BENCH-SCALE TECHNICAL REPORT

TESTS OF THE LED LIGHT ACTIVATED TITANIUM DIOXIDE BENCH-SCALE BALLAST WATER TREATMENT PROCESS

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ABSTRACT

This technical report presents findings from bench-scale tests evaluating the performance of the LED Light Activated Titanium Dioxide Technology, hereafter LED TiO_2, developed by YJB LED Professional Services of Crosslake, Minnesota, USA. Researchers conducted the bench-scale evaluation beginning in July 2019 and ending in September 2019 at the Lake Superior Research Institute (LSRI) of the University of Wisconsin-Superior (UWS) in Superior, Wisconsin, USA. The LED TiO_2 treatment process applies light emitting diodes (LED) to activate a photocatalytic coating that creates a bacteriostatic, fungistatic, and algastatic environment. Biological effectiveness testing was completed with the algae, *Selenastrum capricornutum* and pathogen indicator organisms, *Escherichia coli* and *Enterococcus faecium* in lab water. The system was found to be effective at treating microbes in highly-transparent/low-suspended solids water, but was less effective at treating algae.

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1 INTRODUCTION

A major focus area of the Lake Superior Research Institute's Great Waters Research Collaborative (LSRI-GWRC) is providing unbiased, independent data in support of the accelerated development of technologies having the potential for preventing the introduction and/or controlling the spread of non-indigenous organisms within the Laurentian Great Lakes. This report details the results of the LSRI-GWRC bench-scale evaluation of the LED light activated titanium dioxide technology (hereafter LED TiO_2). Developed by YJB LED Professional Services of Crosslake, Minnesota, USA, the LED TiO_2 treatment process applies light emitting diodes (LED) to activate a photocatalytic coating that creates a bacteriostatic, fungistatic, and algastatic environment. The LED TiO_2 treatment process is a prototype in the early research and development stage that is appropriately-sized for laboratory evaluation.

This technical report presents findings from bench-scale tests of the LED TiO_2 treatment process, which took place from July 2019 to September 2019 at the LSRI of UWS in Superior, Wisconsin, USA. Test objectives included determining biological effectiveness of the LED TiO_2 treatment process in laboratory water with the standard freshwater test organism *Selenastrum capricornutum* and standard pathogen indicator bacteria *Escherichia coli* and *Enterococcus faecium*.

2 TEST METHODS

2.1 TEST PLAN AND SOPS

A Test Quality Assurance Plan (TQAP) 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. The TQAP details sample and data collection and analysis, sample handling and preservation, data quality objectives, and the QAQC requirements. It was approved by both LSRI-GWRC and the technology developer prior to the start of bench-scale test activities. The SOPs followed throughout testing are described in the methods section and listed in the References section of this report.

2.2 TECHNOLOGY DESCRIPTION AND EXPERIMENTAL APPARATUS

The LED TiO_2 technology evaluated by LSRI-GWRC is a prototype in the early research and development stage. The prototype tested represents an in-tank treatment technology, which would be utilized during a voyage from one Great Lakes port to another. The prototype tested (Figure 1) consists of two, 2-gallon plastic containers, one of which is coated internally with the photocatalytic TPX-220 and contains a stack of fibrous filters coated with TPX-220 and a small submersible pump (AC power = 120 volts (V), estimated power consumption of 6 watts (W)). The developer has stated that the TPX-220 is effective against odor causing bacteria, fungi, and algae, by inhibiting their growth. According to the Bench-Scale Testing Service Application submitted by YJB LED Professional Services to GWRC, the photocatalytic TPX-220 coating is composed of titanium dioxide and peroxytitanium acid. The resulting chemical reaction of the TPX-220 and LED light in an aqueous environment generates hydroxyl free radicals and oxygen, which can destroy organic molecules. The filters within the plastic container have a vertical slit cut into them to allow the tubing and pump power cord to go through. Forty-five feet of 3/8” clear plastic tubing (total volume = 1 L), which is coated internally with TPX-220, runs from the pump within the first container and coils around the outside of a second 2-gallon container and returns water back to first 2-gallon container. The end of the tubing coming back to the first container is inserted into a horizontal slit in the top filter in the stack of fibrous filters, through which the water flows via gravity (Figure 1). LED lights which activate the photocatalytic TiO_2 are mounted above (80 W) and below (60 W) both 2-gallon containers (AC power = 120 V). The LED lights and submersible pump plugged into Underwriters Laboratory (UL) approved power strips above and below the prototype treatment system.

