



Treating Algal Blooms with OZONIX®

Technology Demonstration

Outboards Only Marina (Jensen Beach, FL)

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Protected by 14+ Approved United States Patents

7,699,994; 7,699,988; 7,785,470; 7,943,087; 8,318,027; 8,721,898; 8,858,064; 8,936,392;
8,906,242; 8,968,577; 8,999,154; 9,034,180; 9,169,146; 9,266,752

Numerous Patents Pending

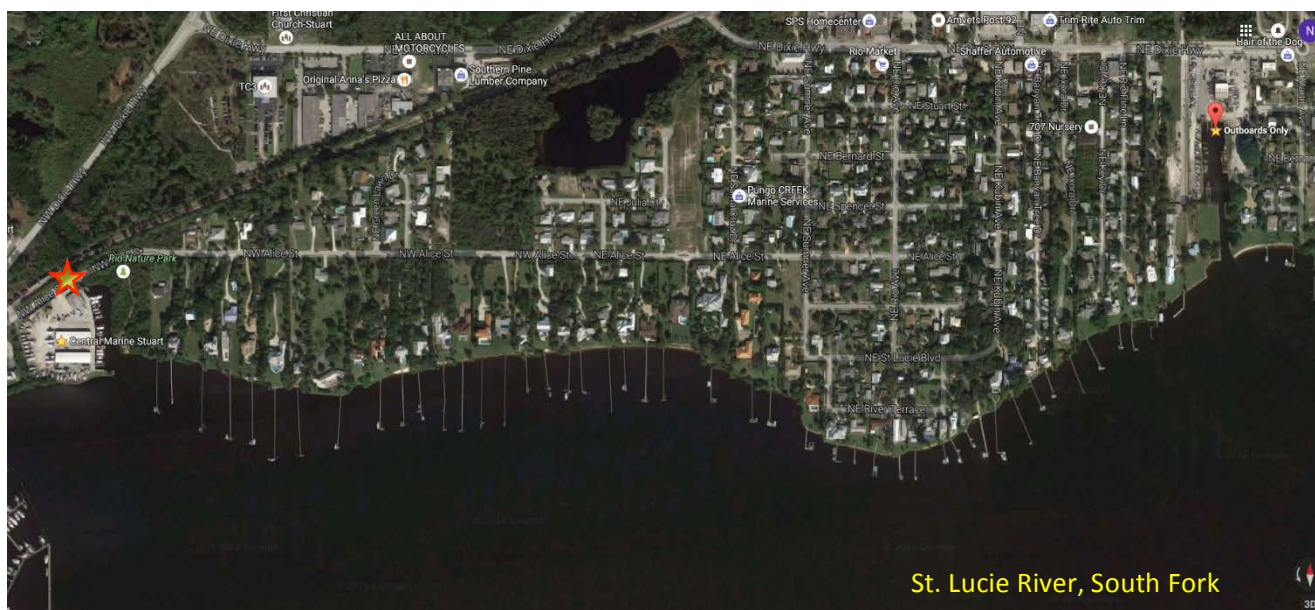
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Site Description:

St. Lucie River, South Fork

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The following series of pictures reveals how in just a matter of weeks, a Marina or stagnant body of water can be overtaken by cyanobacteria. The pictures include an adjacent marina to Outboards Only called Central Marine, which is approximately 1 mile downstream from the Outboards Only location.



St. Lucie River, South Fork



June 3, 2016



June 29, 2016

Central Marine

The following pictures demonstrate the conditions at the Outboards Only Marina the day before the OZONIX® demonstration started.



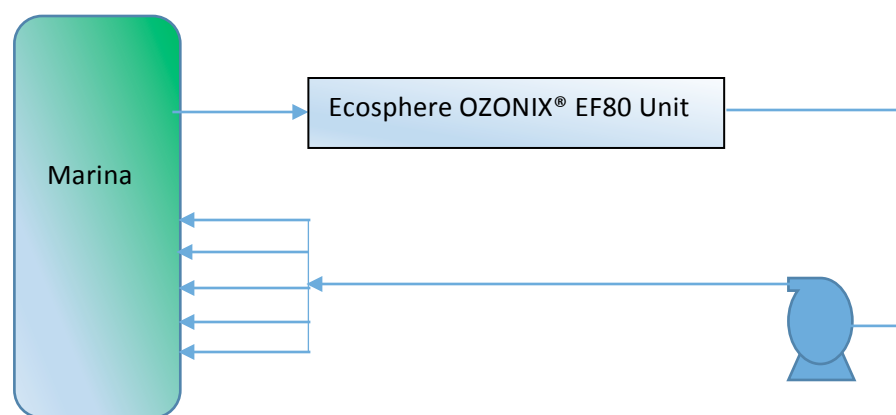
July 09, 2016 – Outboards Only Marina

Objective :

The objective of the current project were two fold. The foremost was to help and save the local businesses from closing down (which had already been closed for a week) and help the neighbourhood community from the unbearable odours of the rotting algae in the marina. Ecosphere also wanted to demonstrate the potential of the OZONIX® technology and their long-standing commitment to the environment through this social cause.

