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(R-18)Use of Bio-Zyme Bacteria from TRM Biologics, Inc.

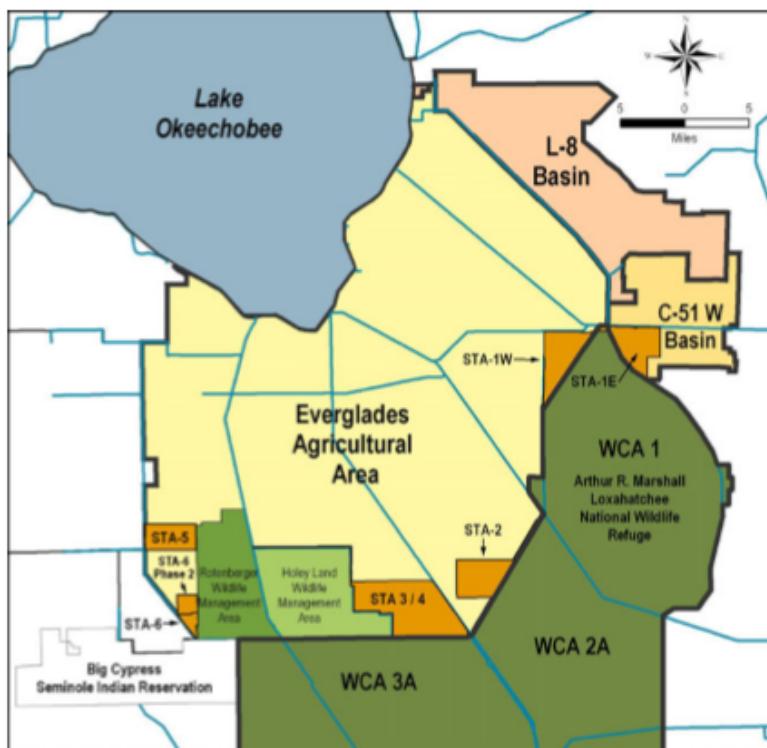
Test Plot: Florida Everglades STA (Stormwater Treatment Area). Bio-Zyme Trial in STA 1W for muck removal.

Introduction

South Florida receives heavy rainfall which is the area's primary source of fresh water. Stormwater runoff carries nutrients including Nitrogen, Phosphorous with both organic and inorganic residuals into the stormwater treatment areas.

These Stormwater Treatment Areas (STAs) are wetlands created to remove and sequester nutrients through plant growth. The accumulation of dead plant material creates muck and slows the effectiveness of the STAs. In the Florida Everglades, there are more than two dozen pump stations, 350 water control structures and more than 600 miles of levees and canals. Repairs, maintenance, erosion control and debris cleanup are essential and ongoing tasks. The Everglades Stormwater Treatment Areas collectively comprise 57,000 acres of effective treatment area.

The location of the six STAs is shown in the drawing below:





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Range of Challenges

The 6 STA zones treat an immense amount of water with a total processed flow of 1.4 million acre-feet of water. They retained a total of 208 tons of total Phosphorous (86% removal).

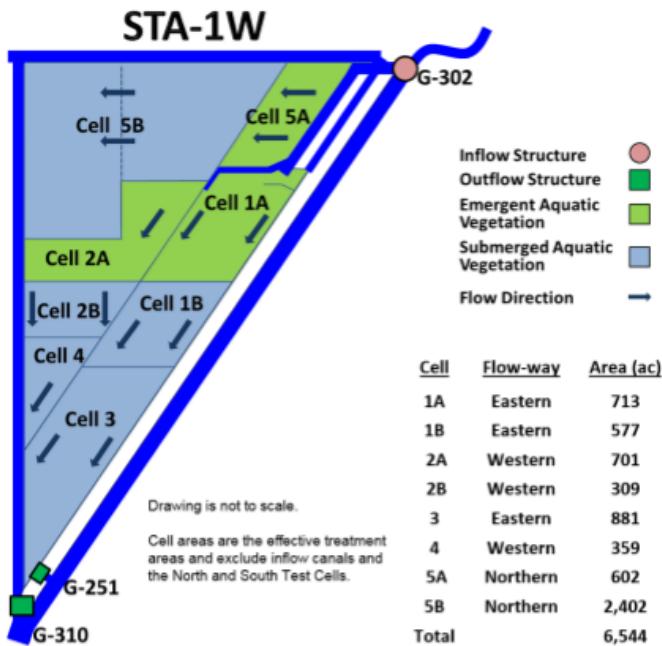
There is a continuous program of planting, seeding, herbicide treatments and manual removal of excess plant material. The maintenance effort is huge and costly. This program is driven by the need to maintain this nutrient trapping process that's performed by the STAs. However the end result of aging in the STA is the build-up of dead plant material and muck.

The first zone of the STA system in operation was STA-1W which began operation in 1994. This STA comprises 6,544 acres of treatment area.

