

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

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How to cite this paper: 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. *American Journal of Plant Sciences*, 8, 1148-1158.

<https://doi.org/10.4236/ajps.2017.85075>

Received: April 8, 2017

Accepted: April 27, 2017

Published: April 30, 2017

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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 ($6.17 \times 10^{-1} \text{ m}^3 \cdot \text{hr}^{-1}$) 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 ($30\% \pm 13\%$) and phosphate concentrations ($23\% \pm 5\%$) 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.

Keywords

Spirogyra grevilleana, Bioremediation, Phytoremediation, Nutrients, *Escherichia coli*

1. Introduction

Freshwater ecosystems have been critical to sustain life and establish civilizations throughout history. Human settlements worldwide concentrated near freshwater ecosystems, with “over half the world’s populations [living] within 20 km of a permanent river” [1]. One of the most widespread global issues of the 21st Cen-

tury is scarcity of clean water. As human populations grow, increased agricultural and industrial production, combined with poor sanitation practices, has led to a widespread increase in water pollution. Biological contaminants, such as *Escherichia coli* (*E. coli*), are contaminating most freshwater lakes [2].

This study focuses on the filamentous green alga, *Spirogyra grevilleana*, as a potential biofilter capable of decreasing of *E. coli* concentrations as well as nutrients levels in freshwater lakes. Potential positive effects of algae on water quality have been established through prior experimentation and research [3] [4]. This study demonstrates that the use of *S. grevilleana* in a novel filtration device can improve water quality relating to concentrations of *E. coli*, nitrates, and phosphates, while at the same maintaining desired dissolved oxygen and pH levels. If successfully scaled up, this device could be used in freshwater lakes to decrease the negative effects of *E. coli* as well as nutrient pollution and subsequent eutrophication. *Spirogyra* is commonly found in freshwater, and will proliferate and form dense mats in lakes and reservoirs with high nutrient levels [5].

Algae produce a variety of secondary metabolites including carotenoids, phenolic compounds, phycobiliprotein pigments, polysaccharides, and unsaturated fatty acids [5]. These natural products can have antioxidant, anticancer, antimicrobial, antibacterial, antiviral, antialgal, and antifungal properties, as well as bioremediation potential [6]. Antimicrobial activity against a multitude of bacteria and fungi has been documented in *Spirogyra* [7]. Extracts from *Spirogyra spp.* contain n-butanol, which has been shown to retard growth of both gram-positive and gram-negative bacteria [8]. Many early civilizations, including those in ancient Egypt, used natural compounds extracted from samples of *Spirogyra spp.* as primitive antibiotics [9]. *Spirogyra spp.* reduces bacterial levels of *E. coli* and other aquatic bacteria (such as *Giardia spp.*), not only by reducing the nutrient levels needed to sustain bacterial populations, but also by secreting antibacterial compounds into the water [10]. *Spirogyra spp.* is easily grown in culture and available for global distribution [11].

According to Rosen, Croft [12], the 1994 National Water Quality Inventory listed bacteria as the leading cause of impairment for rivers and streams. *E. coli* is a species of heterotrophic bacteria classified as coliform [13]. In addition to organic matter, catalysts for *E. coli* growth include high nutrients, warm temperatures, stagnant water conditions, and dense harmful algal mats [14]. *E. coli* is also a widely used indicator of contamination originating from domestic sewage [15]. Although *E. coli* is not generally pathogenic, its presence in surface water at elevated levels often indicates the presence of fecal contamination as well as co-occurring pathogens such as *Salmonella*, *Streptococci*, *Cryptosporidium*, *Giardia*, and *Enterovirus* [16]. Contaminated surface water facilitates direct human exposure to waterborne pathogens [17].

Excess nutrients, primarily nitrogen and phosphorous from fertilizers, stimulate algae productivity, including filamentous green algae, and contribute to harmful algae blooms [18] Excessive algal blooms and aquatic weed infestations can intensify vertical stratification, hypolimnetic anoxia, and formation of car-