Setup:

Ecosphere deployed its OZONIX® EF80 unit (3,360 GPM) for this demonstration at Outboards Only. The treatment was carried out for a week at no cost to the local business. The approach was to treat the algae and toxins in the water by circulating the algae laden water suspended in the marina through the OZONIX® EF80 unit and to also treat the surface algae through the assistance of a high-pressure water cannon; exposing it to Advanced Oxidation by Hydroxyl Radicals (OH⁻) through Ecosphere's proprietary combination of Ozone (O₃), Hydrodynamic Cavitation, Acoustic Cavitation and Electrochemical Oxidation. The following picture demonstrates the setup arrangement.



Overview of the Ecosphere OZONIX® Technology

OZONIX® is a patented chemical free water treatment technology that is categorized as an Advanced Oxidation Process (AOP) that creates highly reactive OH[•] (Hydroxyl Radicals) to disinfect water. OZONIX® was designed to maximize the creation of hydroxyl radicals and associated oxidizing species such as perhydroxyl radicals, nascent oxygen, hydrogen peroxide and ozone by combining Hydrodynamic Cavitation, Ozone (O₃) disinfection, Acoustic Cavitation and Electrochemical Oxidation in one treatment process. As a result, a five (5) to twenty (20) times increase in disinfection potential can be achieved by using OZONIX® when compared to using Ozone (O₃) by itself. Ecosphere has been issued over 14 patents on this technology and has processed over 6 billion gallons of water for reuse across numerous industries and applications such as Oil & Gas, Food & Beverage, Landfill Leachate and more. It has also been used successfully for Hydrogen sulfide treatment on numerous occasions.

Hydrodynamic Cavitation

The first step in the patented OZONIX® process is Hydrodynamic Cavitation. Hydrodynamic Cavitation is a physiochemical process (physical process that acts as a catalyst for a chemical reaction) that creates

the highly reactive Hydroxyl Radicals (Oxidation Potential: 2.80V). The cavitating bubble produces localized hot spots, which causes pyrolytic chemical reactions that create hydroxyl radicals. Disinfection due to hydrodynamic cavitation happens in a matter of nanoseconds. The OZONIX® process maximizes the efficiency of Hydrodynamic Cavitation through the use of patented static mixers. The static mixers use a proprietary hole and shape design that maximize the amount of cavitating bubbles that are being created and result in an increasing amount of hydroxyl radical production.

Ozone (O₃) Injection

After Hydrodynamic Cavitation, Ozone (O₃) is injected into the system. Ozone has an Oxidation Potential of 2.07V allowing it to oxidize a wide range of pollutants. Ozone kills bacteria in the water through a process called lysis; this is a process where Ozone will penetrate the cell wall and oxidize all the essential components such as DNA. OZONIX® injects Ozone into the system using a venturi, which when combined with the other oxidation processes maximizes the mass transfer efficiencies of Ozone, thus allowing less to be used than typical Ozone disinfection processes. It is important to note that while Ozone destroys bacteria, it also eliminates the food source that makes it nearly impossible for cyanobacteria to regrow.

Acoustic Cavitation

After Ozone (O₃) is injected, the next step in the OZONIX® process is the OZONIX® reactor that consists of simultaneous treatment of Acoustic Cavitation and Electrochemical Oxidation. Acoustic Cavitation uses ultrasonic waves to induce cavitation in the water. Similar to Hydrodynamic Cavitation, Acoustic Cavitation produces localized hot spots causing sonochemical reactions to occur thus producing OH-Hydroxyl Radicals. The OZONIX® process uses multiple frequencies and powers to maximize the intensity of cavitation.

Electrochemical Oxidation

The final process in the OZONIX® reactor is Electrochemical Oxidation. Electrochemical Oxidation uses electricity to maximize the production of OH° Hydroxyl Radicals. OZONIX® uses proprietary coated electrode rods to achieve electrochemical oxidation; maximizing the surface of the rods' longevity and their ability to produce Hydroxyl Radicals. This area of the OZONIX® process is also responsible for the removal of nitrogen species, ionic species and other metals.

