(R-9) Utilizing Bacteria Augmentation to Reduce Organic Bottom Sediment and Nutrients v1

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Purpose

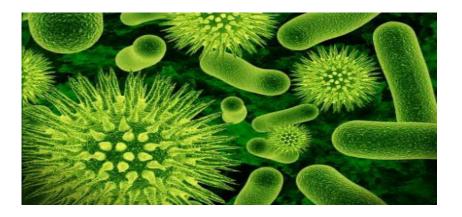
- Provide Background information on Bacterial Augmentation
- Present Observations and Data from: Operational Analyses and Field Trials
- Spark Interest in Bacterial Augmentation as an Integrated Management Component



Bacteria

Bad Actors

- Food Poisoning
- Infectious Diseases
- Forest/Ag Diseases

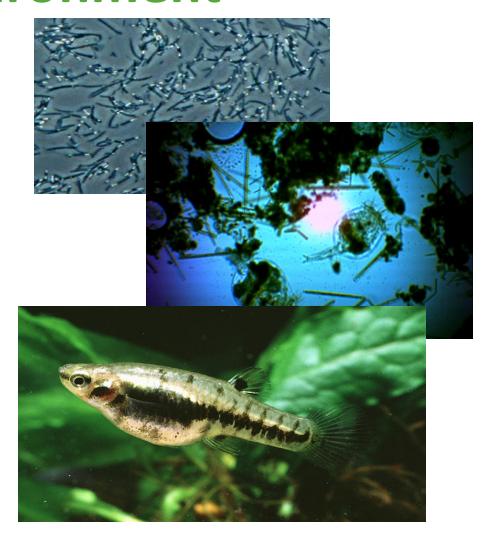


<u>Beneficial Bacteria</u>

- Health Benefits
- Food Production
- Oil Spill Cleanup
- Wastewater Treatment
- Nutrient Cycling
- Decomposition

Bacteria in the Aquatic Environment

- Bacteria is the major "base" of the food chain, from bacteria to protozoa to fish, etc.
- Bacteria conduct critical roles in nutrient utilization of nitrogen (N) and phosphorous (P).
- Bacteria reduce organic matter (sludge) by utilizing carbon compounds.
- Bacteria compete for nutrients.



Product Background: Microbes and a Partial List of Roles.

Nitrosomonas

reduces NH₃ to NO₂

Nitrobacter

- NO₂ to NO₃
- both are soil bacteria; both are required for nitrification and both need aerobic conditions

Aerobacter aerogenes

- aerobic
- oxidizes carbohydrates C+(H₂O) (sugars, starches and cellulose) and short organic acid chains to CO₂ and H₂O
- when O₂ is limited it ferments carbohydrates which become food for *Pseudomonas* sp.
- Bacillus subtilis
 - degrade polymers such as protein, starch, and pectin, therefore, they are thought to be an important contributor to the carbon and nitrogen cycles. Important in digestion in the gut of animals

B. licheniformis

produces a variety of extracellular enzymes that are associated with the cycling of nutrients in nature.

• B. amyloliquefaciens.

- oxidizes carbohydrates, organic acids, fats, oils, proteins and starches
- active in the soil, its enzymes degrade organic material
- denitrifying bacteria (NO₃ to NO₂ to N₂)

Cellulomonas biazotea

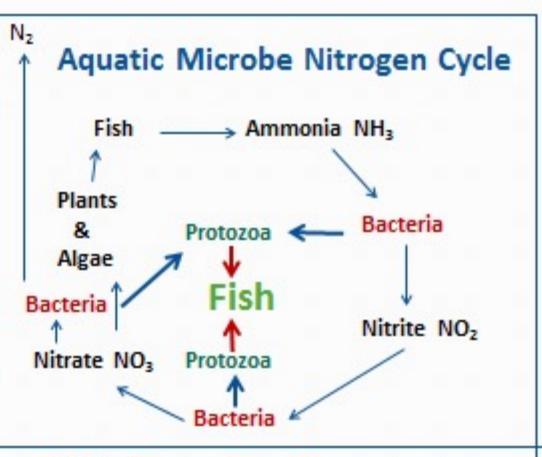
• converts cellulose to soluble carbohydrates which serve for growth of C. biazotea and other bacteria

