity, and temperature were measured (Tables 4-6, Figures 4 and 5). Ozone was measured during treatment to determine the ability of the NBOT BWT to produce and introduce ozone into the treated solution (Table 4). It should be noted that reported total ozone concentrations may be underestimated, as measured ozone concentrations may not include nano-bubble confined ozone. However, the ozone values obtained were similar to those measured by Dr. Peter Moeller, using a different method. ORP was measured to estimate the ability of NBOT to change the oxidation potential of the treated solution; the presence of ozone or hydroxyl radicals should lead to an increase in ORP (Table 4).

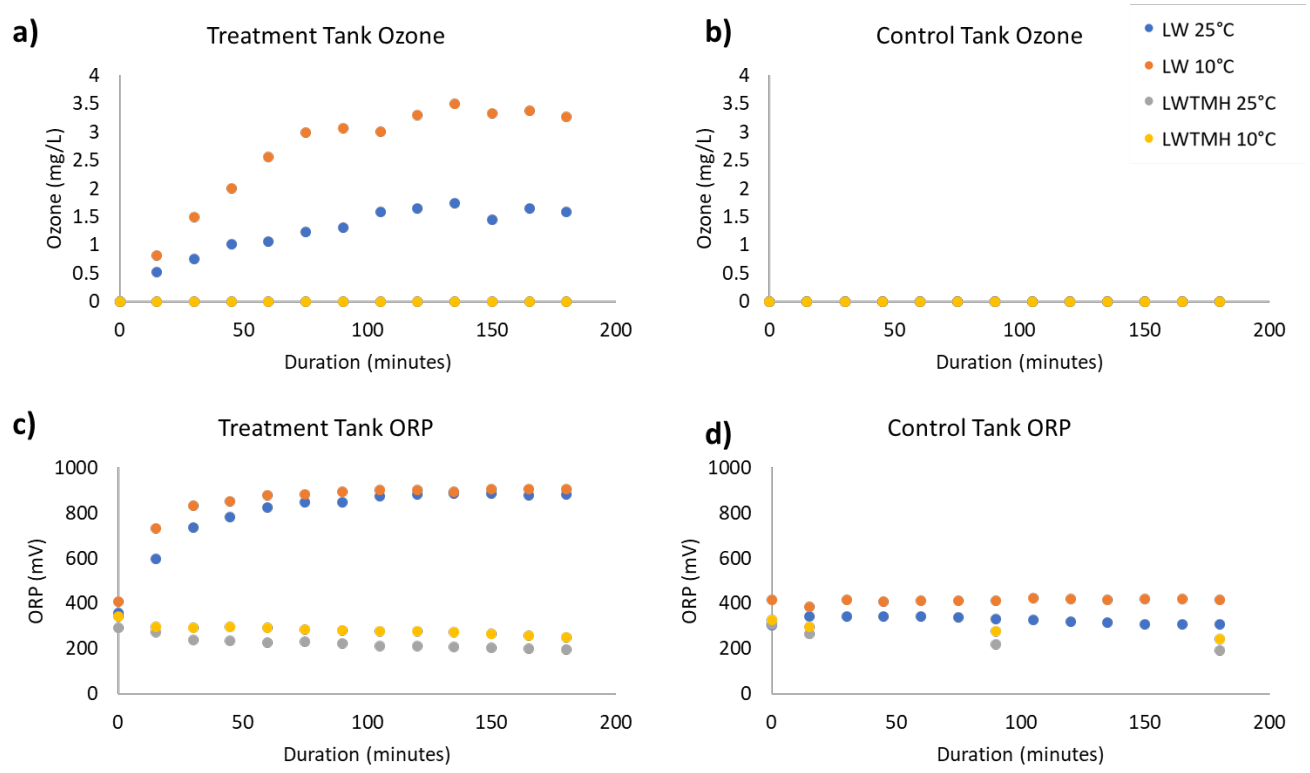

Figure 4. Time series of a) ozone in the treatment tank, b) ozone in the control tank, c) ORP in the treatment tank, d) ORP in the control tank for all NBOT trials.