While plant growth is a key part of the design of any wetland, STA-1W has suffered from excessive organic muck and excess dead and floating plant material. The excess build up prohibits growth of healthy plants. This interferes with nutrient removal as nutrient removal is at its best when the zone is actively growing new, healthy plants.

STA-1W Test Site

This STA was the first in service, it has a buildup of organic sludge and dead plant material that reduces nutrient uptake. The primary focus of vegetation management in STA-1W during WY2016 was the rehabilitation of Cell 1A in the Eastern Flow-way. Eight-hundred acres of primrose willow, water pennywort (*Hydrocotyle umbellata*) and floating cattail tussocks had been treated or mechanically removed in this cell during WY2015. A schematic is shown below:



With the excess accumulation of organic muck, dead plants and less than ideal new plant (emergent) growth for nutrient uptake, the SFWMD agreed to a trial of Bio-Zyme bacterial products in a 50 acre test site.

The decision was made to evaluate the use of Bio-Zyme in Cell 1A. This is the oldest cell in operation existing for over 10 years. Cell 1A would be considered as a bell-weather for how the others will age. In 2016 it's determined that this cell is not aging well. It is 2-3 foot deep but has formed some floating false bottom of unconsolidated material that floats to the surface after the draw-downs which are designed to consolidate this organic sludge material.

Discussion between SFWMD, AVC, and TRM personnel centered on the best possible dosing mechanism. The collective decision was to apply the Bio-Zyme in a 25 acre "open" water site across Cell 1A in STA-1W and not on the surface of the floating material. Due to the shallow depth and high wind/wave action in the 800 acre cell all agreed that Dissolved Oxygen shouldn't be much of a concern.

The goal of the trial was for Bio-Zyme use to eliminate or at least help consolidate organic matter in the sediment so that the layers adhere to one another and a false bottom does not form or float after routine draw-downs. Another fundamental goal was Phosphorous reduction from the water column (incorporation of P into an unavailable form), but for the trial period sludge and muck reduction was the main objective.



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Bio-Zyme Bio-Generator and Dose Procedures

A standard Bio-generator was used to prepare the weekly dosage of Bio-Zyme products.

- 500 gallon polyethylene tank
- Equipped with vigorous diffused aeration
- Weekly, AVC would apply the 500 gal. tank mix to the treatment area, refill and repeat.

The 500 gallons of brewed product were sprayed onto the surface of a 25 acre section of the 50 acre evaluation zone. As the STA receives considerable wind and rainfall the expectation was that by applying the Bio-Zyme in a 25 acre section of the 50 acre evaluation zone the product would naturally migrate and influence the entire 50 acres. An airboat was used to apply the 500 weekly gallons of Bio-Zyme in a zig-zag pattern over the 25 acres.

The project kicked off in July of 2016 and continued for 6 months through December, 2016.

Results of Bio-Zyme Treatment Protocol

The following results were compiled by personnel from Aquatic Vegetation Control and sent to the SFWMD project manager:

To: STA Operations, Management Coordinator, SFWMD

Results (Compiled by Elroy Timmer, Biologist, Aquatic Vegetation Control):

- All of the dozens of tussocks and mud islands that existed before treatment are gone even though the water on the staff gauge is down from 11 feet before treatment to 10 feet 3 months after treatment. With the water drop of 12 inches, it's expected that the tussocks should be more visible but instead they are gone.
- It is hard to even identify the area because of the dramatic change in appearance that has occurred.
- A little vegetation remains in a few spots but that vegetation is lying flat on the water, yellow and unhealthy because the mud supporting the plants no longer exists.

- The water is clear, the bottom is visible 8 – 12 below the surface. Prior to the application the visibility was less than 4 inches. Before the water level dropped there was about 1.5 feet of water above the mud. On average there is still about 15 inches of soil below the clear water. In two years with continued treatment we would expect those 15 inches to be reduced to only a few inches.
- Looking east and north outside of the 50 acre plot the view is still unchanged with mud up to the surface. Floating tussocks are the norm. The water flow is obviously moving south and west. The area outside of the plot to the west has responded well to the treatments. All the tussocks and mud islands are gone to the west as far as you can see.
- The airboat easily glides through the plot area, leaving much less of a black cloud in the water. The muck has been reduced and the consolidated bottom is not as loose as it was pre-treatment.

The amount of removal of dead vegetation and organic muck is best seen in the Google Earth photos below, with 'Before' on the left and 'After' Bio-Zyme on the right:



The clear water zones on the right hand side photo show the powerful change created by the Bio-Zyme dosing. The dosing does not injure plants but plants previously supported only by tussocks now lack support and move with the wind and flow. Plants anchored in firm substrate are unaffected.

This area was inspected again in March of 2019, the incidence of the tussocks and mud islands is still very minimal after a full two years. The water clarity is still very good with visibility to the bottom in 1-1.5 feet. There is a visible difference in the fish population and wetland bird activity is abundant.

A special thank you to SFWMD, AVC, Linda, Elroy, and Travis.