Pseudomonas denitificans and P. stutzsri

- reduces level of nitrate nitrogen under anaerobic conditions
- oxidizes and degrades organic compounds

Nitrogen Assimilation With Bio-Zyme Bacteria

- Nitrosomonas spp.
 - Ammonia (NH₃) to Nitrite (NO₂)
- Nitrobacter spp.
 - Nitrite (NO₂) to Nitrate (NO₃)
- Pseudomonas spp.
 - Nitrate (NO₃) to Nitrogen Gas (N₂)



Bacteria assimilates N in its various forms converting it to fish through protozoa

Carbon utilization - Known Players

• Aerobacter aerogens - B. amyloliquefaciens

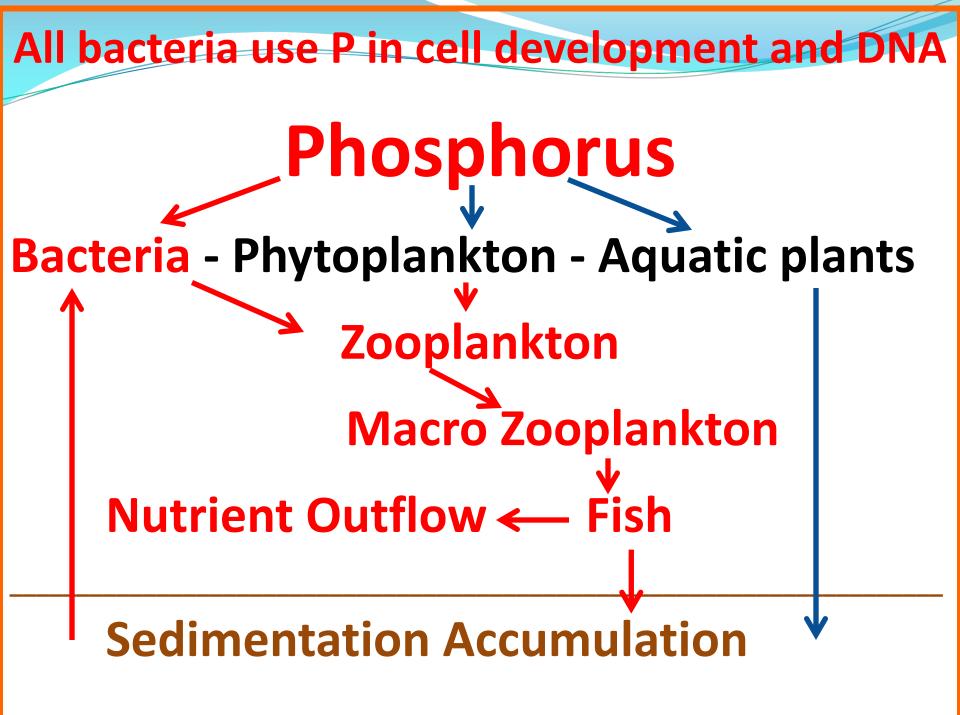
- oxidizes carbohydrates
- Bacillus subtilis
 - degrades polymers such as protein, pectin and starch. It is also a contributor to the carbon and nitrogen cycles.

• Cellulomonas biazotea

converts cellulose to soluble carbohydrates

• Pseudomonas denitificans and P. stutzsri

oxidizes and degrades organic compounds



Nitrogen, Phosphorous and Carbon Utilization

- Bio-Zyme primarily reduces sediment (organic matter) (C) and <u>utilizes C in its metabolism and cell structure</u>.
- This is critical because as Bio-Zyme uses C it must also use N and P. Most living creatures are comprised of approximately 50% C, 14% N and 3% P. These elements are used in cell walls, amino acids, enzymes, energy and essential body components.
- Bio-Zyme carbon utilization therefore provides the "base" of the food chain.
- These bacteria multiply using N, P and C which are transferred from bacteria to protozoa to fish, etc.
- Bio-Zyme only needs to remove about 2 oz. of P from water (0.05 mg/l to 0.005 mg/l per acre foot) for the trophic state index (TSI) of water to be changed from eutrophic to oligotrophic.