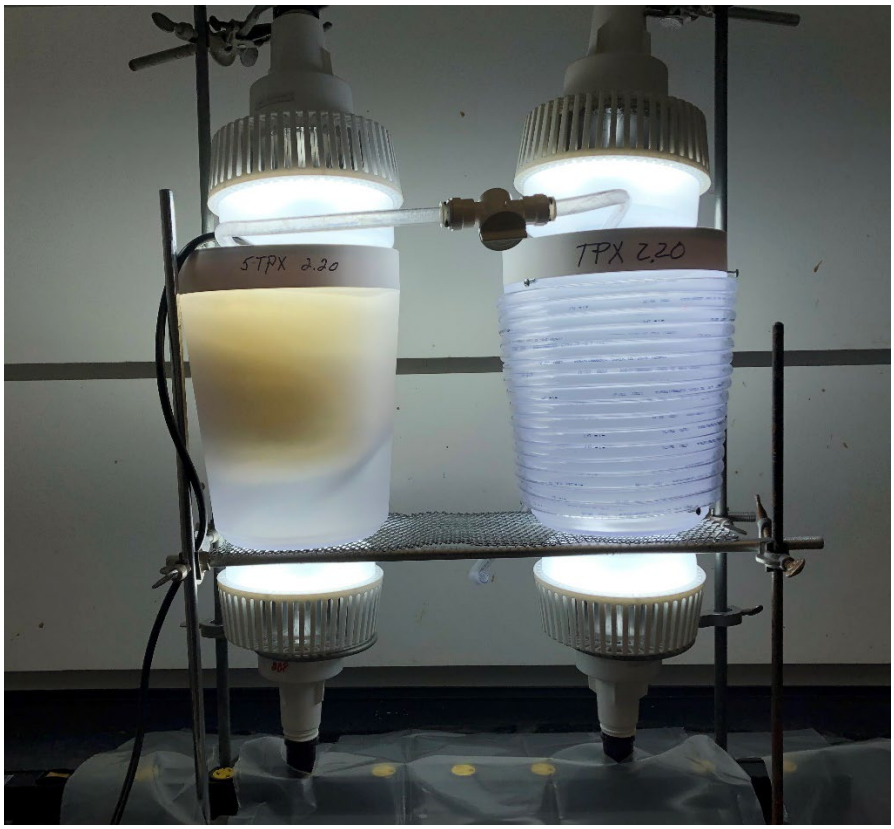

Figure 1. Photograph of the LED TiO_2 System Setup

The LED TiO_2 system functioned as an in-tank treatment in which the LED lights and pump were run continuously throughout the 48-hour testing period. During operation in the LSRI laboratory, water was pumped from the treatment container (Figure 1, left) through the coated tubing and back into the treatment container at a rate of approximately 22 gallons per hour (GPH). The pump is rated to provide a flow rate of 75 GPH, however due to the resistance created by the tubing and the addition of a control valve, the flow rate was reduced to 22 GPH. This rate allows a greater contact time with the photocatalytic coating. At a flow rate of 22 GPH with 2.2 gallons of water in the treatment container and tubing, there were 10 circulation cycles per hour or 480 cycles per 48-hour test period.

2.3 EXPERIMENTAL WATER PREPARATION

Bench-scale tests evaluating the LED TiO_2 treatment process were conducted in LSRI laboratories equipped with adequate ventilation, electrical connections, and climate control. Two experimental water types were prepared as follows:

Laboratory Water (LW): The LW is municipal water from the City of Superior, Wisconsin (sourced from Lake Superior), that is passed through an activated carbon column in order to remove the majority of the chlorine. The remaining residual chlorine is removed through injection of sodium sulfite, and the resulting total residual chlorine concentration is below the limit of detection (i.e., < 3 µg/L Cl_2). The LW

has a very low concentration of organic carbon and suspended solids, and a very high UV transmittance, presenting a less challenging water quality condition to treatment technologies.

Performance Control Water (PCW): The use of PCW is a quality control measure. It is the optimal culture water for the species being tested; therefore, it will vary for each biological effectiveness test conducted. The purpose of the PCW group is to provide information on the health of the test organisms. The PCW for each test organism was:

- LW: *S. capricornutum*
- Tryptic Soy Broth (TSB): *E. coli*
  - Prepared following manufacturer instructions and the LSRI *General Microbiology Laboratory Procedures Handbook.*
- Brain Heart Infusion Broth (BHB): *E. faecium*
  - Prepared following manufacturer instructions and the LSRI *General Microbiology Laboratory Procedures Handbook.*

2.4 BWMS INSTALLATION AND COMMISSIONING

The LED TiO_2 technology was delivered and installed with the assistance of YJB LED Professional Services representative Del Anderson on July 25, 2019. LSRI staff members, Olivia Anders, Alexander Frie, Heidi Saillard and Christine Polkinghorne received hands-on training on the operation of the treatment process and were informed of the recommended operating conditions and safety measures required during operation. LED TiO_2 treatment process was operating at an acceptable level upon completion of the installation and details were recorded on a *Bench-Scale Ballast Water Management System (BWMS) Installation Acceptance Form* on July 25, 2019.

Once the system had been installed, it was inspected by the UWS campus electrician. The wiring on the system as delivered did not meet UL or CSA standards. The developer was contacted and supplied GWRC with commercially ready outlet to socket fixtures and UL approved power strips. GWRC supplied and installed ring stands and clamps to stabilize the prototype treatment system.

2.5 EXPERIMENTAL DESIGN AND TEST METHODS

2.5.1 BIOLOGICAL EFFECTIVENESS TESTING

GWRC's determined the biological effectiveness of the LED TiO_2 system on the freshwater test organisms by following standard operating procedures (SOP) that were developed at LSRI for each type of test organism. The SOPs and test methods were developed with the goal of providing unbiased, independent data in support of the accelerated development of technologies having the potential for preventing the introduction and/or controlling the spread of non-indigenous organisms within the Laurentian Great Lakes via ballast water discharge. The green algae *Selenastrum capricornutum* and two species of indicator bacteria, *Escherichia coli* and *Enterococcus faecium*, were the test organisms used in this evaluation. The test water used was prepared as described in Section 2.3 of this Test Plan. The prepared test water (LW) was placed into a 19 L carboy. Water chemistry measurements were made on the LW prior to organism inoculation, then (during two separate tests) the test organisms were added at

the concentrations shown in Table 1. The inoculated LW was enumerated to determine the initial concentration of organisms, then was split between the control and treatment containers. The bacteria (tested simultaneously) and algae were exposed to the treatment system separately during individual runs of the system. For microbial dose effectiveness tests, a 1 L whole water sample was collected from the carboy prior to spiking to verify the absence of *E. coli* and *E. faecium* in LW. For both algae and microbes, the test organisms were exposed to the treatment by pumping the inoculated water through the LED TiO_2 system for 48 hours during which organism mortality was assessed at 24 hours and 48 hours. Two separate sets of TPX-220 coated treatment buckets, hoses and filters were supplied to GWRC for the testing of the two size classes of organisms. Also, an additional system setup was provided by the developer, which lacked the treatment components (i.e., TiO_2 coated tubing and filter and LED lights) and was used as a control in the testing. The organisms in the control were tested in the same water type and were pumped through the uncoated tubing and back into the uncoated control container in the same manner as the treatment. No filter or LED lights were supplied to be used in the control container. The water recirculated between the control components without passing through a filter.