In both LW tests, ozone increased with treatment time to peak values of 1.74 mg/L and 3.50 mg/L for the 25°C and 10°C tests, respectively (Table 4, Figure 4a). The higher concentration of ozone in lower temperature water is explained well by the solubility of ozone in water, which is inversely related to temperature (Roth and Sullivan, 1981). In both LW tests, ORP also increased with treatment time to a peak value of 886.8 mV and 906.2 mV for the 25°C and 10°C tests, respectively (Table 4, Figure 4c). Dissolved oxygen also increased quickly during treatment, with stable values being reached at ~75 minutes in all experiments. Between the tested temperatures, the 25°C trial had lower maximum DO

(37.0 mg/L) than the LW-10 °C case (45.2 mg/L) (Table 5, Figure 5a), due to lower solubility of oxygen in higher temperature water. In the control tanks, all measured ORP, DO, and ozone concentrations were similar throughout each trial (Figure 4b and 4d, Figure 5b).

In LW-TMH tests, ozone was not detected and ORP measurements displayed a decreasing trend; this possibly occurred due to the measurement of a high mV standard prior to analysis of samples and the resulting "memory" of the ORP probe when measuring solutions of low ionic strength. As such, caution should be used when interpreting ORP results from the LW-TMH tests. DO trends in the LW-TMH tests were similar to those observed in the LW tests, with DO quickly increasing in the treatment tank to a stable value by ~75 mins. As in the LW trials, maximum DO values in the LW-TMH trials were lower in the 25°C trial (34.8 mg/L) than the 10°C trial (42.9 mg/L) (Table 5, Figure 5a). The maximum DO levels in the LW-TMH tests were lower than in the comparable temperature LW tests.

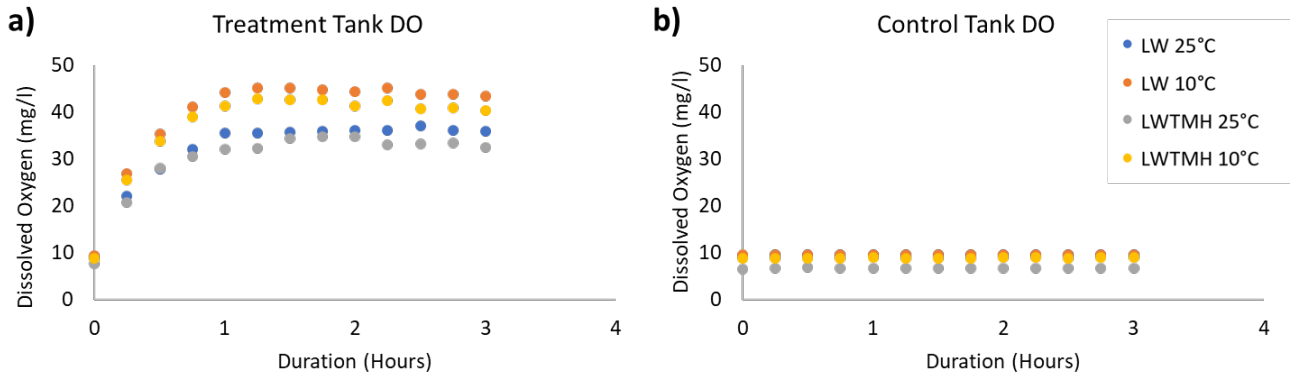

Figure 5. Time series of a) DO in the treatment tank and b) DO in the control tank during NBOT treatment for all NBOT trials.

Table 4. Ozone and ORP measurements collected during treatment of 1000 L tanks with NBOT BWT.