Naturally Occurring Bacteria

- Beneficial bacteria populations have naturally maintained aquatic balances, but now are overwhelmed given:
 - exponential nutrient inputs,
 - habitat disruption,
 - oxygen depletion.
- Muck contains phosphorus, nitrogen, carbon and other components
- Bacteria naturally can utilize muck as it occurs in many lakes without excessive nutrients.

Bacteria Aquatic Management Systems

- Science has Identified key microorganisms.
- R&D has resulted in bacteria production methods and delivery systems.
- These are needed due to increased pressures on waterbodies.

- **Benefits:**
 - Increased clarity
 - Reduced odor
 - P and N reduction
 - Potential herbicide reduction because of lower P and N
 - Sediment reduction
 - Increased O₂ from decrease in organic material in water

Bacteria Product Use

- Currently operational for 15 aquatic management companies in FL and the US. This represents
 20,000 managed waterbodies totaling in excess of
 60,000 surface acres.
- Primary use has been golf course waterways, private lakes and municipal drainage networks in Florida.
- Results are reduced nutrients, shifts in sediments from muck to sand, decreased sprinkler clogging, and aesthetic benefits. All have been observed and documented.

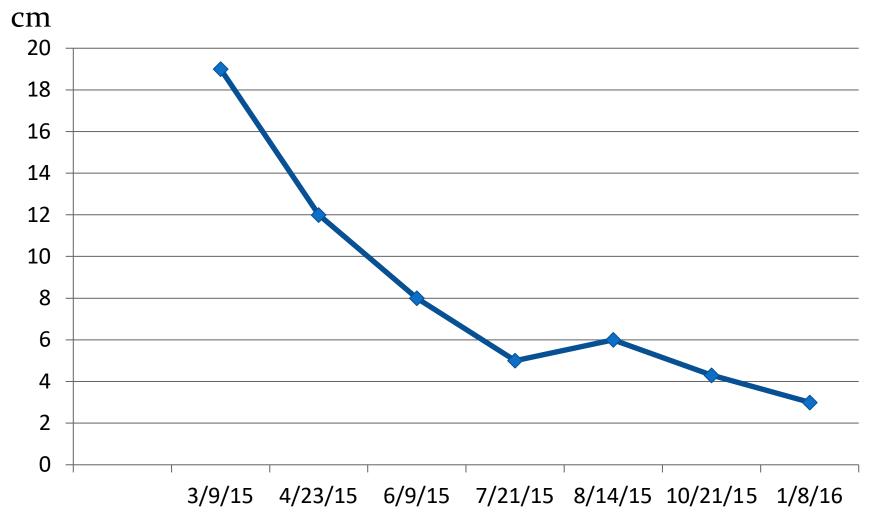
Bio-Incubator - Field Evaluation Methods

- Inoculation Methods
- Bacteria can be incubated in a bio-incubator and distributed directly as living organisms with active enzymes.
 - Temperature can be controlled.
 - Product can be bacteria, food, spores in socks.
 - 4.5 lb. socks are exchanged monthly.

- Monitoring Methods
 - Permanent stations designated.
 - Quarterly sediment depth measurement taken with meter stick/Tube.
 - Depth measured to nearest cm.
 - Muck and Water Sample Analyses.

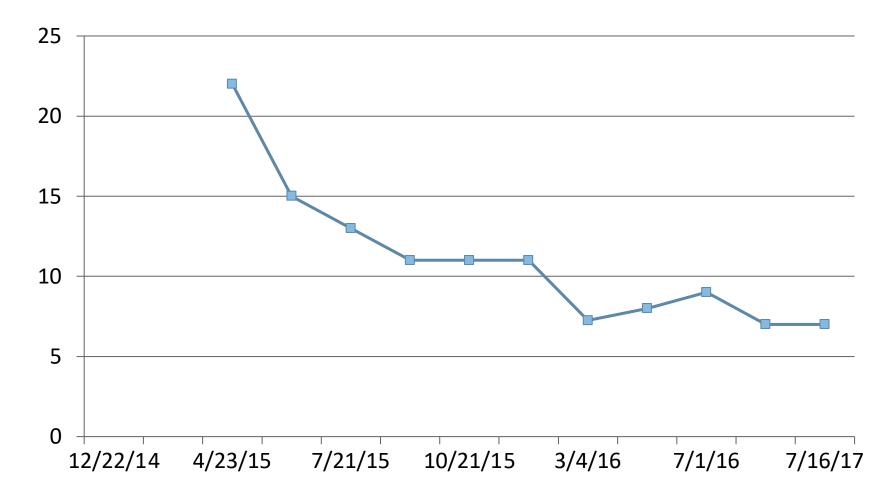
Port Saint Lucie E8 Basin Organic Sediment Removal

