Lyngbya Treatment with AquaSticker

Efficacy Testing in 2023

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Study Summary

- AquaSticker alone did not damage *Lyngbya* cells or filaments throughout the test

- All algaecide treatment combinations with AquaSticker showed more bacterial growth and more damage on *Lyngbya* cells and filaments than algaecide treatment combinations without AquaSticker

- All algaecide treatment combinations with AquaSticker showed greater suppression / degradation of *Lyngbya* than treatment combinations without AquaSticker

- Treatments containing AquaSticker alone and Cutrine Plus alone resulted in increased *Lyngbya* growth at the end of the testing

- The combination of AquaSticker + Cutrine Plus + Hydrothol 191 showed over 10x more suppression / degradation of *Lyngbya* over Cutrine Plus + Hydrothol 191

- The combination of AquaSticker + Cutrine Plus showed 3x better suppression / degradation of *Lyngbya* over Cutrine Plus + Hydrothol 191

- Cutrine Plus alone was determined to be ineffective at suppressing / damaging *Lyngbya* in this test

- Treatments containing Cutrine Plus alone resulted in a 200% increase in *Lyngbya* growth

- A combination of Cutrine Plus + Hydrothol was determined to have minimal effectiveness at suppressing / damaging *Lyngbya* in this test

AquaSticker + Cutrine Plus increased *Lyngbya* degradation by over

300%

while the combination of AquaSticker + Cutrine Plus + Hydrothol 191 increased *Lyngbya* degradation by over

1,000%

Objective

Show that AquaSticker added to an algaecide tank mix is more effective on *Lyngbya* than the algaecide tank mix alone.

Methods

9oz clear plastic cups were labeled and mass was recorded. The preferred media for *Lyngbya* grown in the lab was used. This is BG11 N+ diluted five-fold in dechlorinated tap water with 0.2% vitamin mix added. The cups were filled with 200ml each of the *Lyngbya* media. Pieces of *Lyngbya* were blotted on paper towels to remove excess water. 0.100g±0.003g of black *Lyngbya* was added to each cup. The mass of the cups with media and algae were recorded.

Products were added to 1 ml of water removed from the cup with the corresponding number and resuspended five times to mix. The mixtures were then added to the corresponding cup in a random dropwise manner across the cup surface. The cups were photographed and then placed on a Vivosun heat mat controlled by a Vivosun controller set to 25°C.

The tops of the cups were about 14 inches under the T4 cool fluorescent (6w) grow light. The lights were on a 8h:16h light:dark cycle. To prevent excessive evaporation, the cups were covered loosely with plastic wrap. Every day for the 10 day test period, the cups were uncovered, photographed, and 20ml of media was exchanged with fresh media.

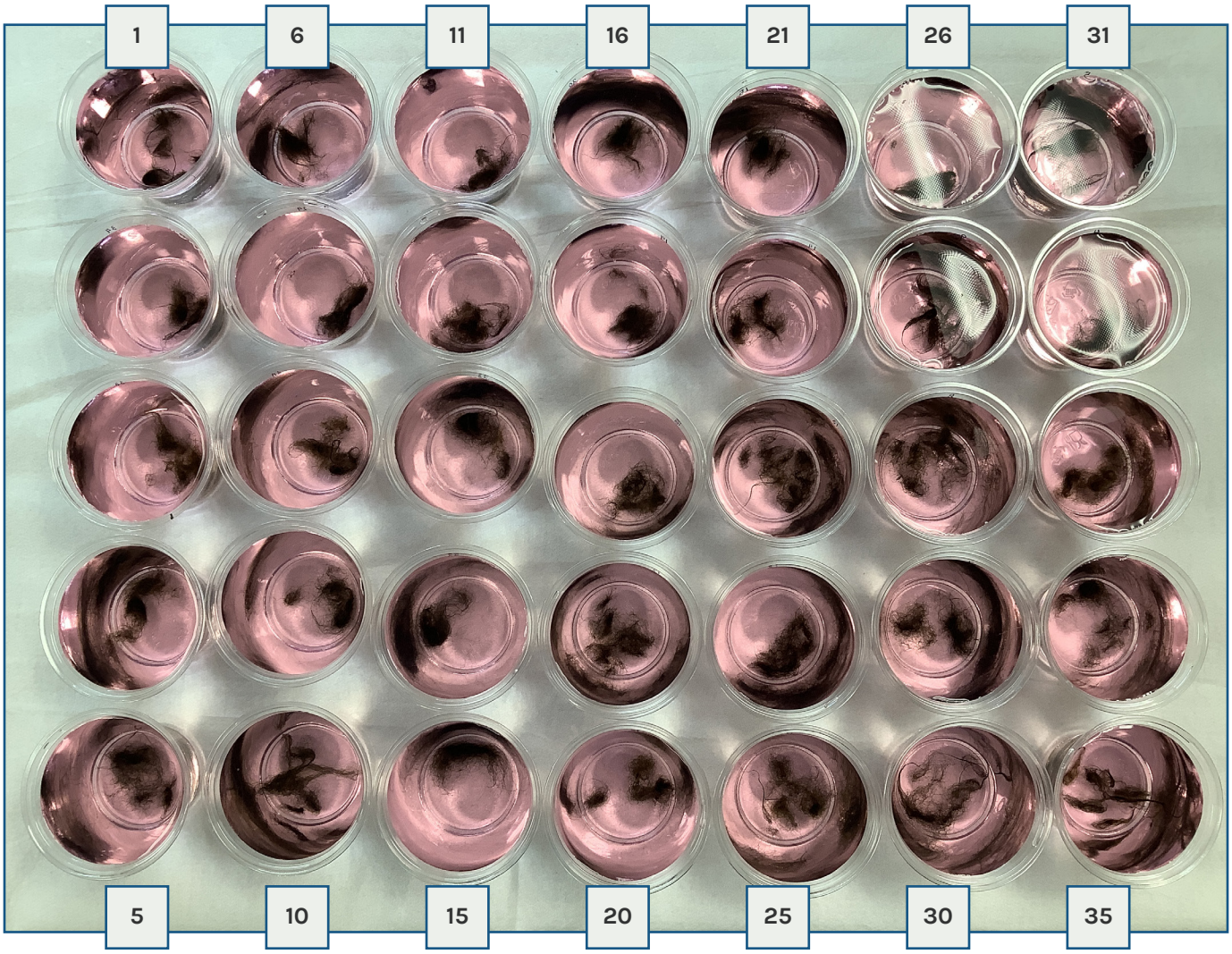
At the end of the test period, the cups were drained of most of the media and placed uncovered back on the mat. The liquid in the cups was evaporated for five days before the final mass of the cups containing dry algae was recorded. Dry algal biomass was determined by subtracting the final cup and algal mass from a sacrificial set prepared initially.