cinogens during water chlorination [17]. Today, eutrophication of natural waters, caused by an increase in dissolved nutrients, is one of the most significant causes of declines in water quality [19]. Although eutrophication is a natural process of aging of lakes and water bodies, human activities can greatly accelerate eutrophication by increasing the rate at which nutrients and organic substances enter aquatic ecosystems [20]. Phosphorus has been shown to be a limiting nutrient controlling phytoplankton growth in the Great Lakes [21]. In Lake Erie, hypoxia has been directly linked to elevated in-lake total phosphorus concentrations and excessive external total phosphorus loading. State laws limited phosphates in detergents in an effort to reduce these effects. Prior to the ban, phytoplankton assemblages shifted towards dominance by taxa favored under nutrient-rich conditions, cyanobacteria, filamentous green algae (esp. *Cladophora*) and red algae, *Bangia* [21] [22]. *Cladophora* can harbor high levels of fecal indicator bacteria and bacterial pathogens [22]. Following the ban, loading of total phosphorus and dissolved oxygen depletion rates declined significantly, total algal biomass decreased, and there were fewer blooms of nuisance species [23]. Algae are currently utilized to improve water quality on a large scale through a device called an “Algal Turf Scrubbers.” An Algal Turf Scrubber usually consists of a series of filtration troughs with thin screens to catch filamentous algae [24]. Adey, Kangas [24] defined algal turfs as “communities of organisms dominated by aggregations of unicellular to branched filamentous algae and cyanobacteria (blue-green algae)”. The original use of ATS, as invented by Walter Adey, was to clean aquariums on display at the Smithsonian [24] [25]. Currently, University of Maryland researcher Patrick Kangas is applying ATS systems to improve water quality in large open-water systems (e.g. Chesapeake Bay). These systems are expensive to install and maintain, and require extensive surface area on both land and water [25]. The dimensions of an average ATS land-based system are 50 m by 1800 m. Approximately 10,000 acres of land-based and floating ATS systems would be needed to significantly remove nutrients from large bodies of water the size of Chesapeake Bay [25].

Flexible, low-cost, and sustainable methods of bacterial and nutrient reduction in freshwater lakes are critically needed. The experiments described in this paper were initiated to test the efficacy of utilizing *S. grevilleana* as an algal filtration method for biological reduction of *E. coli*, nitrate, and phosphate levels. A long-term goal of this work is to produce potable water from freshwater lakes using algal filtration devices.

2. Materials and Methods

An Algal Filtration Device (AFD) (**Figure 1**) was constructed using convex metal mesh tea filters placed on both ends inside of a removable cartridge designed to hold algae. The cartridge and experimental devices were built with transparent PVC pipe so that algae would have solar exposure for photosynthesis. The cartridge containing algae was opened on both ends and inserted into the experimental device. The device was closed and the pump turned on. Lake water was

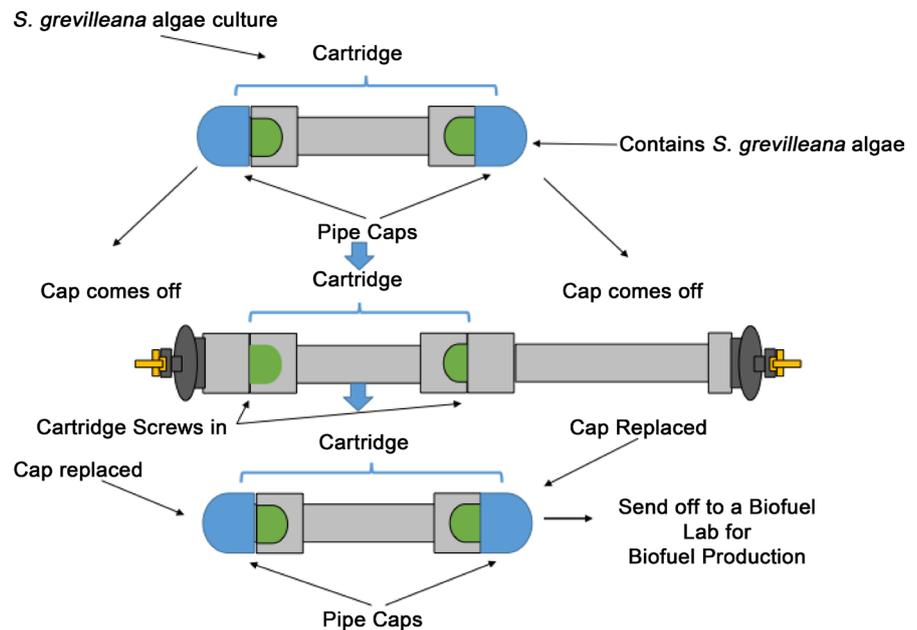


Figure 1. The Algal Filtration Device (AFD) is constructed with clear PVC pipes fitted with two ends, algal cartridge and center connector.

continuously circulated through the AFD utilizing clear plastic tubing attached to a recirculating fountain pump in the sample bucket (Figure 1).

Initially twelve prototype Algal Filtration Devices were constructed using 7.62 cm clear PVC pipes fitted with two ends, algal cartridge and center connector (total length 60.96 cm). A cartridge with domed mesh inserts fitted inside on both pipe ends and clear connectors was constructed to screw into the AFD. Bushing and barbed fittings were added on the other end of the pipe to provide a seal between the pipe and tubing. The male and female connectors were screwed together and tubing secured on each end (Figure 1). The devices were tested for function and seal with distilled water, and then sanitized with by rinsing twice with a 10% chlorine solution.