Table 1. Organism Type and Number per Replicate in GWRC Biological Effectiveness Experiments Using the LED TiO_2 Technology.

Major Taxonomic Group	Type	Species	Exposure Solutions by Water Quality	No. of Organisms per Exposure /Control	No. of Pseudo-Replicates per Exposure /Control
Algae	Green algae	*Selenastrum capricornutum*	1. Untreated LW 2. LW treated with LED TiO_2 3. Untreated PCW	200,000 cells/mL	3
Bacteria	Gram-negative	*Escherichia coli*		≥ 1,000,000 MPN/100 mL	5
	Gram-positive	*Enterococcus faecium*			

The biological effectiveness tests using green algae were conducted by spiking the 19 L carboy containing LW with *S. capricornutum* from 4- to 8-day old cultures to achieve approximately 200,000 cells/mL (LSRI/SOP/GWRC/11 – *Assessing Bench-Scale Dose Effectiveness of Potential Ballast Water Treatment Processes on* Selenastrum capricornutum).

For microbial testing the carboy containing LW was spiked with 1140 µL of each six-hour old culture of *E. coli* and *E. faecium* to bring the density of each microbe to greater than 1,000,000 most probable number (MPN)/100 mL (LSRI/ SOP/GWRC/14 – *Assessing Antimicrobial Effectiveness).*

For both algae and microbe testing, the 19 L carboy was well mixed well following the addition of organisms and three replicate samples were collected to determine the initial concentration of cells, then the inoculated water was poured into the 2-gallon control and treatment containers. The submersible pump was turned on and more inoculated water was added to replace the water in the containers that had been displaced by pumping it into the coiled tubing. Following the addition of the inoculated water to the containers, the LED lights on the treatment system were turned on, marking the start of the 48-hour treatment period. At 24 hours following test initiation, samples were collected for determination of mortality by pipetting from the control and treatment containers. Due to the small volume of water being treated, maximum sample size for each microbe subsample was 10 mL rather than 100 mL at the 24-hour time point.

For the algae test, a PCW sample was prepared by inoculating a 1 L bottle with enough *S. capricornutum* to achieve a concentration of 200,000 cells/mL. The algae PCW was held at room temperature, next to the control apparatus. Triplicate samples were collected from the PCW at 0, 24, and 48 hours. For microbes, a 1 L PCW sample was prepared for each microbe and each was split into 5 replicate samples which were incubated at 25˚C and sampled at 24 hours and 48 hours for enumeration.

2.5.1.1 BIOLOGICAL-EFFECTIVENESS TESTING: WATER QUALITY

Water quality measurements were made throughout the duration of the LED TiO_2 treatment process testing period and involved determination of total suspended solids (TSS), percent transmittance at 254 nm (%T), particulate organic matter (POM), non-purgeable organic carbon (NPOC), dissolved organic carbon (DOC), total alkalinity, total hardness, DO, temperature, specific conductivity, and pH.

TSS analysis was conducted according to LSRI/SOP/SA/66 – *Analyzing Total Suspended Solids (TSS), Particulate Organic Matter (POM), and Mineral Matter (MM)*. Accurately measured sample volumes (± 1%) were vacuum filtered through pre-washed, dried, and pre-weighed glass fiber filters (i.e. Whatman 934-AH). 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. 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).

Analysis of percent transmittance (%T) was conducted according to LSRI/SOP/SA/65 – *Determining Percent Transmittance (%T) of Light in Water at 254 nm*. For analysis of the filtered aliquot, an appropriate volume of sample was filtered through a glass fiber filter (i.e. Whatman 934-AH). A Perkin Elmer Lambda 35 UV-Vis spectrophotometer was used to measure %T of the unfiltered and filtered sample aliquots. Deionized water was used as a reference to adjust the spectrophotometer to 100%T, and then each unfiltered and filtered sample aliquot was measured in a pre-rinsed sample cuvette with a 1 cm path length.

Analysis of NPOC/DOC was conducted according to LSRI/SOP/SA/47 – *Measuring Organic Carbon in Aqueous Samples* on a Shimadzu Model TOC-L Total Organic Carbon Analyzer. 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. An organic carbon stock solution which had a concentration of 1,000 mg/L carbon was used to prepare a working standard of 50 mg/L C which was also acidified to a pH < 2 with concentrated HCl. The standard was used to generate a calibration curve which was then used to determine the concentration of organic carbon in the samples.

Analysis of total hardness was conducted using the Ethylenediaminetetraacetic acid (EDTA) titrimetric method through manual titration according to the method described in LSRI/SOP/GLM/02 – *Procedure for Measuring Total Hardness*. 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 – *Procedure for Measuring Alkalinity*. Total alkalinity is reported as mg/L $CaCO_3$.