Treatment Table

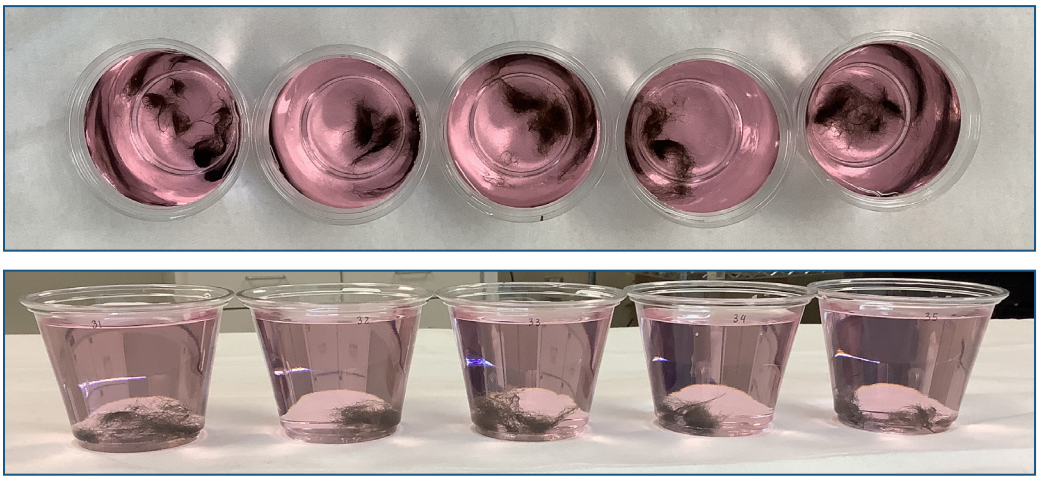
Cup #	Treatment
1-5	AquaSticker (20 lbs/acre)
6-10	Control (1 mL media added)
11-15	Cutrine Plus (3 gal/AF)
16-20	AquaSticker (20 lbs/acre) + Cutrine Plus (3 gal/AF)
21-25	Cutrine Plus (3 gal/AF) + Hydrothol 191 (0.2 ppm)
26-30	AquaSticker (20 lbs/acre) + Cutrine Plus (3 gal/AF) + Hydrothol 191 (0.2 ppm)
31-35	Control – initial sacrifice

Table shows the products used to treat Lyngbya algae. AquaSticker was applied at max label rate (20 lbs/acre), Cutrine Plus was also applied at max label rate (3 gal/AF = 1.0ppm as Cu), and Hydrothol 191 was applied at a low rate (0.2ppm). It is known that Hydrothol 191 alone is not used to treat algae, so Hydrothol 191 was only tested in combination with other products.