Treatment Duration (min.)	LW 25°C				LW 10°C				LW-TMH 25°C				LW-TMH 10°C			
	Treatment		Control		Treatment		Control		Treatment		Control		Treatment		Control	
	ORP (mV)	O_3* (mg/L)	ORP (mV)	O_3* (mg/L)	ORP (mV)	O_3* (mg/L)	ORP (mV)	O_3* (mg/L)	ORP (mV)	O_3* (mg/L)	ORP (mV)	O_3* (mg/L)	ORP (mV)	O_3* (mg/L)	ORP (mV)	O_3* (mg/L)
0	358.2	<0.05	305.7	<0.05	408.8	<0.05	413.9	<0.05	291.0	<0.05	308.1	<0.05	341.5	<0.05	327.6	<0.05
15	597.4	0.52	343.6	<0.23	730.5	0.81	383.5	<0.23	271.8	<0.05	266.4	<0.05	297.6	<0.05	295.9	<0.05
30	737.0	0.75	343.7	<0.23	830.0	1.49	415.1	<0.23	236.9	<0.05	NM	NM	291.4	<0.05	NM	NM
45	780.7	1.01	342.4	<0.23	848.9	2.00	406.9	<0.23	235.8	<0.05	NM	NM	294.8	<0.05	NM	NM
60	823.8	1.07	341.2	<0.23	877.8	2.55	413.5	<0.45	228.0	<0.05	NM	NM	290.7	<0.05	NM	NM
75	845.1	1.23	338.8	<0.23	881.1	2.98	413.1	<0.45	231.1	<0.05	NM	NM	285.6	<0.05	NM	NM
90	848.3	1.31	332.0	<0.23	891.9	3.07	411.7	<0.45	223.5	<0.05	220.5	<0.05	282.2	<0.05	279.1	<0.05
105	874.4	1.58	328.8	<0.23	901.9	3.00	422.7	<0.45	212.9	<0.05	NM	NM	278.3	<0.05	NM	NM
120	881.7	1.65	318.7	<0.23	902.1	3.29	418.0	<0.45	212.2	<0.05	NM	NM	277.1	<0.05	NM	NM
135	885.7	1.74	316.8	<0.23	893.9	3.50	415.6	<0.45	206.6	<0.05	NM	NM	272.6	<0.05	NM	NM
150	886.8	1.44	308.2	<0.23	903.6	3.33	419.2	<0.45	203.3	<0.05	NM	NM	266.8	<0.05	NM	NM
165	878.8	1.65	306.8	<0.23	903.2	3.38	419.1	<0.45	200.9	<0.05	NM	NM	258.5	<0.05	NM	NM
180	883.3	1.58	306.2	<0.23	906.2	3.26	417.6	<0.45	197.5	<0.05	192.6	<0.05	250.2	<0.05	243.2	<0.05

NM= Not Measured

* O_3 reporting limits for below detection limit values vary due to sample dilution. The method detection limit is 0.05 mg/L for an undiluted sample.

For temperature and pH, changes were observed in the treatment tank in all trials (Table 5). Overall, pH decreased (average of 0.37 ± 0.12 -log[H^+]) and temperature increased (average 5.2 ± 1.4°C) within the treatment tank (Table 5).

Treatment tank temperature changes are likely driven by the treatment process, as heat from the microbubble generation process and pumps could be transferred to the solution. The drivers of pH changes are unclear but could be a result of ozone-driven chemistry in the treatment tanks. For example, if NBOT was completely oxidizing organic molecules to CO_2, the pH may decrease. The specific mechanisms of this pH change are beyond the scope of this work.

For conductivity, alkalinity, and hardness, qualitatively, no large changes due to water type or temperature, or between treatment and control tanks were observed (Table 6).

Table 5. Dissolved oxygen, pH, and temperature (Temp.) measurements in treatment and control tanks during treatment.

	LW 25°C						LW 10°C					
	Treatment			Control			Treatment			Control		
Duration (min.)	DO (mg/L)	pH	Temp. (°C)	DO (mg/L)	pH	Temp. (°C)	DO (mg/L)	pH	Temp. (°C)	DO (mg/L)	pH	Temp. (°C)
0	9.0	7.63	24.5	9.3	7.55	23.5	9.4	7.57	11.6	9.5	7.52	11.5
15	22.1	7.57	24.7	9.5	7.60	23.4	26.9	7.56	11.9	9.5	7.53	11.4
30	27.9	7.48	25.0	9.5	7.58	23.4	35.3	7.46	12.3	9.5	7.44	11.4
45	32.0	7.54	25.4	9.5	7.66	23.5	41.1	7.42	13.0	9.5	7.46	11.8
60	35.5	7.43	25.8	9.5	7.61	23.5	44.2	7.52	13.4	9.5	7.51	11.8
75	35.6	7.36	26.0	9.5	7.55	23.4	45.2	7.55	14.0	9.6	7.66	12.2
90	35.7	7.41	26.3	9.5	7.62	23.4	45.1	7.51	14.8	9.5	7.65	12.2
105	36.0	7.38	26.8	9.6	7.59	23.4	44.7	7.51	15.0	9.6	7.58	12.3
120	36.1	7.40	27.2	9.6	7.61	23.4	44.3	7.40	15.7	9.6	7.48	12.5
135	36.2	7.35	27.5	9.5	7.52	23.3	45.1	7.45	16.2	9.6	7.64	12.6
150	37.0	7.34	27.6	9.5	7.62	23.3	43.9	7.41	16.8	9.5	7.52	12.7
165	36.2	7.32	28.1	9.5	7.49	23.3	43.9	7.37	17.2	9.6	7.49	12.9
180	35.9	7.29	28.0	9.5	7.42	23.1	43.4	7.31	17.9	9.5	7.49	13.4