Overview of OZONIX® U.S. Patent No. 8,999,154 – “Apparatus for treating Lake Okeechobee water”

Ecosphere's patented OZONIX® process relates to fluid treatment and, in particular, to Patent No. 8,999,154, which identifies the use of an Advanced Oxidation Process (AOP) to destroy aerobic and anaerobic bacteria in lakes, canals, rivers and streams, based on the principle of degradation/disinfection using a combination of Ozone (O₃) Injection, Hydrodynamic Cavitation, Acoustic Cavitation and Electrolysis.

The purpose of the Outboard Only Marina demonstration project was also to teach the methods outlined in U.S. Patent No. 8,999,154 to increase ORP to aquatic-safe levels so that algae cannot thrive. It is important to note that OZONIX® does not require the use of liquid chemicals and does not generate any sort of secondary byproducts that have to be handled and/or sent to landfills for further treatment.

Sampling and analysis:

Samples in sufficient quantities were taken BEFORE and AFTER the OZONIX® demonstration at the Outboards Only Marina. The staff had to wear protective masks in the first couple of days of treatment to get even closer to water due to the putrid smell. The samples were refrigerated and sent for analysis to GreenWater Laboratories located in Palatka FL.



GreenWater Laboratories/CyanoLab is the only private full-service laboratory of its kind in the United States, with a strict focus on cyanobacteria/algae and the toxins they produce. As treatment started, the odor diminished substantially with staff no longer having to masks second or third day of treatment.

Samples were tested for identification of algal species and the associated toxin levels in the marina. Samples were prepared & analyzed by Green Water Laboratories via enzyme linked immunosorbent assay (ELISA) method. The samples were also scanned at 100X for the presence of potentially toxigenic (PTOX) cyanobacteria using a Nikon Eclipse TE100 inverted microscope equipped with phase contrast optics. Higher magnification was used as necessary for identification and micrographs. Measurements were also done for various parameters such as temperature, pH, oxidation-reduction potential (ORP) and dissolved oxygen levels. Coincidentally, the County (Martin County Engineering Department) was also doing air quality analysis in the area and around the algal blooms across Martin County during the same time as our demonstration. These test were conducted by Limited Environmental Sampling and outsourced to Enviro Team North America, LLC.

Results

The Outboard Only Marina was 385ft long, 112 ft wide and 5-6 feet deep, holding over 1.61-million-gallons of algae infested water. It had accumulated a 4-12 inch thick layer of algae on its surface, in just



a 3-4 week time period. The following pictures give an idea of how bad the algae had grown in such a short time period before Ecosphere could deploy its equipment

The algae layer was so thick in the initial period of treatment that we were having difficulties flowing continuously at over 40 BPM (Barrels Per Minute). However, as time progressed and the algae started to break down due to OZONIX® treatment, we could increase the flow rate to over 80 BPM in just a couple of days. The following pictures show how the flow changed from greenish in the initial period of treatment to clear color after a couple of days' treatment through the OZONIX® EF80.

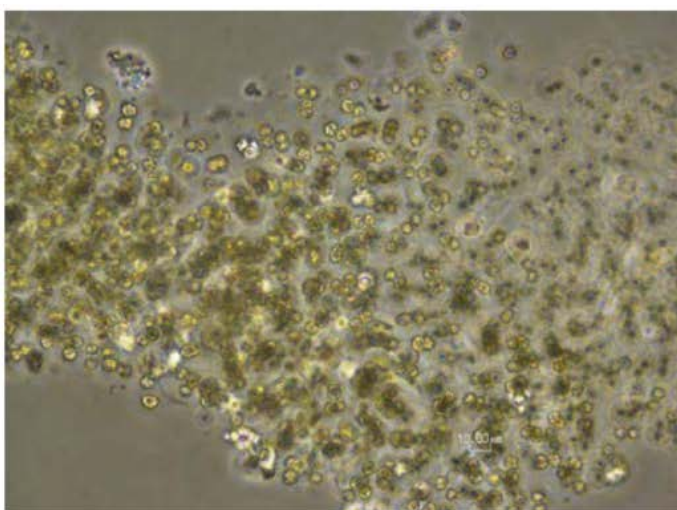


One can also note the change in the algae content and the change in the color of the water itself as the OZONIX® EF80 treatment progressed.

The sample analysis by the third party analysis from GreenWater laboratories identified several colonies of the potentially toxigenic cyanobacterium *Microcystis* and filamentous cyanobacterium *Planktothrix/Oscillatoria* in the raw water as shown below. These cells were completely destroyed and their appearance changed drastically with treatment from the OZONIX® EF80.