Results

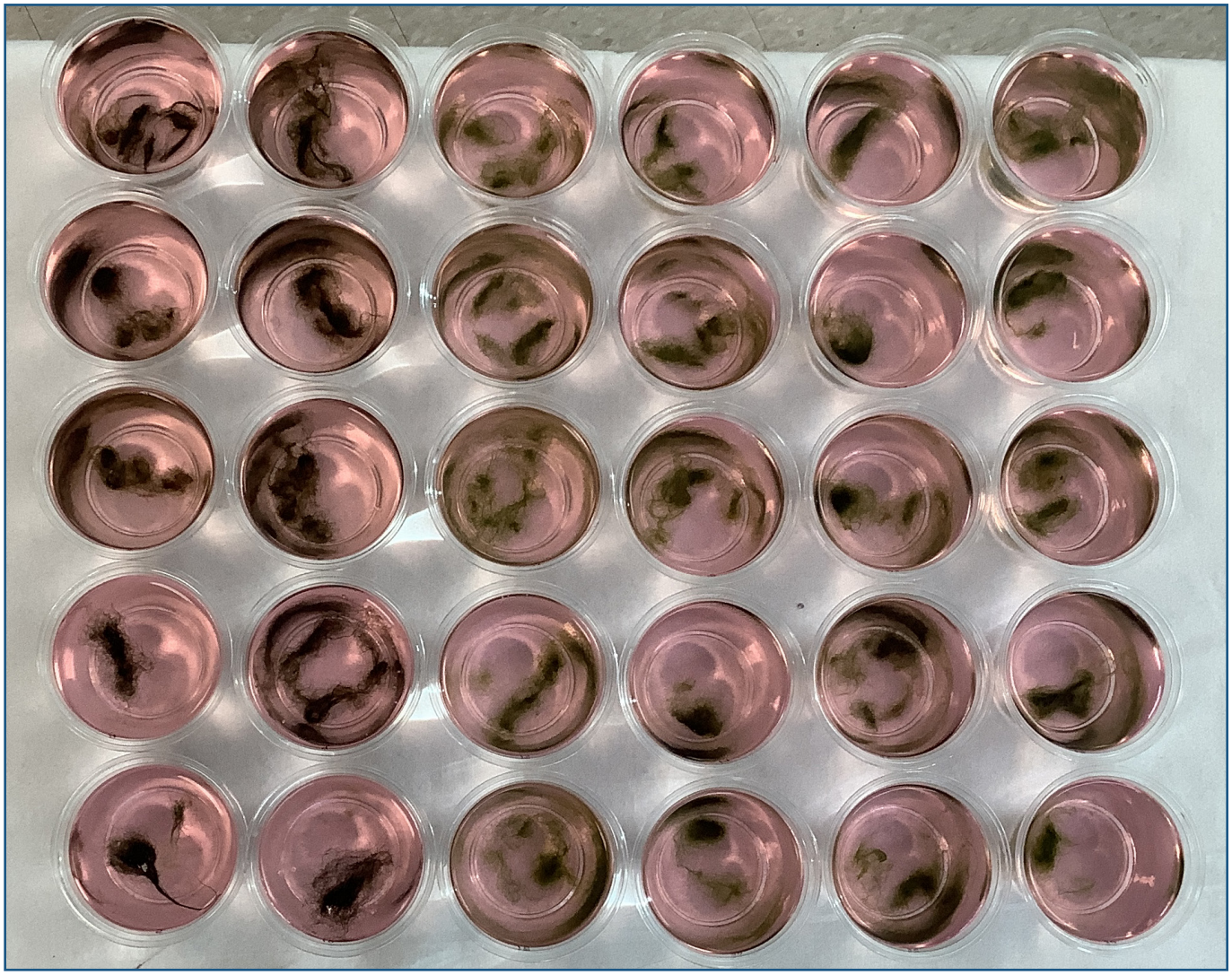


Starting in the upper left and going down are cups one through five. The next column to the right has cup six at the top going down to cup 10. The rest of the columns are similarly orientated. The treatments in columns are: left most AquaSticker (1-5), then next to the right is the column of control cups (6-10). The column to the right of the control cups are treated with Cutrine Plus (11-15). The next column to the right are treated with AquaSticker and Cutrine Plus (16-20). The next column to the right are treated with Cutrine Plus and Hydrothol 191 (21-25). The next column of cups to the right are treated with AquaSticker, Cutrine Plus, and Hydrothol 191 (26-30). The right-most column of cups are the controls that were sacrificed immediately after the cups were treated (31-35).