Twelve replicate sample buckets were tested for two levels of algal biomass cartridges with 16 L of water collected from a St. Johns, GA Recreations Lake near Atlanta, GA (33°59'29.04"N; 84°15'26.64"W). Buckets were sealed, transported to the lab, and lids were removed overnight. Each sample bucket was then outfitted with a circulating pump and an algal filtration device (Figure 2).

Each of the experimental devices was unscrewed in the center of the device on the cartridge end and 10 test tubes (15 ml) of *S. grevilleana* were added to each cartridge. For Phase 2, 30 test tubes containing ~15 ml of *S. grevilleana* culture were added to each treatment cartridge. No algae were added to the control cartridges.

The cartridges were screwed on to one end of the device, then the threads of the center connectors were covered in plumbing tape and screwed shut for a tight seal. One of the recirculating pumps was affixed to the bottom of the experimental sample bucket using suction cups on the bottom of the pump, and then connected to the closest barbed fittings on the experimental device using clear

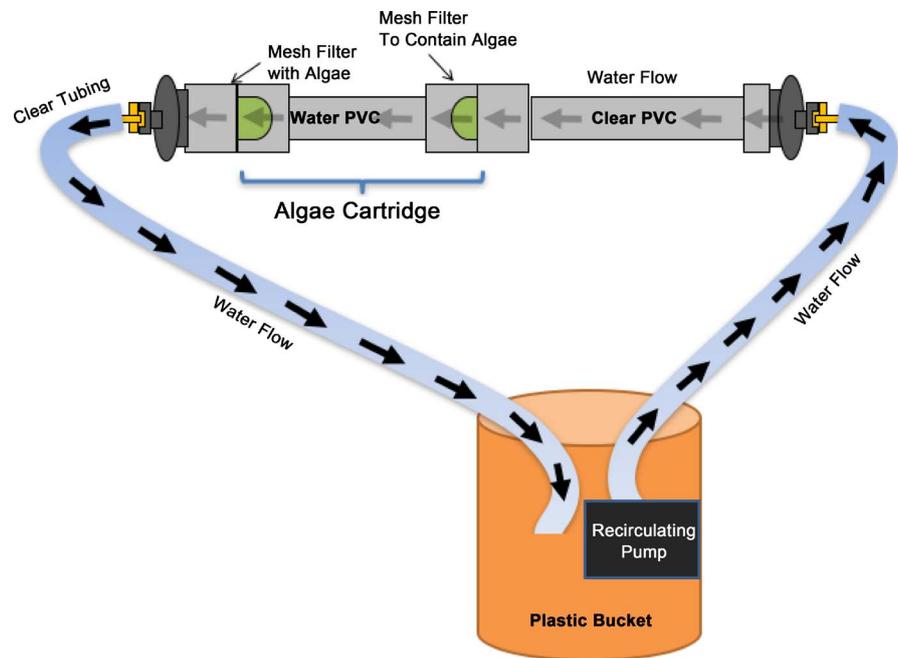


Figure 2. Each experimental bucket had recirculating pump to move water through clear tubing connected to the Algal Filtration Device (AFD) and additional tubing to return filtered water to the bucket.

rubber tubing. An additional tube was attached to the far end of the experimental device and fed back into the sample bucket. Tubing and recirculating pumps were connected for the remaining sample buckets using the same method. The pumps were plugged in and turned on for the duration of testing.

E. coli, nutrients, dissolved oxygen and pH were measured bi-weekly. *E. coli* testing was conducted using 3 M Petrifilm *E. coli*/Coliform Count Plates [16]. Three replicate sample coliform plates were made from each sample bucket, plus a negative control plate. Using a sterile glass 2 mL pipette, 1 mL of distilled water was pipetted onto each 3 M *E. coli* plate and plates were enumerated following a 48 hour incubation at $35^{\circ}\text{C} \pm 1^{\circ}\text{C}$. Nitrate and phosphate levels were read using a Hach DR890 [26]. Surface levels of dissolved oxygen were determined using the PASCO PASSPORT connected to the SPARK system. An Oakton pH Eco Tester 2 meter pH was used to determine surface pH for each sample bucket.

3. Results and Conclusions

This experiment utilized devices that were designed to contain *S. grevilleana* and form an algal filter for filtering lake water. All the experimental and control devices for both phases were built utilizing the same specifications. Phase 1 experimental devices contained 10 test tubes of *S. grevilleana* per device. Phase 2 experimental devices contained 30 test tubes of *S. grevilleana* per device. In Phase 1, the algae thinly covered the mesh filter; a doubling of algal biomass was tested in Phase 2, which created a thicker algal mat that densely covered the mesh filter.