Analysis of DO was conducted using a Hach LDO HQ30d Dissolved Oxygen meter and LDO101 electrode, which was calibrated daily following LSRI/SOP/GLM/30 – *Calibrating, Maintaining and Using the HQ30d and HQ40d Meter and LDO101 Optical Electrode to Measure Dissolved Oxygen in Water Samples.* Temperature was measured using a Fisher digital thermometer that was verified quarterly following LSRI/SOP/GLM/17 – *Procedures for Thermometer Verification and Calibration*. Specific conductivity was measured using an Oakton Model CON 110 Conductivity/TDS/Temperature Meter that is calibrated on a monthly basis following LSRI/SOP/GLM/26 - *Procedures for Calibrating and Using the Oakton CON 110 Conductivity/TDS/Temperature Meter*. Its accuracy was also verified daily prior to sample analysis using a Daily Check Standard (0.0100M potassium chloride). pH analysis was conducted using an Orion 3 Star meter and Orion 8157BNUMD pH probe. The meter and probe were calibrated daily following LSRI/SOP/GLM/05 – *Procedure for pH Meter Calibration and Operation of pH Meters Utilizing Automatic Temperature Compensation (ATC)*. A check buffer of 8.00 was also measured after calibration to verify the accuracy of the calibration.

2.5.1.2 BACTERIAL ENUMERATION

From each whole water sample, subsamples were collected at designated analysis periods (0, 24, and 48 hours) and placed in sterile 120-ml sample vessels. *E. coli* and *Enterococcus* were enumerated according to LSRI/SOP/SA/56 – *Detection and Enumeration Total Coliforms and E. coli Using IDEXX Colilert®* and LSRI/SOP SA/62 *Detection and Enumeration of Enterococcus using IDEXX Enterolert®.* The Colilert and Enterolert assays have a detection limit of 1 MPN *E. coli*/100 mL and 1 MPN *Enterococcus*/100 mL, respectively. Both tests use Defined Substrate Technology® (DST) in which the bacteria metabolize the enzymes in the specific media causing the sample to fluoresce. Results are given as MPN, a common method of obtaining quantitative data on concentrations of discrete items from positive/negative (incidence) data, and in this case correlates well with colony forming units (CFU). Microbial density (as MPN/100 mL) over the 48-hour test period were calculated and reported.

2.5.1.3 ALGAL ENUMERATION

Whole water algae samples collected at 0, 24, and 48 hours were analyzed by staining a subsample of *S. capricornutum* cells from each sample with the vital stain SYTOX® Green. The *SOP GWRC/11* was

followed for staining and counting. Counting was conducted by enumerating the number of live and dead cells within a known area using a compound microscope equipped with epifluorescence able to excite samples at 450-490 nm under 400x magnification.

2.6 DEVIATIONS

During the course of conducting biological effectiveness testing with the LED TiO_2 technology there were several deviations that occurred from the TQAP. Those deviations are listed in Table 2 along with corrective actions that were taken as a response to the deviation and perceived impact of the deviation on the test results.

Table 2. Deviations Encountered during LED TiO_2 Treatment Process Bench-Scale Testing.

Test Impacted	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
Microbial Effectiveness Algae Dose-Effectiveness	Conductivity meters were not successfully calibrated within a month prior to measuring. Multiple analysts (ALF, CNP, OGA) attempted the calibrations but the low check standard did not pass for any analyst. The low check standards were remade but still were above the acceptable range. Because the high check standard did pass, it was deduced that the 100 µS/cm purchased standard might be low and offsetting the calibration.	The standard supplier, VWR, was called and a replacement 100 µS/cm calibration standard was sent at no cost to LSRI. Upon arrival ALF reperformed the calibration with the new solution and both the low and high check standards passed. This result suggests the previous batch of 100 µS/cm standard was bad.	Conductivity measurements in low conductivity ranges may be slightly high.	N	Alexander Frie
Microbial Effectiveness	POM standard not run with samples as the POM standard was expired. An update to the SOP requires POM standard is analyzed daily with samples.	New POM standard purchased.	Since all values were below the detection limit for POM there is no impact.	N	Alexander Frie
Microbial Effectiveness	Two *E. coli* duplicate analyses and two *E. faecium* duplicate analyses had RPDs > 30% leading to unacceptable average in regards to acceptance criteria listed in SOPs. However, the analyst	LSRI will be reviewing the DQO portion of the SOPs to determine the most appropriate way to present this DQO acceptance criteria in the future. Analyst	None; 95 % Confidence Intervals (as determined by IDEXX Quanti-Tray/2000© method) overlapped for all	N	Heidi Saillard

Test Impacted	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
	believes the DQO listed in the SOPs need to be reevaluated as all duplicate samples analyzed had overlapping 95% confidence intervals (CI). The CIs provided by IDEXX for use of Quanti-Tray/2000 indicate no significant differences in any of the duplicate samples analyzed.	suggests change to acceptable if "Upper and Lower 95% confidence intervals of the resulting duplicate analyses MPN values overlap".	duplicate samples analyzed, indicating no significant differences between samples.		
Algae Effectiveness	Initial concentration of *S. capricornutum* in the control and treatment stock was less than 200,000 ± 20%	Perform further counts of the initial inoculum to ensure initial counts are accurate prior to making calculations of how much inoculum to add.	Minimal impact, challenge to the treatment system was lower than if algae concentration would have been in acceptable limit.	N	Christine Polkinghorne
Algae Effectiveness	One duplicate DOC sample was outside of the relative percent difference range established in the data quality objectives. The analyst (ALF) believes this may have resulted from an accidental spike of the sample in question as the value was much different from the expected value.	QA/QC discussion in report includes a discussion of this data point. Sample was not able to be reanalyzed because the whole sample was spiked.	Minimal impact, because this sample was a duplicate, another sample was measured to verify that the water quality parameters were met.	N	Alexander Frie

3 LED TIO_2 TREATMENT PROCESS OPERATIONAL PERFORMANCE

During the testing period, no operational issues occurred with the LED TiO_2 technology.