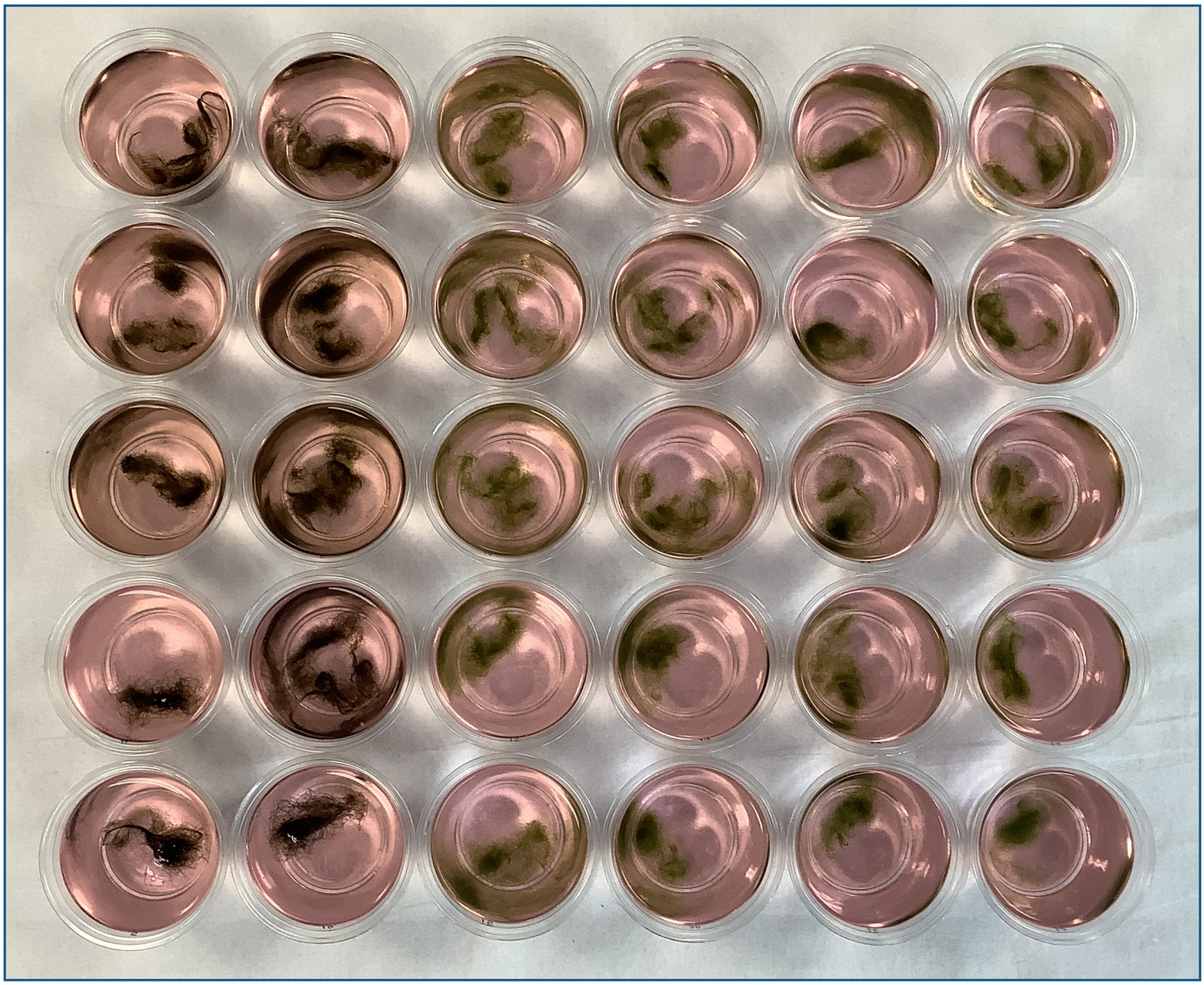


LEFT: The five cups that were sacrificed immediately after the treatments were applied to the cups (31-35).

They were set up the same as the other cups and had one milliliter of media applied before the cups were drained and set to dry.



By **day three** there was a noticable difference between the cups with the AquaSticker and control cups containing black colored algae while the other treated cups had green colored algae.



By the end of the test there was a noticeable contrast between the color of the algae in the cups. The cups treated with AquaSticker and the control cups contained algae that was black in color. The rest of the treated cups contained algae that was a light green color. Generally, algae becoming more pale in color indicates that the algae are dying or dead.

AquaSticker – Some growth of bacteria on healthy looking *Lyngbya* filaments

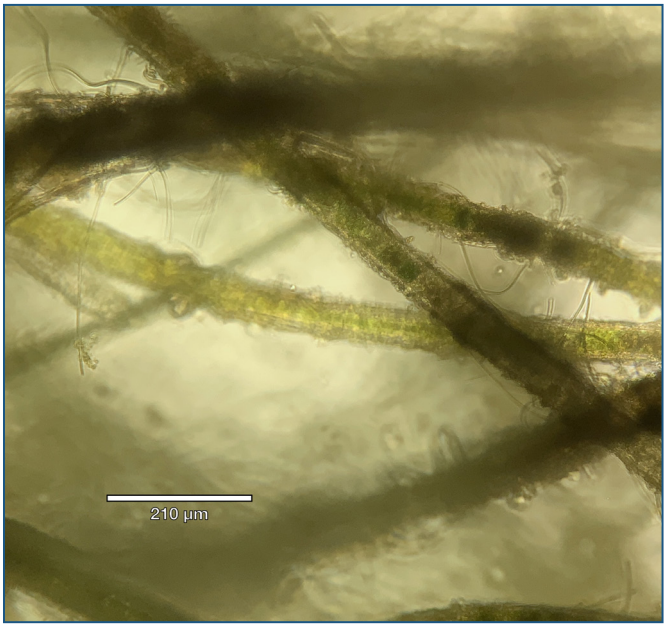


Figure 1. Test 3_0 showing increase in filamentous bacteria on *Lyngbya* filament.

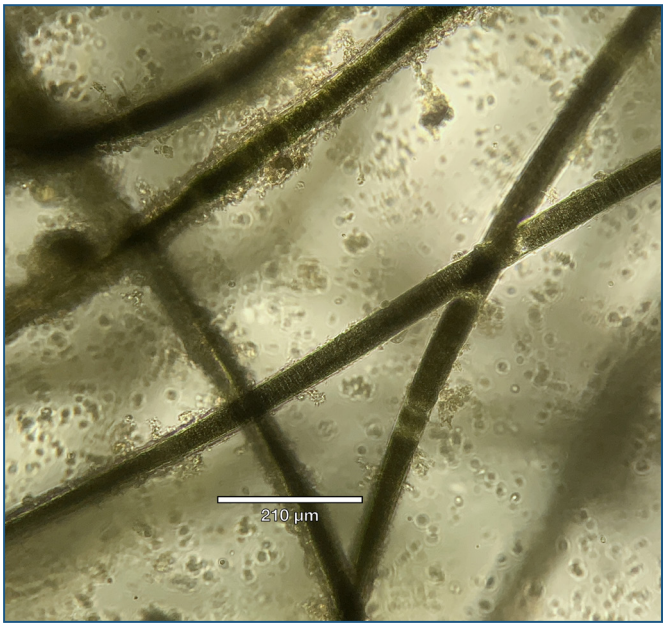


Figure 2. Test 4_1 showing slight increase in bacteria on *Lyngbya* filament.