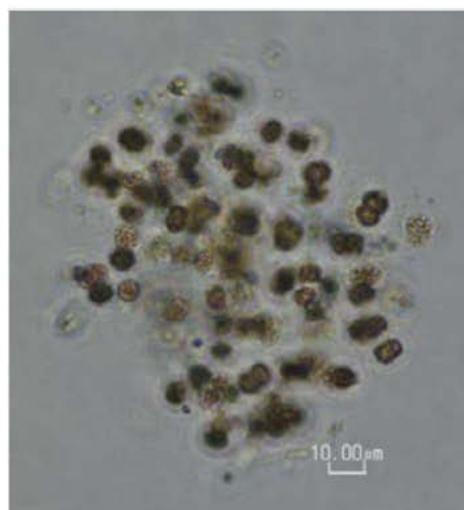


Planktothrix/Oscillatoria sp. at 400x (Raw C)



Microcystis sp. at 400x (Raw C)

Before Treatment



Microcystis sp. at 400x (Treated C)

After Treatment

Air and water quality analysis of the samples from the County study as well as Ecosphere demonstration results are shown below.

Air and Water Quality Toxins Analysis

Location	Microcystins in water (µg/L)		Microcystins in air (ng/m³)	Microcystis Cells in air
	Before Treatment	After Treatment		
Central Marina	33100	No Treatment	0.6361	ND
Outboard Only Marina	8850	< 1.5 With Ozonix® Treatment	0.0311 95% reduction in airborne toxins (samples taken after two days of treatment)	ND

As can be seen from the above table, the algal toxin, Microcystin (MC) was found in the water samples and its level was significantly higher (100-1000 times) than the established WHO guidelines for recreational exposure to cyanobacteria for high risk environment (>100,000 cyanobacterial cells/ml corresponding to >20 µg/L for MC-LR). At these levels, the County declares these sites unfit for recreational use due to the high levels of toxins found in the Marina(s).

One can observe the decrease in the toxin levels caused by the OZONIX® treatment. The concentration of microcystin (MC) went down from 8850 µg/L before treatment to < 1.5 µg/L (non-detect) resulting in a 99.983% decrease in the toxin levels in over 1.6 million gallons of water in matter of days (< 50 hrs of actual run time) and making the water safe for recreational use.

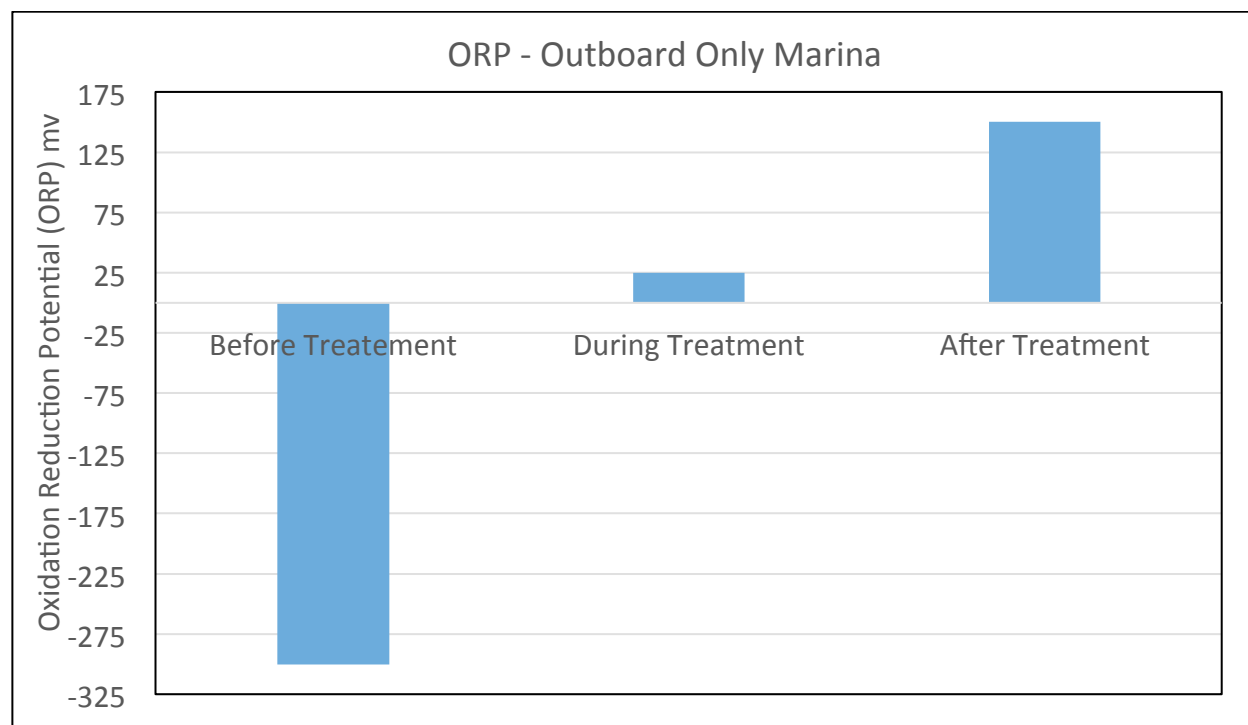
It can also be noted that the Microcystin levels in air were 95% lower at the Outboards Only Marina (after 2 days of treatment) than the Central Marine Marina (that received no treatment) due to the degradation of microcystin into easily degradable byproducts such as diols, aldehydes, fatty acids and smaller carboxylic acids as is widely known in literature. These byproducts are easily degradable in nature and are part of essential elements for life in small quantities.