4 RESULTS

Findings from the LED TiO_2 biological effectiveness tests, which were conducted on one species of algae, and two species of standard pathogen indicator bacteria in LW are presented in the following subsections.

4.1 BIOLOGICAL EFFECTIVENESS

4.1.1 GREEN ALGAE (*SELENASTRUM CAPRICORNUTUM*)

As detailed in the "Methods" section, water chemistry and water quality were measured on stock solutions of the water prior to initiation of testing. Water chemistry was additionally measured at the completion of the 48-hour exposure period. Water chemistry measurements of the stock solutions met the water chemistry acceptance requirements listed in the test plan and shown in Table 3 (LSRI, 2017). The results of the water chemistry measurements taken during the tests with *S. capricornutum* are shown in Table 4. The treatment system caused the treated water to be warmer than the control water but there were no significant (p<0.05) differences in water chemistry parameters between the control and treatment samples.

Table 3. Reference Limits for Water Type Prepared for GWRC Bench-Scale Evaluation

Parameter	Units	Water Type	Acceptable Range for Initiating Bench-Scale Testing
Temperature	°C	LW	22 to 28
pH	NA	LW	6.5 to 9.0
Specific Conductivity	µS/cm	LW	120 to170
Salinity	ppt	LW	<1
Dissolved Oxygen (DO)	mg/L	LW	4 to 12
Total Suspended Solids (TSS)	mg/L	LW	Less than reporting limit
Particulate Organic Matter (POM)	mg/L	LW	Less than reporting limit
Dissolved Organic Carbon (DOC)	mg/L	LW	Less than detection to 2
Non-Purgeable Organic Carbon (NPOC)	mg/L	LW	Less than detection to 2
Percent UV Transmittance at 254 nm (%T)	%	LW	93 to 100 (filtered and unfiltered)

Table 4. Temperature, pH, DO, Conductivity, Hardness and Alkalinity of Stock and Exposure Solutions Measured during Dose Effectiveness Tests with LED TiO_2 Treatment Process involving *S. capricornutum* in LW at 25°C ± 3°C.

Exposure	Sample Time (Hrs.)	Temp (°C)	pH	DO (mg/L)	Conductivity (µS/cm)	Hardness (mg/L CaCO3)	Alkalinity (mg/L CaCO3)
Stock (Control and Treatment LW)	0	25.4	7.54	6.5	153.5	54.8	52.6
PCW (LW)	0	24.3	7.57	7.0	162.3	52.4	53.2
Control (LW)	48	21.2	7.44	6.0	153.8	NM	NM
Treatment (LW)	48	24.1	7.74	7.1	164.7	NM	NM
PCW (LW)	48	20.8	7.49	8.8	155.5	NM	NM

NM = Not Measured.

Water quality measurements taken on stock solutions at the initiation of tests with *S. capricornutum* are presented in Table 5. All water quality values were within acceptance limits for testing (Table 3).

Table 5. Water Quality Values Measured in Stock Solutions during Dose Effectiveness Tests of the LED TiO_2 Treatment Process involving *S. capricornutum* in LW and PCW at 25°C ± 3°C.

Stock Solution Water Type	Total Suspended Solids (TSS; mg/L)	Percent Transmittance, Filtered/Unfiltered (%)	Non-Purgeable Organic Carbon (NPOC; mg/L)	Dissolved Organic Carbon (DOC; mg/L)	Particulate Organic Matter (POM; mg/L)	Mineral Matter (TSS-POM) (MM; mg/L)
LW	<1.25	98.4/98.3	0.87[J]	0.90[J]	<1.25	<1.25
PCW (LW)	<1.25	98.2/98.2	0.91[J]	0.83[J]	<1.25	<1.25

J = Value between limit of detection (0.70 mg/L) and limit of quantitation (2.3 mg/L).

Initial target density range of *S. capricornutum* in the 19 L carboy was 160,000 to 240,000 cells/mL. The initial cell density in the 19 L carboy was lower than the targeted concentration (146,667 cells/mL), which posed a slightly reduced challenge to the treatment system (Table 6). Densities of live cells in the PCW and control did not change significantly during the 48-hour treatment period, nor was there a substantial mortality in the PCW or control during that period. Approximately 60% of the initial cell density of *S. capricornutum* was recovered in the treatment sample following the 48-hour treatment period. Of the recovered cells observed following 48 hours of treatment by the LED TiO_2 treatment process, 18.1% were dead while 81.9% were live. The mortality in the recovered cells from the treatment sample after 24 hours treatment was 7.3%.

Table 6. Cell Density (± Standard Deviation) and Percent Mortality of *S. capricornutum* during Dose Effectiveness Testing with the LED TiO_2 Treatment Process.

Water Type	Exposure	0 Hour (cell/mL)		24 Hour (cell/mL)		48 Hour (cell/mL)		Percent Mortality at 48 Hours
		Live	Dead	Live	Dead	Live	Dead	
LW	Initial 19 L Carboy	146,667 ± 8611	0 ± 0					
	Control			150,000 ± 18,028	0 ± 0	164,833 ± 66,933	667 ± 1,155	0.3
	Treatment			85,000 ± 61,667	6,667 ± 5773	72,222 ± 1,470	16,111 ± 4006	18.1
	PCW	161,905 ± 9511	0 ± 0	155,556 ± 5,092	0 ± 0	153,631 ± 11,147	1,786 ± 619	1.2

4.1.2 MICROBES (*ESCHERICHIA COLI* AND *ENTEROCOCCUS FAECIUM*)

Water chemistry was measured on stock solutions of the water prior to initiation of testing with microbes. Water chemistry was also measured at the termination of the 48-hour exposure period. The results of water chemistry measurements made during the tests with *E. coli* and *E. faecium* are presented in Table 7. As with the algae testing, the temperature of the treatment exposure was elevated from the control exposure. Additionally, the DO and pH of the treatment sample were lower than the control, likely due to differences in microbial densities.