Control – Healthy *Lyngbya* filaments with low growths on bacteria on the filaments

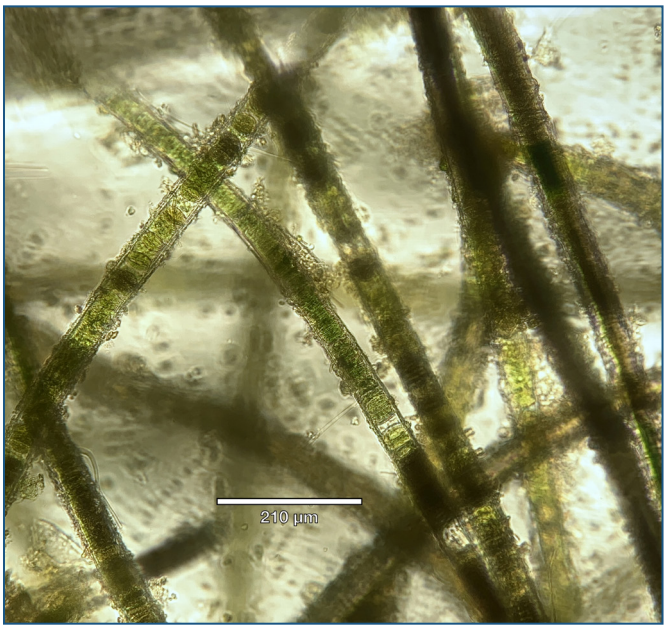


Figure 3. Test 8_2 showing slight bacteria on *Lyngbya* filament.

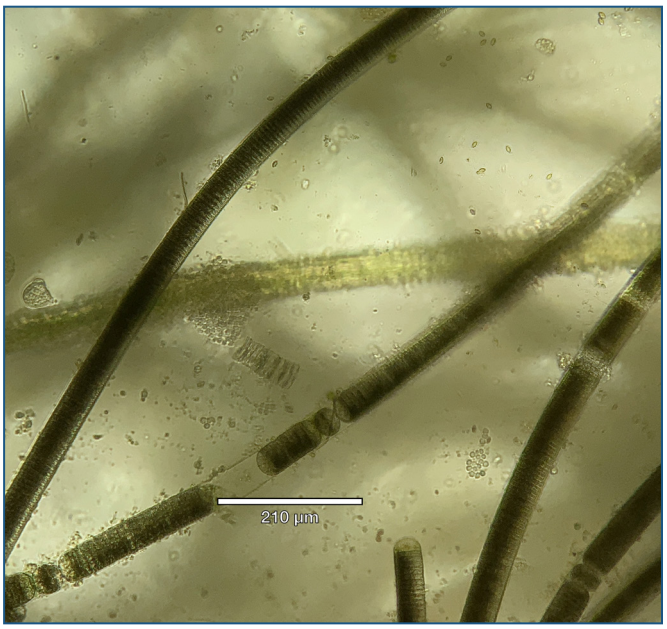


Figure 4. Test 9_2 showing slight bacteria on *Lyngbya* filament.

Cutrine Plus – Damaged cells, but mostly intact filaments

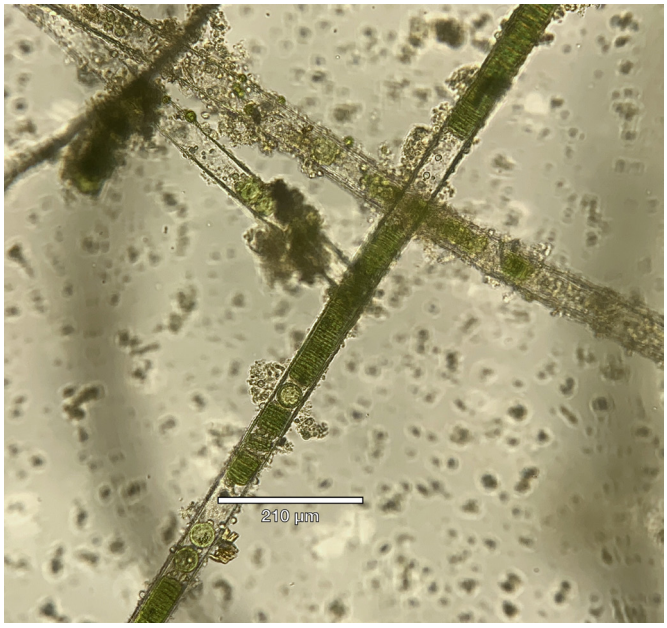


Figure 5. Test 11_2 showing slight increase of bacteria on *Lyngbya* filament and damaged cells.

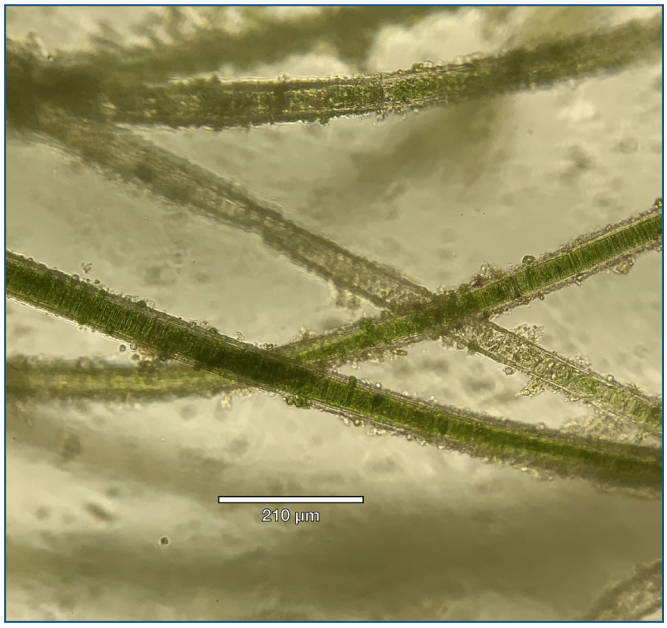


Figure 6. Test 11_3 showing slight increase of bacteria on *Lyngbya* filament and light colored cells.

