

Utilizing *Spirogyra grevilleana* as a Phytoremediatory Agent for Reduction of Limnetic Nutrients and *Escherichia coli* Concentrations

Malcolm Barnard^{1,1}, James W. Porter^{2,2}, and Susan Wilde^{3,3}

¹University of North Carolina at Chapel Hill Marine Sciences

²University of Georgia Odum School of Ecology

³University of Georgia Warnell School of Forestry and Natural Resources

November 30, 2022

Abstract

The freshwater alga *Spirogyra grevilleana* was used in an experimental biofiltration system to reduce levels of *Escherichia coli*, nitrates, and phosphates. Water collected from a 2.32 ha lake in Atlanta, Georgia, USA was pumped at a constant rate (m³ hr⁻¹) through the algal filtration devices with low and high concentrations of *S. grevilleana*. Effluent water was tested over time for *E. coli*, nitrate, phosphate, dissolved oxygen, and pH levels. Both concentrations of *S. grevilleana* reduced *E. coli* by 100% and significantly reduced nitrate concentrations (30% ± 13%) and phosphate concentrations (23% ± 5%) while maintaining dissolved oxygen and pH at normal levels. Utilizing *S. grevilleana* in an algal filtration device could potentially provide a sustainable, flexible, and low-cost method of *E. coli* reduction in freshwater lakes worldwide. Initial results indicate that the use of *S. grevilleana* in conjunction with an algal filtration device is potentially capable of creating potable water.

Utilizing *Spirogyra grevilleana* as a Phytoremediatory Agent for Reduction of Limnetic Nutrients and *Escherichia coli* Concentrations

Malcolm A. Barnard (malcolm.a.barnard@gmail.com)^{1,2}, James W. Porter², and Susan B. Wilde²

¹University of North Carolina, Chapel Hill, North Carolina, USA and ²University of Georgia, Athens, Georgia, USA

Introduction

One of the most widespread global issues of the twenty-first century is scarcity of clean drinking water. Lakes and rivers worldwide are becoming contaminated by chemical and biological pollutants, such as *Escherichia coli*, thus decreasing potability of the water. Overuse of fertilizers and subsequent runoff have led to increased *E. coli* and nutrient levels in many lakes and rivers worldwide; consequently, these sources are highly polluted by increased concentrations of *E. coli*, nitrates, and phosphates. Implementing sustainable and affordable methods of bioremediation and phytoremediation is critical to providing clean drinking water to global communities.

This study uses *Spirogyra grevilleana*, a species of filamentous green algae, as a potential algal filter to naturally decrease the effects of *E. coli* and dissolved nutrients in freshwaters. There are over 400 species in the genus *Spirogyra* worldwide, making this genus ideal for global applications. *Spirogyra* spp. reduce bacterial levels of *E. coli* and other aquatic bacteria by reducing nutrient levels needed to sustain bacterial populations, and also by secreting antibacterial secondary metabolites into the water.

Based on prior experimentation and research, *S. grevilleana* has been shown to improve water quality in freshwater lakes. The construction of an Algal Filtration Device containing *S. grevilleana* as an algal filter could provide a nonchemical and sustainable solution to increased *E. coli*, nitrate, and phosphate levels.

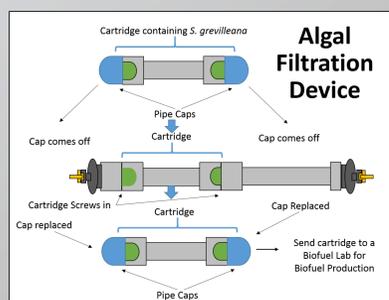
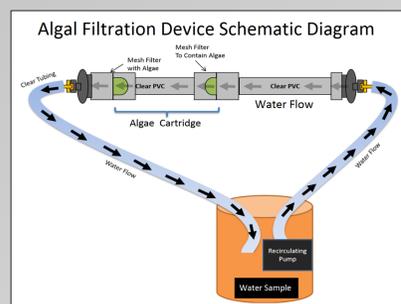


Figure 1: The Algal Filtration Device. Algal Filtration Device was constructed of clear pipe and common plumbing fittings. The device utilizes a removable cartridge with internal filters on both ends to contain the algae from 900 mL of concentrated *S. grevilleana* culture. The cartridge ends are capped prior to use. The caps are removed and the cartridge is screwed into the Algal Filtration Device. The algal cartridge can be replaced as needed and residual algae sent to a lab for biofuel production.