Results of independent testing by the Florida Department of Environmental Protection (DEP) during and after the Ecosphere Treatment at Outboard Only Marina are also shown below.

Florida Department of Environmental Protection	
Sample Collection	Water Quality Toxin Analysis - Mycrocystin in Water (µg/l)
07/11/2016 @ 16:20 - During Treatment (Treatment started on 07/09/2016)	149
07/18/2016 @ 11:50 - 3 days after Ecosphere Treatment	< 1.0 (below detection limit of the instrument)

The results from the Florida Department of Environmental Protection (DEP) further validate the efficacy of Ecosphere's multi-patented and proven OZONIX® technology for treating algal blooms.

During the demonstration, Ecosphere also measured the oxidation reduction potential (ORP) levels of the marina before and after treatment. ORP measurements reveal the surrounding environment of the water bodies. It represents the level of presence of oxidizing and reduces species presents in the water body under consideration. For example, presence of oxidizing environments such as dissolved oxygen levels, presence of other oxidizing species such as hydrogen peroxide, presence of ozone, presence of hydroxyl radicals etc. The results from the ORP measurements from the Outboard Only Marina are shown in the following figure.



It can be seen that the ORP levels were around negative (300) mv before Ecosphere deployed its OZONIX® equipment to the Marina for treatment. This represents highly anaerobic, septic conditions. The dissolved oxygen in the Marina was depleted due to the rapid and outrageous growth of cyanobacteria “blue-green” algae. The degradation of the dead algae by anaerobic bacteria was causing elevated levels of hydrogen sulfide and the rotting smell of the dead algae and the dead fish in the marina; making it impossible to live in the neighborhood.

One can easily appreciate the change in the environment of the Marina as it started replenishing the water by adding oxygen and oxidizing species back into the water and simultaneously killing the dead and live algae mats present on the surface of the Marina as the water and algae passed through the OZONIX® system. The recirculation and spraying of the ozonated water on the surface also settled any airborne particulates and toxic gases released from disturbing the stagnant algal mass.

Effectiveness can also be seen through the ORP level increase from -300mv to +25 mv to + 150 mv in less than 50 hrs of operation, making the water safe for use and recreational purposes again. In just a matter of days, a community went from not being able to breathe to the Ecosphere staff swimming in the water after the treatment was completed.



Ecosphere had also collected samples before and after the OZONIX® treatment of the Outboard Only Marina and monitored and tested important water quality parameters such as temperature, pH, ORP and DO. The following table shows this data. It can be seen from the table that the Outboards Only

Sample	Water Quality Parameters Tested			
	Dissolved Oxygen (DO), mg/L	Oxidation Reduction Potential(ORP), mv	pH	Temperature, °F
Before Treatment	0.9 - 1.0	- 300 mv	7.22	92.1
After Treatment	2.5 - 2.6	+ 150 mv	7.58	88.3

Marina went from being anoxic to being sufficient in dissolved oxygen to support life. The DO went up from 0.9 mg/L to over 2.5 mg/L while ORP simultaneously went up from -300 mv to over 150 mv. Other parameters did not change much indicating no adverse effect of our treatment on the quality of water. It was fulfilling to see fish swimming and life coming back to Outboard Only Marina after Ecosphere's Treatment.

In Phil Norman's (Owner, Outboards Only Marina) own words - ***"The water had never been so clear in the last eight years. I could never see the bottom of the Marina before you guys (Ecosphere) came in".***



If given the opportunity, we plan to do the same on the C-44 canal and throughout the surrounding rivers using OZONIX® Algae Response Vessels to address the growing algae bloom issue in the St. Lucie River and Indian River Lagoon ecosystem.



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