AquaSticker + Cutrine Plus – Damaged cells and noticeable bacterial growth along the outside of the *Lyngbya* filaments

Figure 7. Test 16_3 showing increase of bacteria on *Lyngbya* filament and damaged cells.

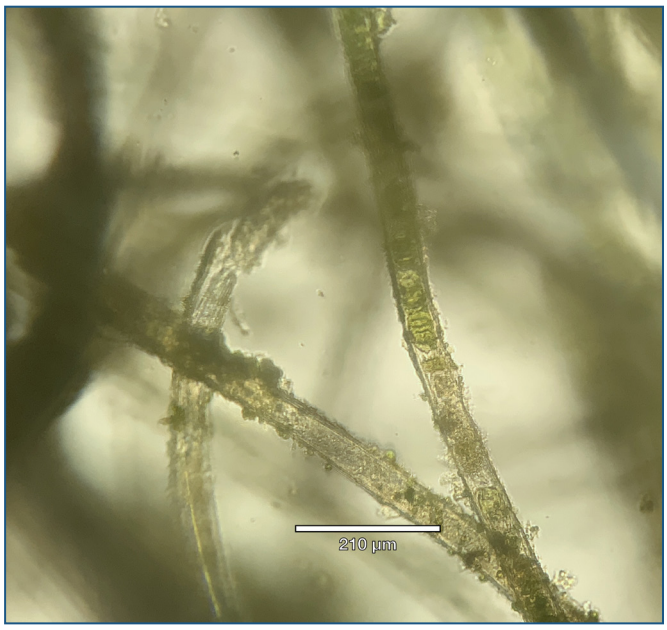


Figure 8. Test 18_1 showing increase of bacteria on *Lyngbya* filament and damaged cells.

AquaSticker + Cutrine Plus – Damaged cells and noticeable bacterial growth along the outside of the *Lyngbya* filaments

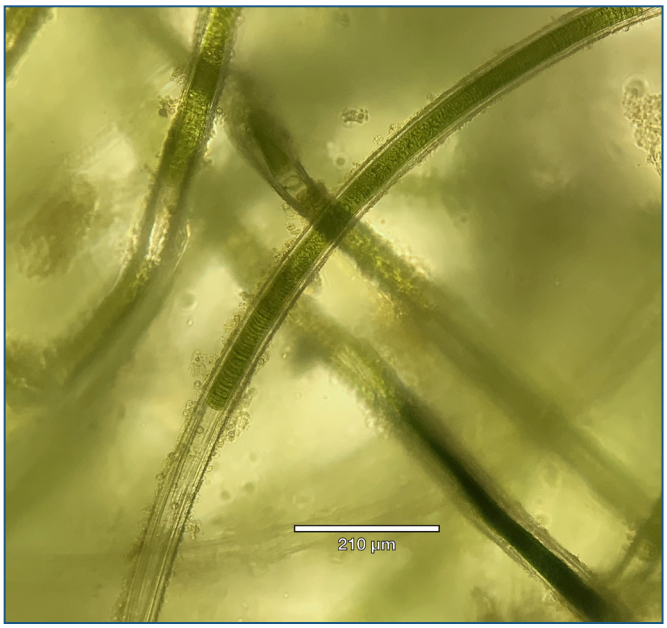


Figure 9. Test 20_0 showing increase of bacteria on *Lyngbya* filament and slightly damaged filaments.

Cutrine Plus + Hydrothol 191 – Damaged cells, similar to Cutrine Plus alone

Figure 10. Test 21_0 showing slight increase of bacteria on *Lyngbya* filament.

Figure 11. Test 24_1 showing slight increase of bacteria on *Lyngbya* filament and slightly damaged filaments.

AquaSticker + Cutrine Plus + Hydrothol 191 - There was visible microscopic damage to the *Lyngbya* filaments in the AquaSticker + Cutrine Plus + Hydrothol 191 treated cups

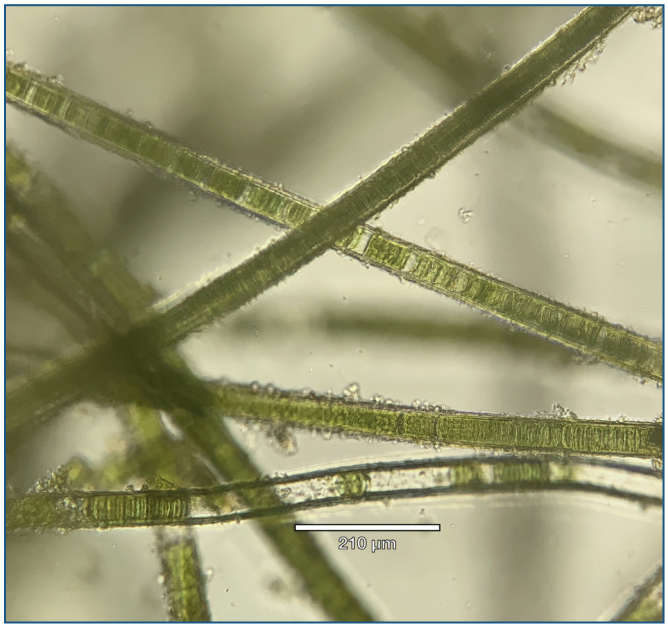


Figure 12. Test 26_3 showing increase of bacteria on Lyngbya filament and slightly damaged cells and filaments.