(Each point is an average of 3 plots with 4 measurements per plot)



Evaluation-PGA Pond Organic Sediment Removal

cm (Each point is an average of 3 plots with 4 measurements per plot)



Institute of Food and Agricultural Sciences Analytical Services Laborat



Livestock Waste Testing Laboratory, Gainesville, FL

Livestock Waste Testing Lab 631 Wallace Building Gainesville, FL 32622 (352) 392-1950 FAX (352) 392-1960

Typical Muck Analysis

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9007

Livestock Waste Analysis Grower Report

Clarence Elroy Timmer 482 SW Deer Run Port St Lucie, FL 34953

PHONE: 561-249-4628

Lab #	9007
Sample Label	IS #1
Date Collected	April 29, 2016
Date Delivered	May 4, 2016
Date of Report	May 16, 2016
County of Sampl	Palm Beach
Collected By	0

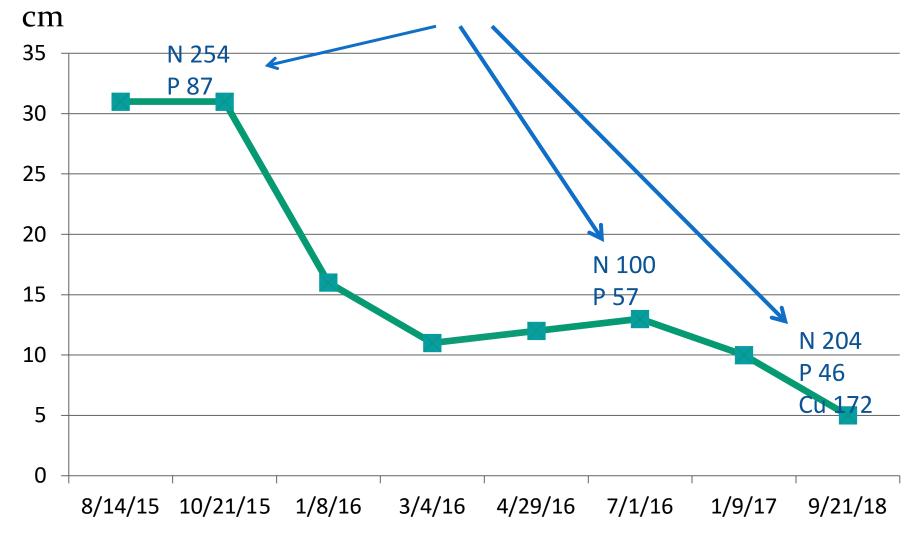
Sample Type: Dairy waste collected from lagoon. Crop or Use: #N/A Application Equipment:Applied through center pivot Incorporation #N/A Previous Applications #N/A

Nutrient Constitue	nt Raw Sample	Adjusted For Application Losses of N	Units	N-raw				188
Nitrogen (N):	188	134	lbs/acre-in	N-adj			134	
Phosphorus (P2O	5) 91	91	lbs/acre-in					
Potassium (K2O): 47	47	lbs/acre-in	P₂O₅		91		
pH as Sa Moisture C	ontent: 78.3%			K2O	47			
	Solids: 21.7% al Ash 18.8%			0	50	100	150	200
						lbs/acre-in		
Number	Sample Id	(Cu mg/kg		Mn mg/l	ĸg	Zn mg	g/kg

Ibis Country Club Lake 10 - Organic Sediment Removal

(Each point is an average of 3 plots, 4 measurements per plot)