Table 7. Temperature, pH, DO, Conductivity, Hardness and Alkalinity of Stock and Exposure Solutions Measured during Dose Effectiveness Tests with LED TiO_2 Treatment Process involving *E. coli* and *E. faecium* in LW, and PCW at 25°C ± 3°C. Stock solutions are measured prior to the start of the test and do not have average values. Time is Incubation Time Post-Treatment.

Exposure	Sample Time (Hrs.)	Temp (°C)	pH	DO (mg/L)	Conductivity	Hardness (mg/L CaCO3)	Alkalinity (mg/L CaCO3)
Stock (Control and Treatment LW)	0	23.3	7.16	6.3	157.0 µS/cm	55.6	55.8
PCW (BHB)	0	22.3	7.31	6.8	12.57 mS/cm	NM	NM
PCW (TSB)	0	22.1	7.05	7.0	12.31 mS/cm	NM	NM
Control (LW)	48	21.8	7.61	6.6	176.6 µS/cm	NM	NM
Treatment (LW)	48	26.9	7.01	2.0	169.9 µS/cm	NM	NM
PCW (BHB)	48	23.9	6.32	8.0	13.47 mS/cm	NM	NM
PCW (TSB)	48	23.8	6.98	0.2	13.79 mS.cm	NM	NM

NM = Not Measured.
BHB = Brain Heart Infusion Broth.
TSB = Tryptic Soy Broth.

Water quality measurements were made on stock solutions prior to the initiation of the microbe tests (Table 8). All water quality values were within the acceptance limits for test initiation (Table 3).

Table 8. Water Quality Values Measured in Stock Solutions during Dose Effectiveness Tests of the LED TiO_2 Treatment Process involving *E. coli* and *E. faecium* in LW and LW-TMH at 25°C ±3°C. The PCW, BHB and TSB, are not measured for Water Quality Values.

Stock Solution Water Type	Total Suspended Solids (TSS; mg/L)	Percent Transmittance, Filtered	Non-Purgeable Organic Carbon (NPOC; mg/L)	Dissolved Organic Carbon (DOC; mg/L)	Particulate Organic Matter (POM; mg/L)	Mineral Matter (TSS-POM) (MM; mg/L)
LW	<1.25	97.2	1.29[J]	1.13[J]	<1.25	<1.25

J = Value between limit of detection (0.70 mg/L) and limit of quantitation (2.3 mg/L).

Results of dose effectiveness tests involving *E. coli* and *E. faecium* in LW and LW-TMH are shown in Table 9**.** Initial concentrations (0 hour) of both species in the inoculated LW prior to the start of testing and in the control pseudo replicates were > 1.0E+06 MPN/100 mL for all tests indicating initial target concentrations were achieved. After 24 hours of treatment, both *E. coli* and *E. faecium* densities were >2.4E+06. Density of both *E. coli* and *E. faecium* were lower in the treated water than the control water at the end of the 48-hour exposure to the LED TiO_2 technology. The density of both microbes in the performance control samples had increased significantly at the end of the 48-hour period, indicating that the organisms were healthy at test initiation.

Table 9. Average Density (MPN/100mL) ± Standard Error of the Mean (SEM) of Bacteria *E. coli* and *E. faecium* During Biological Effectiveness Tests of the LED TiO_2 Treatment Process.

Water Type	Species	Exposure	0 Hour	± SEM	24 Hour	± SEM	48 Hour	± SEM	% Reduction from 0 HR
LW	*E. coli*	Initial 19 L Carboy	7.6E+06	5.4E+05					
		Control			2.3E+07	3.5E+06	2.5E+07	1.8E+06	
		Treatment			> 2.4E+06	NC	3.7E+06	4.1E+05	51
		Performance Control	8.5E+06	7.7E+05	3.1E+11	2.9E+10	5.5E+11	1.5E+11	
	E. faecium	Initial 19 L Carboy	5.6E+06	4.4E+05					
		Control			5.7E+06	6.6E+05	6.1E+06	6.9E+05	
		Treatment			> 2.4E+06	NC	3.4E+05	4.1E+04	94
		Performance Control	5.2E+06	7.3E+05	1.1E+11	2.3E+10	8.0E+10	1.2E+10	

NC = Not calculated because all replicates were above the method's limit of enumeration.

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 LED TiO_2 technology are summarized in Table 10. Two parameters did not meet the DQOs for this project. All other parameters met the DQOs stated in each referenced SOP.

The parameters that did not meet the DQOs were the precision of NPOC measurements and completeness of unfiltered %T measurements. The precision of NPOC measurements failed due to an analyst error and should not have impact on the overall conclusions of this report. This is discussed in detail in Table 2. The completeness of unfiltered % T failed due to an analyst error, which resulted in no unfiltered measurements being analyzed for the microbial dose-effectiveness test. The DQO failure does not impact the conclusions of this testing.

Table 10. Data Quality Objectives (DQOs), Criteria, and Performance Measurement Results from Water Chemistry and Water Quality Analyses Conducted during LED TiO_2 Treatment Process Dose Effectiveness Testing.