Figure 13. Test 27_2 showing increase of bacteria on Lyngbya filament and slightly damaged cells and filaments.

Figure 14. Test 27_3 showing increase of bacteria on *Lyngbya* filament and slightly damaged cells and filaments.

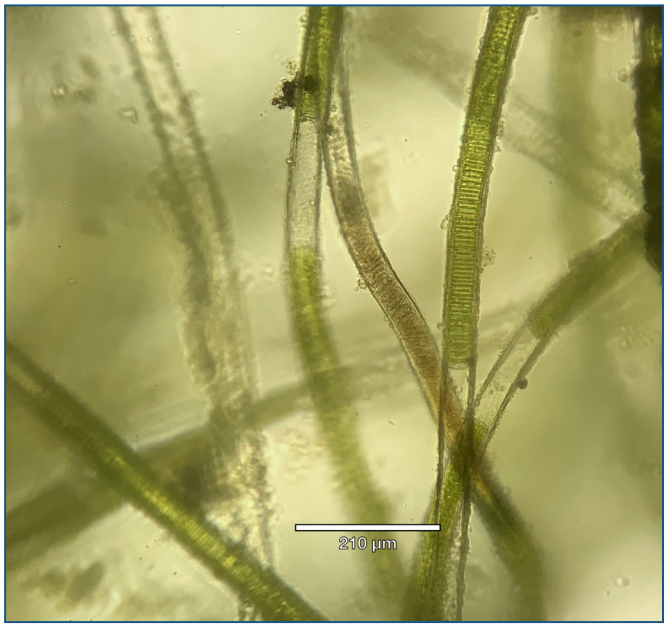
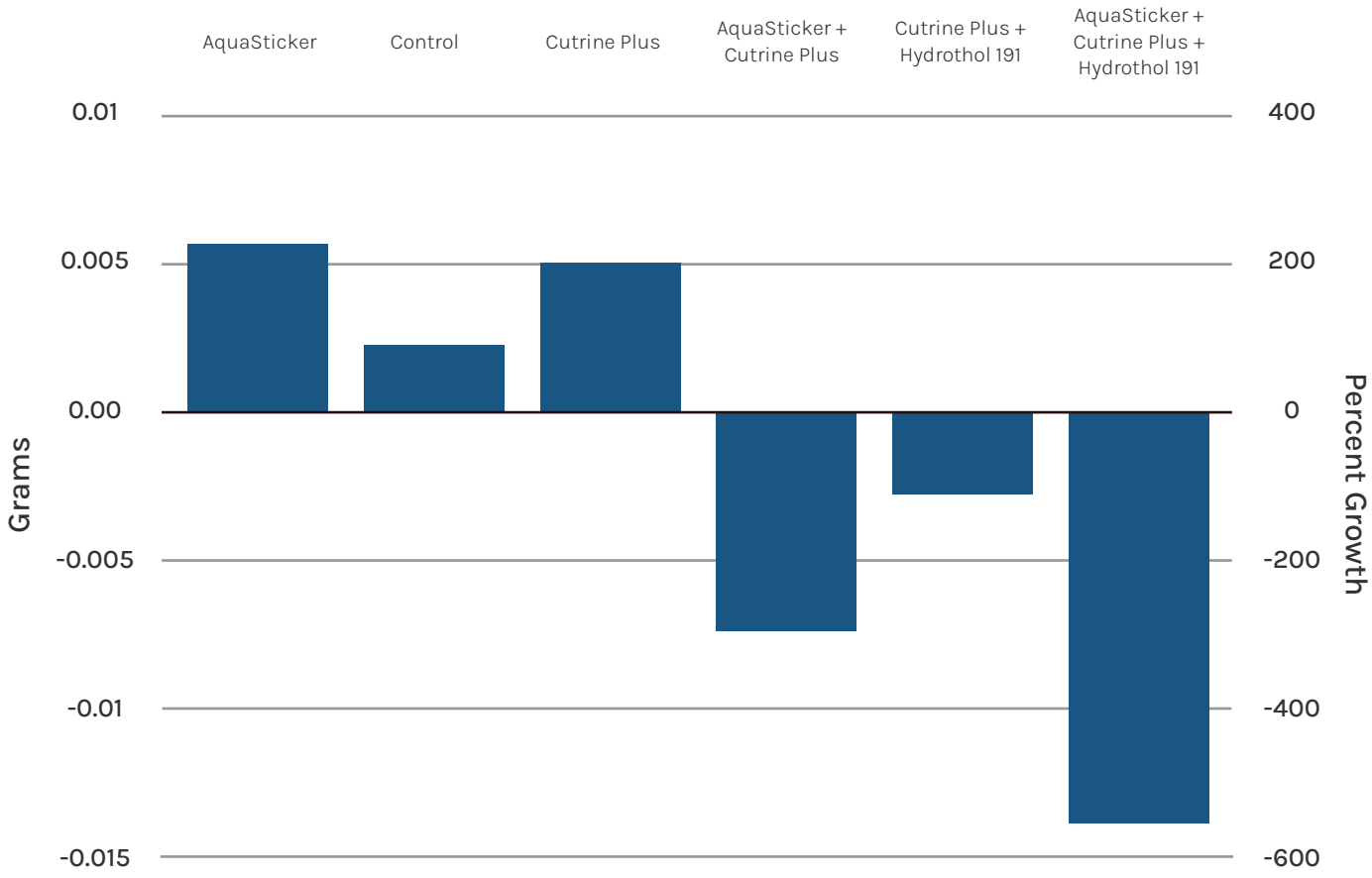


Figure 15. Test 29_2 showing increase of bacteria on *Lyngbya* filament and slightly damaged cells.