	LW-TMH 25°C						LW-TMH 10°C					
	Treatment			Control			Treatment			Control		
Duration (min.)	DO (mg/L)	pH	Temp. (°C)	DO (mg/L)	pH	Temp. (°C)	DO (mg/L)	pH	Temp. (°C)	DO (mg/L)	pH	Temp. (°C)
0	7.6	7.36	24.7	6.5	7.31	24.6	8.8	7.52	12.7	8.7	7.37	13.2
15	20.7	7.28	25.2	6.6	7.25	24.5	25.5	7.39	13.4	8.8	7.44	14.1
30	28.1	7.29	25.6	6.8	7.31	24.6	33.8	7.33	13.7	8.8	7.49	13.4
45	30.5	7.15	26.0	6.7	7.28	24.5	39.0	7.21	14.4	8.8	7.47	14.0
60	32.1	7.19	26.3	6.6	7.22	24.5	41.4	7.30	14.9	8.9	7.53	13.9
75	32.3	7.21	26.7	6.7	7.33	24.4	42.9	7.17	15.5	8.8	7.45	14.3
90	34.3	7.06	27.0	6.6	7.23	24.5	42.6	7.22	16.3	8.8	7.53	15.1
105	34.8	7.08	27.7	6.7	7.22	24.5	42.6	7.10	16.2	8.8	7.41	14.4
120	34.8	7.00	28.2	6.7	7.24	24.6	41.4	7.10	17.1	8.9	7.48	14.8
135	33.0	7.10	28.3	6.7	7.30	24.6	42.4	7.02	17.6	8.9	7.51	14.8
150	33.2	7.01	29.0	6.7	7.25	24.5	40.8	7.00	18.0	8.8	7.47	14.9
165	33.5	7.04	29.3	6.7	7.38	24.6	41.0	7.01	18.6	8.9	7.49	15.0
180	32.4	7.03	29.2	6.7	7.30	24.5	40.4	6.99	19.0	8.9	7.52	15.4

Table 6. Conductivity, alkalinity, and hardness summary statistics from during treatment periods in both control and treatment tanks.

	Avg. During Treatment (min, max)		
Test	**Conductivity (µS/cm)**	**Hardness (mg/L as $CaCO_3$)**	**Alkalinity (mg/L as $CaCO_3$)**
Control LW 25°C	132.5 (132.2, 133.1)	49.9 (48.1, 52.2)	49.3 (48.6, 50.3)
Treatment LW 25°C	133.6 (133.0, 135.2)	51.4 (49.3, 52.4)	50.2 (48.4, 51.7)
Control LW 10°C	131.7 (131.0, 132.8)	49.5 (47.9, 52.2)	49.5 (47.8, 51.3)
Treatment LW 10°C	131.5 (130.0, 132.5)	52.4 (49.5, 57.4)	48.6 (47.2, 50.0)
Control LW-TMH 25°C	137.4 (135.3, 139.2)	50.2 (48.4, 52.6)	51.8 (50.0, 52.4)
Treatment LW-TMH 25°C	137.0 (136.4, 137.9)	49.5 (48.6, 50.4)	49.9 (47.8, 51.2)
Control LW-TMH 10°C	138.7 (137.8, 139.6)	52.1 (48.6, 54.8)	48.9 (48.6, 49.0)
Treatment LW-TMH 10°C	139.6 (139.1, 140.1)	53.1 (52.2, 54.0)	48.6 (47.2, 50.2)

4.4 POST-TREATMENT AQUATIC DEGREDATION

Ozone, ORP, pH, DO, conductivity, and temperature were measured post treatment (Table 7 and Table 8). Because ozone was below the detection limit of the method (BDL) at the conclusion of both LW-TMH experiments, ORP and ozone were not measured after 30 minutes post treatment.