Figure 2: Algal Filtration Device Methodology. The Algal Filtration Device operates by pumping water through the algal cartridge using a recirculating pump and tubing. When the device is activated, the circulating water causes the algae to form an algal mat on the filter inside the cartridge. Repetitive and continuous filtration of the water through the algal mat kills *E. coli* and filters out nitrogen and phosphorus.



Affiliated Citations

- Barnard, M.A., Porter, J.W. and Wilde, S.B. (2017) Utilizing *Spirogyra grevilleana* as a phytoremediatory agent for reduction of limnetic nutrients and *Escherichia coli* concentrations. *Amer. Jour. Plant Sci.* 8:1148-1158. doi: 10.4236/ajps.2017.85075
- Barnard, M.A. 2014. Algal system for improving water quality. United States Patent Application US/2016/0122217. Patent Pending.
- Barnard, M.A. 2014. Algal system for improving water quality. Canadian Patent Application CA/2951817. Patent Pending.

Methodology

16.0 ± 0.1 L samples of water were collected from a 2.32 ha freshwater lake in Atlanta, Georgia, USA. The baseline dissolved oxygen was measured using a PASCO dissolved oxygen meter (n=1) that was calibrated with the Winkler Method. The baseline pH was measured using a Hach pH meter (n=1) and the nitrate-nitrogen and phosphate levels were measured by a Hach handheld DR890 photometric colorimeter (n=3). The baseline *E. coli* levels were measured using a plate method (n=3) including incubation for 48 hours at 35.0 ± 1.0°C, as detailed in US EPA Method 1604. Each sample was outfitted with a pump and an Algal Filtration Device. Lighting was provided 16 hr d⁻¹ to simulate sunlight in the laboratory setting. The pumps were activated and run continuously throughout the experiment. The samples were monitored over three weeks with *E. coli* tested twice per week and the other indicators tested three times per week using the same experimental methods as the baseline testing.

Results

Percent Change from Baseline

Parameter	Percent Change
<i>E. coli</i>	- 100%
Nitrates	- 23.3%
Phosphates	- 29.8%
Dissolved Oxygen	- 10.9%
pH	+ 19.6%

Figure 3: Percent Change from Baseline. Overall, the algal filtration device utilizing *S. grevilleana* catalyzed a full reduction of *E. coli* levels by 100%, as well as decreased the levels of nitrates by 23.3% and phosphates by 29.8%. Dissolved oxygen and pH were maintained at safe levels.

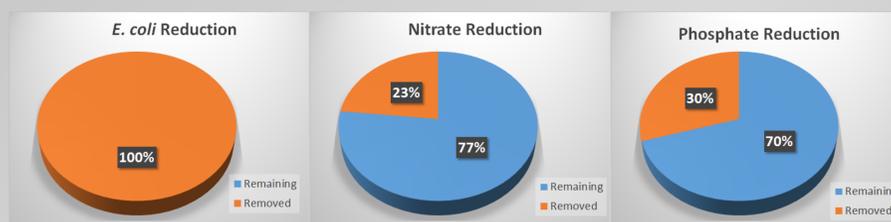


Figure 4: *E. coli*, Nitrate, and Phosphate Reduction. Filtering the water through the algal filtration device resulted in a reduction of *E. coli*, nitrates and phosphates from initial levels.