AquaSticker + Cutrine Plus – Damaged cells and noticeable bacterial growth along the outside of the *Lyngbya* filaments

Figure 16. Test 30_3 showing increase of bacteria on *Lyngbya* filament and slightly damaged cells and filaments.

Dry Weight Over Initial and Percent Growth Over Control



Dry weights over initial sacrificed control cups (left labels) show that the control cups and those treated with AquaSticker or Cutrine Plus had some growth over the initial amount of algae added. The control cup should have grown during the nine days of the test and did as shown in the chart. The cups treated with AquaSticker alone and Cutrine Plus alone both had *Lyngbya* growth that was twice as much as the growth of the control cups over the course of the test. The cups treated with AquaSticker and Cutrine Plus showed that the algae did not grow and may have begun to degrade over the course of the test. Cutrine Plus combined with Hydrothol 191 showed some suppression of *Lyngbya* growth, however, this was a minor suppression compared to AquaSticker combined with Cutrine Plus. The best suppression/degradation of *Lyngbya* was in the cups treated with a combination of AquaSticker, Cutrine Plus, and Hydrothol 191. This combination was about twice as effective as AquaSticker combined with Cutrine Plus without Hydrothol 191.

Discussion:

Lyngbya is a benthic filamentous cyanobacterium that is difficult to control. This is due to the thick sheath surrounding the filaments as well as the attached growth of some diatoms in certain environments. Both the thick sheath and presence of attached epiphytic algae, such as diatoms, act as physical barriers to chemical control agents protecting the cells from damage and from up taking inhibiting metals, such as copper ions. Most of the fastest control options focus on chemical herbicides/algaecides. For example, treating an area of *Lyngbya* first with a peroxide algaecide and then coming back an hour later and applying a copper algaecide. This takes more time than just applying one algaecide and it also increases the risk of releasing toxins from the cyanobacterial cells. A lot of literature recommends raking *Lyngbya* mats out of the pond to counter the risk of released toxins, however, this is physically intensive and takes a lot of time. This method also needs to be repeated frequently to keep controlling new growth. Adding a compound to provide better treatment that can be applied as a tank mix and kills *Lyngbya* without releasing toxins and also decreases growth would be more effective than currently available management methods.

AquaSticker is a biocatalysts that we developed to aid in herbicide/algaecide treatments on plants/algae. It is a dry product that can be added into a tank mix to increase the effectiveness of the herbicide/algaecide mix on the target organisms. Some ways this may occur is: 1) through increasing the viscosity of the mix to keep the aqueous chemicals near the target organism(s), 2) increase the rate at which the chemicals get into the target cells, 3) increase damage on already weakened/damaged areas of filamentous algae or on plant structures, or 4) alter the biofilm on the surface of algae/plants and make it less synergistic with the host. In this test, we found that adding AquaSticker (20 lbs/acre) in combination with Cutrine Plus (3 gal/AF) and Hydrothol 191 (0.2ppm) resulted in *Lyngbya* dry mass that was lower than the initial dry mass at the start of the test. This indicates that this treatment prevented *Lyngbya* growth over the nine days of the test and also may have resulted in degradation of some of the algal biomass, which would result in lower ending dry mass compared to the initial dry mass.
The combination of AquaSticker, Cutrine Plus, and Hydrothol 191 has been previously recommend to applicators in the field. The use of just AquaSticker combined with Cutrine Plus has also been used in the field and can be effective with higher efficacy in soft water. It is known that Hydrothol 191 is seldom used in the field alone to treat algae, and so was not tested alone here, but can sometimes provide an increase in treatment efficacy on some types of algae. In areas with lower *Lyngbya* biomass than the 0.1g used in this test, there may be an increased thickness of the bacterial biofilm coating the filaments. This alone could be detrimental to the algae since it can inhibit the exchange of nutrients with the algae and also algal waste products with the environment. Although toxin release was not tested here, increasing the bacteria-induced degradation of the dead/damaged *Lyngbya* filament has the potential to lower the release of toxins, since degradation would occur slower than cell lysis that is seen with peroxide algaecides and you have the potential to recruit bacterial and fungal species that can detoxify any release toxins. In areas with thick mats and high biomass, there will be a need for repeat treatments which is the same as with any algaecide treatment. Adding products containing bacteria, such as Temperature Driven Solutions, after the tank mix treatment could help to improve bacterial degradation of *Lyngbya* which could help to delay time needed between treatments as the bacteria would leave less available nutrients for the undamaged *Lyngbya* to regrow.

Conclusions:

Lyngbya is a difficult to control filamentous cyanobacteria. This study shows that combining commonly used pesticides with the Naturalake Biosciences' biocatalyst AquaSticker can improve efficacy. Based on this testing, the combination of AquaSticker (20 lbs/acre), Cutrine Plus (3 gal/AF), and Hydrothol 191 (0.2 ppm) sprayed over the *Lyngbya* was the most effective combination at suppressing and degrading the algal cells. The combination of AquaSticker (20 lbs/acre) and Cutrine Plus (3 gal/AF) was also observed to be a more effective treatment on *Lyngbya* than using combinations without AquaSticker. It is important to note this study shows that AquaSticker has no algacidal effect alone and did not damage *Lyngbya* cells when used alone.

Notes

Study crafted and completed by

Naturalake Biosciences