Post-treatment degradation of ozone in the LW experiments was relatively rapid. At four hours post treatment, the ozone concentrations had decreased to <0.05 mg/L in the LW 25°C test and to 0.45 mg/L in the LW 10°C test (Table 7). Alternatively, ORP degradation was inconsistent between experiments with a large decrease being observed in the LW 25°C test but only a small decrease observed in the LW 10°C test. This could also be due to the "memory" of the ORP probe when measuring low ionic strength solutions after measuring a standard, given this, as with the during-test ORP data, the post treatment ORP measurements should be interpreted cautiously. At the 24-hour post-treatment time point, ozone measurements from both LW experiments were BDL. Degradation of DO was slower, after 48 hours post-treatment and for all test cases, treatment replicates still had higher concentrations of DO than the control replicates (Table 8). Post treatment, conductivity values were similar to those observed during treatment and temperature stabilized near the incubation temperature (Table 8).

Table 7. Average (±standard deviation) ozone concentration and ORP measured in samples after NBOT treatment.

Post Treatment Duration (min.)	LW 25°C				LW 10°C				LW-TMH 25°C				LW-TMH 10°C			
	Treatment		Control		Treatment		Control		Treatment		Control		Treatment		Control	
	ORP (mV)	O_3^* (mg/L)	ORP (mV)	O_3^* (mg/L)	ORP (mV)	O_3^* (mg/L)	ORP (mV)	O_3^* (mg/L)	ORP (mV)	O_3^* (mg/L)	ORP (mV)	O_3^* (mg/L)	ORP (mV)	O_3^* (mg/L)	ORP (mV)	O_3^* (mg/L)
30	828.7 (9.4)	0.68 (0.11)	334.5 (2.5)	<0.23	879.9 (4)	2.4 (0.09)	384.1 (2.9)	<0.45	189.2 (2.3)	<0.05	174.8 (5)	<0.05	229.8 (1.5)	<0.05	214.5 (3.1)	<0.05
60	792.1 (11.4)	0.35 (0.01)	329.4 (1.0)	<0.23	868.2 (4.3)	1.64 (0.03)	381.7 (3.2)	<0.23	NM	NM	NM	NM	NM	NM	NM	NM
120	626.2 (51.8)	0.07 (0.01)	260.1 (0.8)	<0.05	844.5 (18.1)	1.01 (0.02)	343.7 (14.9)	<0.23	NM	NM	NM	NM	NM	NM	NM	NM
240	245.8 (2.7)	<0.05	238.9 (4.0)	<0.05	794.7 (20.1)	0.45 (0.02)	365.3 (27.1)	<0.05	NM	NM	NM	NM	NM	NM	NM	NM
1440	401.2 (3.4)	<0.05	394.8 (0.2)	<0.05	296.9 (21.1)	<0.05	315.6 (0.6)	<0.05	NM	NM	NM	NM	NM	NM	NM	NM

NM= Not Measured

* O_3 reporting limits for below detection limit values vary due to sample dilution. The method detection limit is 0.05 mg/L for an undiluted sample.

Table 8. Temperature (Temp.), pH, DO, and Conductivity (Cond.) summary statistics from post treatment incubations.