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: TSS/POM: 67% %T: 67% NPOC/DOC: 67% Hardness: 67% Alkalinity: 67%	TSS: Not Measured* %TF: 0.40% ± 0.42% %TU: 1.99% NPOC: 100% ± 84.9% DOC: 17.7% ± 10.2% POM: Not Measured* Hardness: 2.5% ± 3.5% Alkalinity: 2.4% ± 0.69%
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: 1	Filter blank (%T): 99.2%
	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/POM Filter Blanks Analyzed: 2	Filter blank (TSS): < 0.63 mg/L ± 0
				Filter blank (POM): < 0.63 mg/L ± 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: 6	Blank (NPOC): < 0.70
	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: 2	Filter blank (DOC): < 0.70
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: 50%	NPOC/DOC: 101.4% ± 2.1%
	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: TSS: 100% POM: 50% NPOC: 100%	TSS: 6.4% ± 5.8% D POM: 1.4% D
				NPOC Reference Standard 3.4% ± 2.8% D NPOC 10 mg/L Standard: 2.7% ± 1.8% D

Data Quality Indicator	Evaluation Process/Performance Measurement	Data Quality Objective	Performance Measurement Result	
	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)	Number of Analysis Days Containing a Reference Standard: 2 Number of test types: 1	Hardness: DQO met 100% of the time 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	TSS: 100%	
			%T, Filtered: 50%	
			%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	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 Determined 2 February 2019	

*** Precision within TSS and POM measurements was not measured due to all duplicates being below the detection limit, making this value incalculable.**

5.2 ALGAE TESTING

During *S. capricornutum* testing, data quality was measured by analyzing a minimum of 10% of samples in duplicate and by having a second individual conduct quality assurance counts on a minimum of 10% samples. For all testing with *S. capricornutum*, the minimum number of duplicate and quality assurance samples were met or exceeded (Table 11). The initial concentration of the algae in the carboy used to fill the control and treatment containers averaged 146,667 live cells/mL which does fall below the 200,000 ± 20% cell/mL starting concentration requirement, however this should not have had a significant effect on the results of the test. If anything, the challenge to the system was lower than it would have been with 200,000 ± 20% cell/mL starting concentration.

Table 11. Average Relative Percent Difference (RPD) for *S. capricornutum* counts conducted during LED TiO_2 Treatment Process Bench-Scale Testing.

Test Date	Duplicate or Quality Assurance Count	Percent of Samples with QA counts	DQO	Relative Percent Difference	
				Live	Dead
17 September 2019	Duplicate	12.5%	RPD ≤ 20%, when greater than 10 cells of live/dead are counted	28.2	88.9*
	Quality Assurance	12.5%		1.9	1.1

* This difference is large due to one sample having one dead cell and the duplicate having no dead cells

5.3 MICROBE TESTING

During microbe testing, data quality was measured by analyzing a minimum of 10% of samples in duplicate, analyzing both qualitative and quantitative positive controls, and by having a second individual conduct quality assurance counts on a minimum of 10% samples. The minimum number of quality assurance samples were met or exceeded (Table 12) for each data quality indicator. Data quality objectives for precision exceeded the <30% average RPD objective in listed both the *E. coli* and *E. faecium* analysis methods. Two of the *E. coli* duplicate analyses and two of the *E. faecium* duplicate analyses had relative percent differences (RPD) greater than 30%. Although this is out of the acceptance range listed in the methods data quality objectives, the 95 % Confidence Intervals (as determined by IDEXX Quanti-Tray/2000© method) overlap for all duplicate samples analyzed, indicating that there were no significant differences between any of the duplicate samples analyzed during this test. LSRI will be reviewing the DQO portion of the SOPs to determine the most appropriate way to determine acceptance values for duplicate agreements. All data quality objectives for bias, accuracy and completeness (i.e., method blanks and quantitative positive and negative controls) were within acceptable limits for microbe testing (Table 12).

Table 12. Data Quality Objective Summary for Bench-Scale Tests using *E. coli* and *E. faecium*.

Data Quality Indicator	Evaluation Process/ Performance Measurement	Data Quality Objective	Performance Measurement Result
Precision	Samples (10%) are analyzed in duplicate – with performance measured by average relative percent difference (RPD) of all duplicate analyses.	<30% average RPD	***E. coli:*** 4 of 38 (10.5%) reported samples analyzed in duplicate; **Average RPD =** 35.5^{CI} (n=3; one NC)
			E. faecium: 4 of 38 (10.5%) reported samples analyzed in duplicate; **Average RPD =** 38.2^{CI} (n=3; one NC)
Bias, Operator	Samples (10%) are counted by two separate analysts with performance measured by average relative percent difference (RPD) of all second counts.	<20% average RPD	***E. coli*:** QA counts for 16 of 58 (27.6%) reported samples and QA samples; RPD =0%
			E. faecium: QA counts for 16 of 58 (27.6%) reported samples and QA samples; RPD =0%
Bias, Positive Control	Qualitative positive control samples (American Type Culture Collection) are analyzed on each analysis date or IDEXX-QC samples are analyzed as a quantitative positive control at least once per ballast water treatment system test.	Results must be greater than the limit of detection.	***E. coli:*** Qualitative Positive controls all >1 MPN/100 mL n=2
			E. faecium: Qualitative Positive controls all >1 MPN/100 mL n=2
Bias, Negative Control	Qualitative negative control samples (American Type Culture Collection) are analyzed on each analysis date or IDEXX-	Results must be less than the limit of detection.	***E. coli:*** Qualitative Negative controls all <1 MPN/100 mL n=3