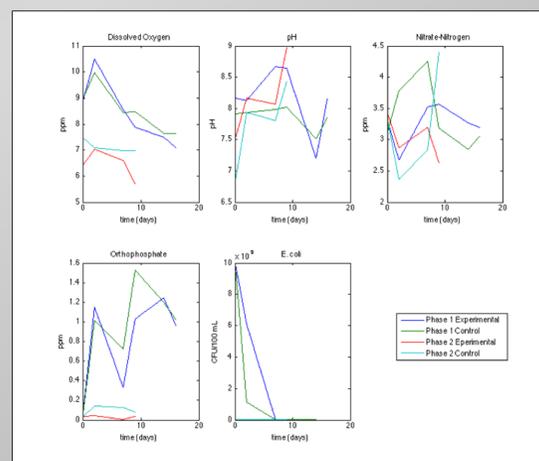


Figure 5: Time Series of Dissolved Oxygen, pH, *E. coli*, Nitrate, and Phosphate Fluctuations. Filtering the water through the algal filtration device resulted in a water quality augmentation.

Summary and Conclusions

The lab-tested filtration device resulted in 100% reduction in *E. coli* levels, as well as 23% reduction in nitrate levels and 30% reduction in phosphate levels. Use of *S. grevilleana* as an algal filter is an effective method of *E. coli* and nutrient reduction in freshwater lakes. The Algal Filtration Device is sustainable, economical, and portable, making this approach suitable to mitigate pollution and to improve access to clean drinking water sources.

Comparing Results to US EPA Potable Water Standards

Parameter	EPA Standard	This Experiment	Analysis
<i>E. coli</i> / Coliform	<5% Positive Plates	0% Positive Plates	In Range
Nitrates	<10 mg L ⁻¹	2.63 mg L ⁻¹	In Range
pH	6.5-8.5	8.97	High

Figure 6: Potential for Production of Potable Water. Overall, the resultant water is potable compared to US EPA potable water standards. pH is slightly elevated but does not affect potability. pH levels could be adjusted if needed using a buffer, such as Sodium Phosphate (Na₃PO₄). Utilization of this algal filtration device to remediate water collected from polluted freshwater lakes could potentially provide access to water for communities currently lacking close proximity to potable water.

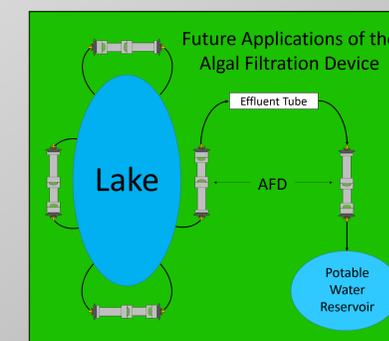


Figure 7: Future Applications of the Algal Filtration Device. Potential usage for the Algal Filtration Device includes employing multiple devices to filter a freshwater lake to remove *E. coli* and to reduce nitrate and phosphate levels. The number of devices installed would be dependent on lake volume. During lake filtration, an effluent tube containing numerous Algal Filtration Devices would remove a portion of water for additional filtration before being deposited into a potable water reservoir for connection to community pipes or wells for localized sources of drinking water.

$$t \left(1 - \ln F - \frac{V}{F} \right) \ln V = \frac{V}{F} \left(\frac{V}{F} \ln V - t \right) \left(\ln V - \ln F + V \left(\ln V - \ln F + \frac{Ft}{V} \right) \right) + \frac{V}{F} \ln V - t$$

Figure 8: Equation 1 for Scalability. This equation could be used to scale filtration effects of the Algal Filtration Device for any given volume of water. In the equation, t is time (day), V is volume (m³), and F is the flow rate integrated from time 0 to time t (m³).

$$F = \int_0^t Q dt, \text{ where } Q = \sum Q_i$$

Figure 9: Equation 2 for Scalability of Multiple Devices. This equation allows for multiple devices to be figured into Equation 1 (Figure 8). Q is the total volumetric flow rate comprised of multiple individual filters (Q_i). F is the integrated flow rate from Equation 1.

Acknowledgements

This research was supported by an *Action for Nature International Eco Hero Award*. Equipment and supplies were donated by the Hach Company, 3M and Georgia Adopt-A-Stream. Support was also provided by Dr. Alan P. Covich of the University of Georgia's Odum School of Ecology, Dr. Todd Rasmussen of the University of Georgia's Warnell School of Forestry and Natural Resources, and by the science department at Johns Creek High School in Johns Creek, Georgia, USA.