	LW 25°C								LW 10°C							
	Avg. Treatment (min, max)				Avg. Control (min, max)				Avg. Treatment (min, max)				Avg. Control (min, max)			
Time (hrs.)	Temp (˚C)	pH	DO (mg/L)	Cond. (µS/cm)	Temp (˚C)	pH	DO (mg/L)	Cond. (µS/cm)	Temp (˚C)	pH	DO (mg/L)	Cond. (µS/cm)	Temp (˚C)	pH	DO (mg/L)	Cond. (µS/cm)
0-4	26.2 (25.0, 27.3)	7.39 (7.33, 7.42)	31.8 (28.0, 34.8)	134.1 (133.3, 134.6)	24.0 (23.4, 24.8)	7.7 (7.63, 7.75)	9.2 (9.1, 9.4)	132.6 (131.7, 133.3)	14.5 (11.9, 16.5)	7.38 (7.34, 7.43)	39.9 (36.8, 43.1)	133.5 (132.3, 134.7)	11.8 (11.0, 12.1)	7.56 (7.43, 7.61)	9.6 (9.4, 9.7)	132.8 (132.1, 133.5)
24	25.0 (25.0, 25.0)	7.44 (7.42, 7.48)	20.1 (17.8, 21.5)	134.7 (134.6, 134.9)	25.2 (25.1, 25.3)	7.74 (7.70, 7.77)	8.5 (8.5, 8.6)	133.4 (133.3, 133.5)	10.4 (10.4, 10.5)	7.41 (7.39, 7.42)	30.8 (30.2, 31.4)	134.9 (134.0, 135.4)	10.3 (10.2, 10.4)	7.59 (7.55, 7.64)	10.0 (9.9, 10.0)	132.8 (132.1, 133.2)
48	25.4 (25.3, 25.5)	7.50 (7.48, 7.52)	14.3 (13.3, 14.9)	134.6 (134.5, 134.7)	25.6 (25.5, 25.8)	7.73 (7.68, 7.78)	8.2 (8.2, 8.3)	133.6 (133.6, 133.7)	10.2 (10.2, 10.2)	7.47 (7.45, 7.48)	25.9 (24.8, 27.3)	135.0 (134.4, 135.5)	10.4 (10.3, 10.5)	7.61 (7.53, 7.69)	10.3 (10.2, 10.3)	133.7 (133.0, 134.9)

	LW-TMH 25°C								LW-TMH 10°C							
	Avg. Treatment (min, max)				Avg. Control (min, max)				Avg. Treatment (min, max)				Avg. Control (min, max)			
Time (hrs.)	Temp (˚C)	pH	DO (mg/L)	Cond. (µS/cm)	Temp (˚C)	pH	DO (mg/L)	Cond. (µS/cm)	Temp (˚C)	pH	DO (mg/L)	Cond. (µS/cm)	Temp (˚C)	pH	DO (mg/L)	Cond. (µS/cm)
0-4	27.4 (25.8, 28.9)	7.03 (6.95, 7.09)	31.2 (28.1, 33.0)	137.3 (134.6, 138.5)	25.0 (24.6, 25.3)	7.35 (7.26, 7.39)	6.8 (6.7, 7.0)	138.7 (136.4, 139.8)	14.9 (11.7, 17.2)	7.06 (7.02, 7.08)	39.5 (37.4, 41.5)	140.8 (139.5, 142.2)	12.7 (11.0, 14.0)	7.50 (7.42, 7.56)	8.9 (8.8, 8.9)	140.4 (139.8, 141.1)
24	25.2 (25.2, 25.2)	7.11 (7.09, 7.15)	22.3 (21.4, 23.3)	138.1 (137.9, 138.2)	25.3 (25.2, 25.4)	7.37 (7.31, 7.44)	7.1 (7.0, 7.2)	139.4 (138.8, 139.7)	10.5 (10.4, 10.6)	7.04 (7.01, 7.08)	35.2 (34.7, 35.9)	142.3 (142.2, 142.5)	10.3 (10.2, 10.3)	7.54 (7.47, 7.59)	9.2 (9.1, 9.2)	141.6 (141.0, 142.6)
48	25.2 (25.1, 25.2)	7.14 (7.11, 7.21)	16.9 (15.4, 18.5)	138.6 (138.4, 138.9)	25.2 (25.1, 25.3)	7.44 (7.41, 7.49)	7.4 (7.3, 7.4)	139.4 (138.9, 139.7)	10.3 (10.2, 10.4)	7.08 (7.05, 7.10)	32.4 (31.6, 33.3)	142.1 (141.7, 142.4)	10.2 (10.1, 10.3)	7.57 (7.54, 7.60)	9.3 (9.3, 9.4)	141.1 (140.6, 141.7)

5 QUALITY ASSURANCE/QUALITY CONTROL – DATA QUALITY OBJECTIVES

5.1 WATER CHEMISTRY AND WATER QUALITY

The data quality objectives (DQO) for water quality and chemistry analyses conducted during the evaluation of the NBOT are summarized in Table 9. Quality control requirements are specified in each SOP outlined in Section 3, and those requirements are used to determine whether the DQO's for the overall evaluation were met. Quality objectives were not met for POM analysis. Relative differences in duplicate values of POM were greater than the quality objective and no reference POM standards were analyzed over the course of the project. These QA/QC failures should not impact the overall conclusions of this report, but the accuracy of the POM analysis was not verified at any point during the tests.