Data Quality Indicator	Evaluation Process/ Performance Measurement	Data Quality Objective	Performance Measurement Result
	QC samples are analyzed as a negative control at least once per ballast water treatment system test.		***E. faecium:*** Qualitative Negative controls all <1 MPN/100 mL n=4
Bias, Method	Sterilized water (similar matrix sample) analyzed using same method as samples on each analysis date.	Results must be less than the limit of detection.	***E. coli:*** All method blanks <1 MPN/100 mL, n=4
			E. faecium: All method blanks <1 MPN/100 mL, n=4
Bias, Diluent Blank	One per analysis day, diluent (e.g., sterile deionized water) blank run analyzed using same media as samples	Results must be less than the limit of detection.	***E. coli:*** All diluent blanks <1 MPN/100 mL, n=4
			E. faecium: All diluent blanks <1 MPN/100 mL, n=4
Accuracy	IDEXX-QC samples are analyzed as a quantitative positive control at least once per ballast water treatment system test.	***E. coli:*** 237 MPN/100 mL; Acceptable Range: 24-492 MPN/100 mL	***E. coli:*** Quantitative analyses within IDEXX acceptance range (n=1) 307.6 MPN/100 mL
		E. faecalis: 116 MPN/100 mL; Acceptable Range: 53-179 MPN/100 mL	***E. faecalis:*** Quantitative analyses within IDEXX acceptance range (n=1) 90.8 MPN/100 mL
Representativeness	All samples are collected, handled, and analyzed in the same manner.	Not Applicable – Qualitative.	All microbial samples were collected, handled, and analyzed in the same manner (using the appropriate LSRI/GWRC SOPs).
Comparability	Routine procedures are conducted according to appropriate SOPs to ensure consistency between tests.	Not Applicable – Qualitative.	The LSRI/GWRC SOPs listed in Section 2.4.1.2 were used for all microbial analyses conducted.
Completeness	Percentage of valid (i.e., collected, handled, analyzed correctly and meet DQOs) microbial samples measured out of the total number of microbial samples collected. Performance is measured by percent completeness (%C).	>90% Complete	***E. coli*:** 55 of 58 samples (Control, Treatment, PCW, QA) = 95% Completeness
			E. faecium: 55 of 58 samples (Control, Treatment, PCW, QA) = 95% Completeness
Sensitivity	The limit of detection (LOD) for the analytical method used is reported.	Dependent upon the analytical technique used. Adjusted for volume used.	***E. coli* LOD:** <1 MPN/100 mL
			***E. faecium:* LOD:** <1 MPN/100 mL

NC - Not Calculable; values greater than range of Quanti-Tray
CI – 95 % Confidence Intervals (as determined by IDEXX Quanti-Tray/2000© method) overlap, indicating no significant difference between duplicate samples

6 CONCLUSIONS AND DISCUSSION

Over the course of several weeks, LSRI-GWRC evaluated the LED TiO_2 technology, developed by YJB LED Professional Services of Crosslake, MN, USA. The LED TiO_2 treatment process as installed in LSRI's laboratory was an early prototype of an in-tank, recirculating ballast water treatment technology, wherein the treatment would occur during the entire 48-hour voyage time of a vessel. Overall, the testing unit provided by LED TiO_2 treatment process operated well with no technical difficulties.

The LED TiO_2 treatment process was evaluated with respect to freshwater biological effectiveness to green algae and microbes in laboratory water. Deviations to LSRI SOPs and/or the LSRI-GWRC test plan that occurred during testing were minor and did not impact the results.

The effectiveness testing with the green algae *S. capricornutum* found that there was a 60% recovery of algae cells in the treated samples. It is likely that the decrease was due to the "C-shaped" *S. capricornutum* cells being trapped by the TPX-220 coated filter that was part of the treatment process. It is not possible to say what portion of the trapped cells were alive or dead. For this reason, we recommend any future biological effectiveness testing be conducted with a control that utilizes the same filter that the treatment process uses but without the TPX-220 coating. Of the algae cells that were recovered in the treated samples, 18% of the cells were dead. The control samples demonstrated that the pump did not impact the survival of the organisms and that the organisms were healthy during testing.

Effectiveness testing with microbes showed a 51% reduction in *E. coli* and 94% reduction in *E. faecium* from the starting concentration after 48 hours of treatment with the LED TiO_2. Survival rates in the control samples for both *E. coli* and *E. faecium* demonstrated that the pump did not impact survival and that the organisms were healthy during testing.

During biological effectiveness testing, algae were observed to be physically removed by the TPX-220-coated filter. The treatment process may benefit from additional testing to determine whether organic matter trapped in the filter reduces the reactive surface area of the filter, which may impact biological effectiveness over time. Additional research may be needed to identify a method to remove trapped organisms, organic matter, and other particulates from the filter following repeated use. Following each biological effectiveness trial, it was noted that there were particulates in the bottom of the treatment container, which were not identified but could have been from degradation of the fibrous filter during the 48-hour run time. The purpose of the TPX-220-coated filter was to increase the surface area for exposure of organisms to hydroxyl radicals, and the impact of any degradation of the fibrous filter on the biological effectiveness of the treatment process was outside the scope of this research.

Results from this laboratory-based testing, although limited to only two trials and three species, indicate some potential effectiveness of LED TiO_2 technology for treatment of Great Lakes ballast water after 48 hours of continuous operation. Under testing conditions, the LED TiO_2 treatment process reduced concentrations of both algae and microbes as compared to pre-treatment concentrations. The data indicate that *at this stage in development*, the technology is not treating to the level required by the United States Coast Guard Ballast Water Discharge Standard. The discharge standard requires less than

10 live cells/mL in the ≥10 µm to <50 µm size class (nominally protists such as green algae), less than 250 MPN/100 mL *E. coli*, and less than 100 MPN/100 mL *E. faecium* in ballast water discharge. Additional research and development are needed to increase biological effectiveness of this treatment process, but this initial independent testing suggests some freshwater effectiveness particularly with organisms <10 µm (i.e., pathogen indicator organisms).

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