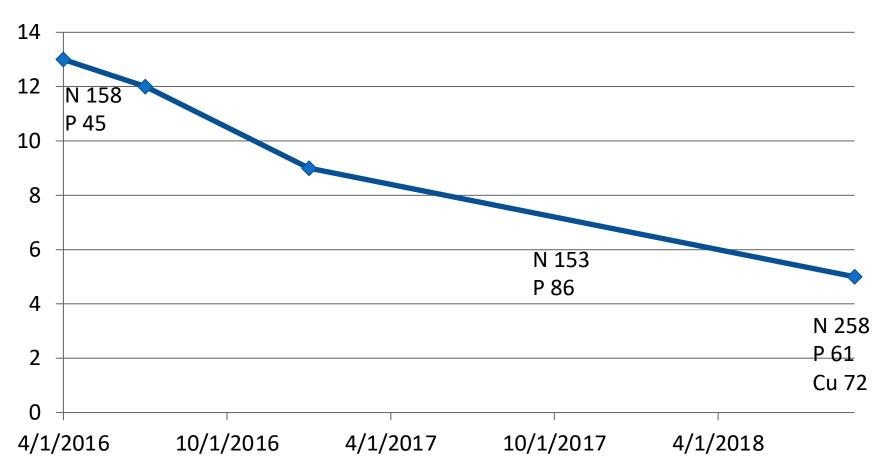
Nitrogen (N), Phosphorus (P) (lb./acre in.) in muck sample



Ibis Country Club Lake 5 – Organic Sediment Removal

(Each point is an average of 3 plots, 4 measurements per plot)

Nitrogen (N), Phosphorus (P) measured in lb./acre in.



CM

Bio-Incubator Installation at City of Port St. Lucie Peacock Lake



PEACOCK LAKE

- Lake (100 acres) 18 foot depth.
- Lake is an enclosed system with no Stormwater Inputs.
- *Microcystis* bloom in December 2016.
- Objective: reduce total phosphorus.
- Weekly treatments of Bio-Zyme using bulk powder solids spread by airboat.
- Currently treated by a 200 gallon bio-incubator releasing every 72 hours as needed.

Peacock Lake 100 acres-

	Date	Bio-Zyme	Laboratory	Total Phosphorous
		Bulk		rate mg/l
	1/9/17			
Total Phosphorus		5 lbs.		
reduced from:	1/18/17		Pace	7 0.05
	1/23/17	5 lbs.		
	1/30/17	5 lbs.		
	2/6/17	50 lbs.		
	2/21/17		Flowers	0.04U
0.05 mg/l - 0 mat	3/27/17	50 lbs.		
_	3/30/17		Jupiter Env.	0.024
0.015 mg/l - 6 mat <0.005 mg/l - 18 mat	4/9/17		Jupiter Env.	0.021
	5/23/17			
	5/31/17	10 lbs.		
	6/8/13	10 lbs.		
	6/13/17			
	6/20/17			
	6/27/17	10 lbs.		
	7/3/17	10 lbs.		4
	7/3/17		Jupiter Env.	0.015
	7/11/18	300 lbs.	Jupiter Env.	× <0.005

20 feet deep

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Peacock Lake

Intake float

6 feet deep





Goal to Improve Water Quality and Reduce the Use of Copper

Clear Waters, Inc.