Table 9. Quality control and quality assurance data from the water chemistry analyses. Bold text indicates exceedances.

Data Quality Indicator	Evaluation Process/Performance Measurement	Data Quality Objective	Performance Measurement Result	
Precision	Samples (10%) were collected and analyzed in duplicate with performance measured by average relative percent difference (RPD).	< 20% average RPD	Percentage of Samples Collected and Analyzed in Duplicate:	Duplicate Relative Percent Difference
			%TF: 12.5%	%TF: 0.9 ± 1.3%
			%TU: 12.5%	%TU: 3.1 ± 4.7%
			NPOC: 12.5%	%NPOC: 13.6 ± 10.2%
			DOC: 12.5%	%DOC: 10.6 ± 14.4%
			POM: 12.5%	**POM: 27.0 ± 21.9%**
			TSS: 12.5%	TSS: 18.6 ± 6.6%
			Hardness: 12.5%	Hardness: 3.9 ± 3.6%
			Alkalinity: 12.5%	Alkalinity: 2.7 ± 1.4%
			Ozone: 12.7%	Ozone: 6.0 ± 8.6%
			ORP: 12.7%	ORP: 0.6 ± 0.3%
Bias, Filter Blanks	%T filter blanks were prepared by filtering deionized water samples (one per analysis date)	> 98% average %T	Number of %T Filter Blanks Analyzed: 4	Filter blanks (%T): 99.6 ± 0.4%
	TSS/POM filter blanks were prepared by filtering deionized water samples (one per analysis date) and then drying, weighing, ashing and weighing the filter	< 0.63 mg/L average TSS/POM	Number of TSS Filter Blanks Analyzed: 4	Filter Blanks (TSS)= <0.63 ± 0
			Number of POM Filter Blanks Analyzed: 4	Filter Blanks (POM)= <0.63 ± 0
	NPOC blanks were prepared by acidifying a volume of deionized water to 0.2% with concentrated hydrochloric acid	< 0.70 mg/L average NPOC	Number of NPOC Blanks Analyzed: 16	Blanks (NPOC)= <0.70 ± 0
	DOC filter blanks were prepared by filtering deionized water samples (one per analysis date)	< 0.70 mg/L average DOC	Number of DOC Filter Blanks Analyzed: 4	Filter Blanks (DOC)= <0.70 ± 0

Data Quality Indicator	Evaluation Process/Performance Measurement	Data Quality Objective	Performance Measurement Result	
Accuracy	Samples (10%) were spiked with a total organic carbon spiking solution with performance measured by average spike-recovery (SPR).	75% - 125% average SPR	Percentage of NPOC/DOC Samples Spiked: 12.5%	NPOC/DOC Spike Recovery= 98.1 ± 2.4
	Performance was measured by average percent difference (%D) between all measured and nominal reference standard values.	< 20% average D	Percentage of Analysis Days Containing a Reference Standard:	Reference Standard Percent Difference
			TSS: 100%	TSS: 5.7 ± 4.7%
			POM: 100%	POM: NA
			NPOC: 100%	NPOC: 6.9 ± 1.6%
			ORP:100%	ORP 14.5 ± 5.0 %
	A hardness/alkalinity reference standard was analyzed once per bench scale test type per analyst. Performance was measured by ensuring the titrated value was within the acceptance range for the standard.	Within acceptance range (lot dependent)	Percentage of Analysis Days Containing a Reference Standard: 50%	Hardness: DQO met 100% of the time
			Test types: 1	Alkalinity: DQO met 100% of the time
Representativeness	All samples were collected, handled, and analyzed in the same manner.	Not Applicable – Qualitative.	All water chemistry/quality samples were collected, handled, transported and analyzed in the same manner using the appropriate SOPs.	
Comparability	Routine procedures were conducted according to appropriate SOPs to ensure consistency between tests.	Not Applicable – Qualitative.	The SOPs listed in the methods and references section were used for all water chemistry and water quality analyses.	
Completeness	Percentage of valid (i.e., collected, handled, analyzed correctly and meeting DQOs) water chemistry samples measured out of the total number of water chemistry samples collected. Performance is measured by percent completeness (%C).	> 90% C	Ozone: 100%	
			ORP: 100%	
			TSS: 100%	
			%T, Filtered: 100%	
			%T, Unfiltered: 100%	
			NPOC: 100%	
			DOC: 100%	
			Hardness: 100%	
			Alkalinity: 100%	
Sensitivity	The limit of detection (LOD) and limit of quantification (LOQ) for each analyte and analytical method utilized was determined annually unless a reporting limit was used based on the amount filtered as was the case with TSS/POM.	Not Applicable	Ozone LOD: 0.05 mg/L (Baird et al., 2017)	
			TSS/POM RL: 1.25 mg/L based on filtering 800 mL of sample	
			NPOC/DOC LOD: 0.70 mg/L	
			NPOC/DOC LOQ: 2.3 mg/L	
			NPOC/DOC Determined: 2 February 2019	