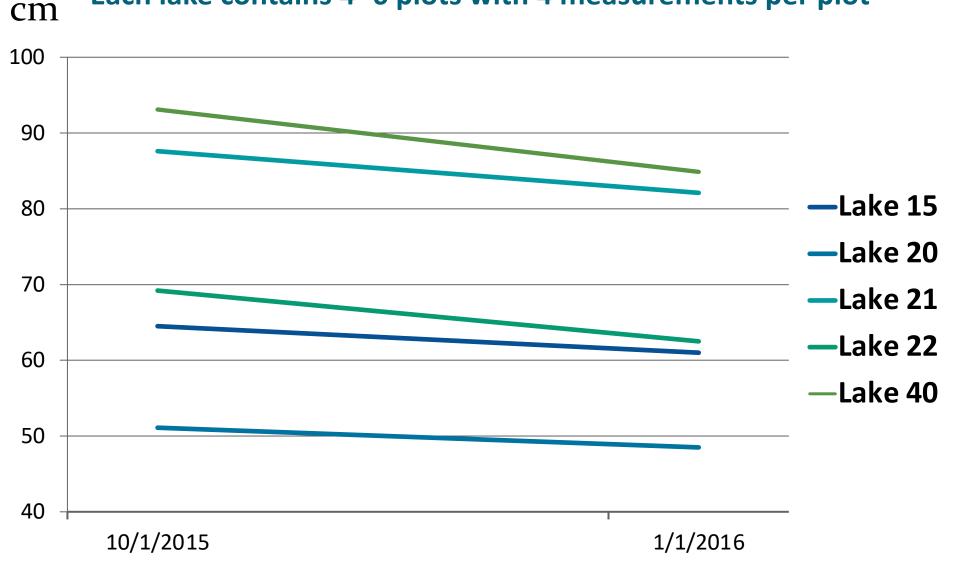


After 6 Months with Bio-Incubator

Players Club POA

Organic Sediment Removal **1.8 cm Per Month** Each lake contains 4- 6 plots with 4 measurements per plot

Clear Waters, Inc.



STAs or Constructed Wetlands Have Been Used for

Years as Key Components of Restoration.

Benefits

- Nutrient Removal
- Water Storage
- Wildlife Habitat
- Research Opportunity
- Recreation Opportunity
- Public Education (PR)
- Urban Greenspace
- Downstream Restoration

Concerns

- Large Land Tracts
- Require Large Capital Investment
- Maintenance Expense
- Accumulate "muck"
- Accumulate Nutrients
- Require periodic drawdowns
- May require periodic mechanical removal of "muck"

South Florida Water Management District Muck Reduction Trial

- AVC performed a muck reduction Field Trial in STA 1 West in 2016-17.
- The STA contained large areas of floating tussocks and waist deep muck.
- Some tussocks were vegetated but most bare mud above the waterline with sparse vegetation.
- The visible water was 1-3 inches deep above the muck.



STA Field Trial and Results

- Treatment prescription was 500 gallons of incubated Bio-Zyme (12.5 lbs. + 2.5 gal, Nitrifier) per week via airboat.
- Each week a 25 acre plot within the 50 acre trial area was treated.
- Duration was 9 months July to March of 2017.
- The initial area impacted by the trial appears to encompass over 350 acres. Later the result spread to about 800 acres.
- Most of the area still remains tussock free with reduced muck 3 years after treatment

Floating Tussocks Before Treatment



Prior to initial application Mud to the surface

March 2017 – After Treatment



March 2017 (After Treatment)



Summary; Supported Observations

- Decreased depth of organic sediment layer.
- Increased water clarity and light penetration.
- Elimination of floating tussocks
- The bottom sediments became consolidated and firm compared to mush and soup.
- More fish were observed

In Closing: The Potential is Exciting! Innovation - Repurposing Known Technology

- Lake Okeechobee
 - Flocculent Management.
 - Organic Sediment and Nutrient especially P. Reduction.
 - Fish Spawning Area Restoration.
 - Increase Light Penetration to Promote SUV Growth.
 - Aquatic Plant Management Reduction of decaying plant material from treatment.

In Closing: The Potential is Exciting! Innovation - Repurposing Known Technology

- STAs
 - Organic Sediment Reduction/Consolidation.
 - Extend Cell Life Span and Reduce Management Costs.
 - Nutrient reduction
- Private Land Discharges
 - Pretreatment Option.
- Lagoons City Water Lakes
 - Reduce Lake Discharge Nutrients that Feed Toxic Algae.

Acknowledgements

• Aquatic Vegetation Control. Inc.

Elroy Timmer, Senior Scientist	60 yrs.	561-248-4628
Linda DeFee	10 yrs.	561-845-5525
 www.avcaquatic.com 		
Aquatic Balance		
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 www.aquaticbalance.net 		
Clear Waters Inc.		
Trace Wolfe, President	45 yrs.	386-767-4928
 www.clearwaterslakemamt.com 		

Large data set and 1000's of sites are available for inspection

Thank You.

- TRM, Inc.
- trmbiozyme.com
- Martin County
- NPBCID
- Port St. Lucie
- SFWMD