6 CONCLUSIONS

The LSRI-GWRC water-only evaluation of the in-tank NBOT BWT met the stated objectives, as outlined in the Test Plan (Schaefer et al., 2019). The reported deviations and quality control failures do not impact LSRI-GWRC's ability to draw conclusions on the performance of NBOT BWT during testing. The system was fully operational during all reported tests and was operated in accordance with the developer's instructions.

Objectives 1 and 2: Determination of the DO, O_3, and ORP concentration in simulated Great Lakes ballast water over time, and determination of the impact of temperature and water quality on the generation of ozone during NBOT treatment.

The data generated during this evaluation support the NBOT BWT's mechanism of action as stated by the developer. In LW, the NBOT BWT increased dissolved O_3, DO, and ORP levels, reaching an equilibrium state for dissolved O_3 in under three hours of treatment at both ~25°C and ~10°C. The maximum O_3 concentration achieved at 25°C was 1.74 mg/L. At 10°C, the maximum concentration achieved was nearly twice that, at 3.50 mg/L O_3. Test temperature and water quality both had substantial impacts on the concentration of dissolved O_3 and the ORP in treated water. In colder water, O_3 is more soluble, additionally, the lower temperature likely slowed the decomposition of O_3 to oxygen and hydroxyl radicals. In LW-TMH water, no increase in O_3 or ORP were observed within the three-hour system run time. However, DO was observed to increase in LW-TMH at both 10°C and 25°C, which indicates that the technology was effectively creating nanobubbles. The absence of an increase in O_3 while DO was increasing suggests that the presence of O_3-reactive species (i.e., dissolved and particulate organic compounds) was a large enough sink to consume all O_3 introduced by NBOT.

Objective 3: Determination of the aquatic degradation of O_3 following NBOT treatment, and the impact of temperature and water quality on the rate of degradation.

The aquatic degradation of O_3 after NBOT treatment was determined in LW, and the impact of water temperature on the rate of O_3 degradation was substantial. In LW at 25°C, O_3 concentration was below the limit of detection within four hours after treatment. In LW at 10°C, dissolved O_3 concentration degraded at a slower rate, and within 24 hours following treatment the O_3 was below the limit of detection. During the LW-TMH trials, all O_3 measurements were below the limit of detection. Therefore, aquatic degradation was not assessed for this test water type.

LSRI-GWRC did not determine biological effectiveness or chronic residual toxicity of the NBOT BWT, as proposed in the Test Plan (Schaefer et al., 2019). Following water-only testing and consultation with the developer, the laboratory evaluation was concluded on this version of the system. This decision was made because the system was not achieving the dissolved O_3 concentrations that the developer had hoped for. LSRI-GWRC agreed to evaluate a different version of the NBOT BWT, a 2.5-horsepower model, which the developers hypothesize will produce higher dissolved O_3 concentrations.

When relating the results from these four trials to the potential application of ozone technology as a ballast water treatment for U.S. and Canadian Laker vessels, the higher solubility of O_3 in colder water

temperatures is a benefit for much of the Great Lakes shipping season. The Great Lakes shipping season is approximately March through January, and ballast water temperatures can be 10°C or lower for much of the season (excepting the summer months). During LSRI-GWRC testing, the maximum dissolved O_3 concentration reached during treatment was two times higher at 10°C as compared to 25°C. However, Great Lakes ballast water often contains high concentrations of organic compounds that were shown in LSRI-GWRC's testing to react quickly with dissolved O_3 resulting in no measurable O_3 after three hours of continuous treatment. The impact of these more challenging water qualities on ozone technology is substantial, and the developers hypothesize that a newer model of NBOT will overcome this challenge.

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