E. coli was reduced by almost 100% for experimental and control groups in both phases (Figure 3). The experimental groups in both phases had a higher

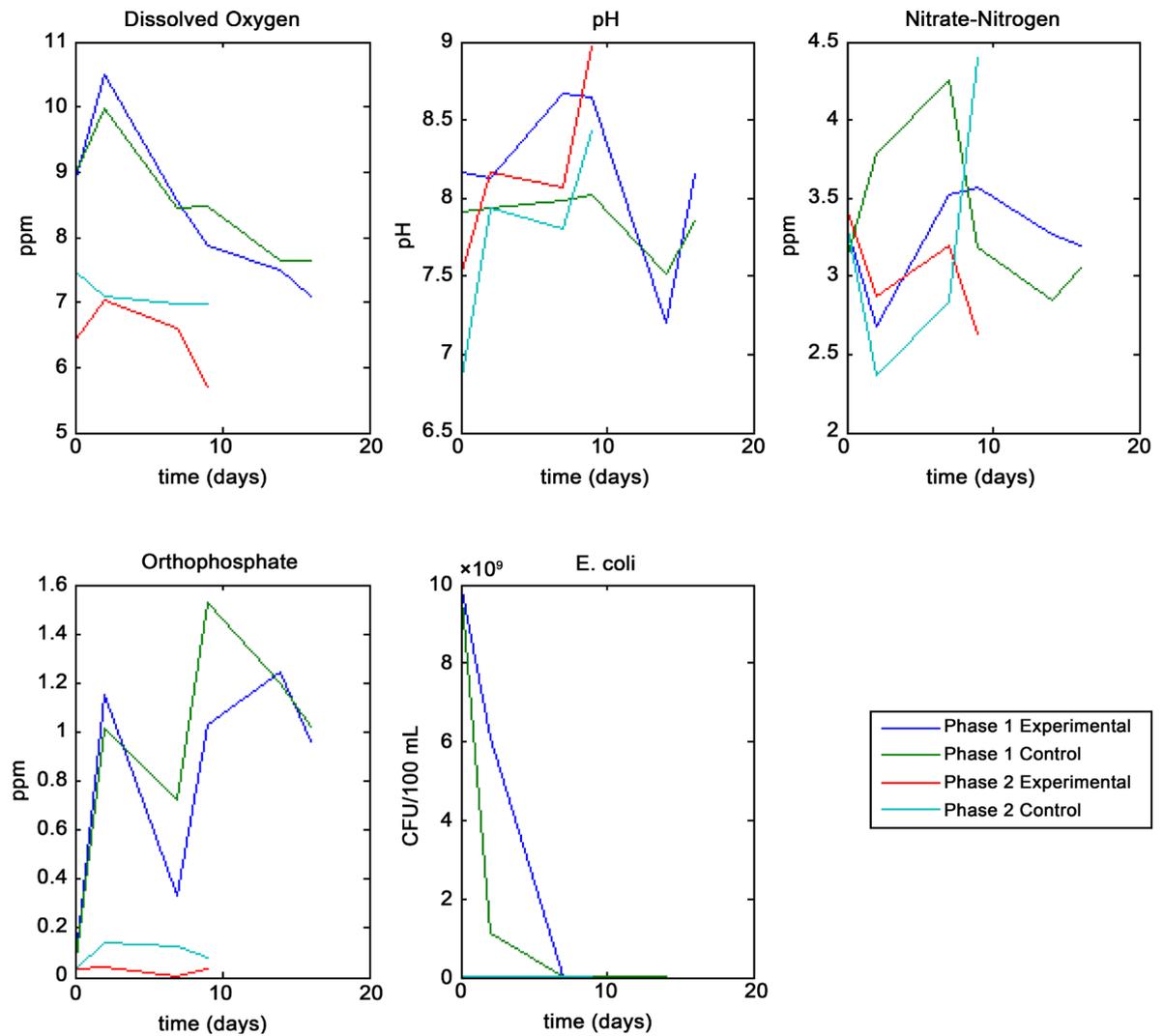


Figure 3. Changes in water quality parameters over the course of the experiment.

Table 1. Percent Change in Dissolved Oxygen, pH, Nutrients and *E. coli* from initial to final reading.

Parameter	Phase 1		Phase 2	
	Experimental	Control	Experimental	Control
Dissolved Oxygen	-20.0%	-14.7%	-10.9%	-6.7%
pH	-0.8%	-0.8%	+19.6%	+24.0%
Nitrates	+23.2%	-2.6%	-23.3%	+23.4%
Phosphates	+13.5%	+159.1%	-29.8%	+185.2%
<i>E. coli</i>	-100% ⁺	-100% ⁺	-100% ⁺	-100% ⁺

initial rate of *E. coli* removal than the control groups during the peak periods of *E. coli* reduction. The algal filtration device with *S. grevilleana* efficiently reduced *E. coli* levels, more rapidly than the control device until those levels were so low that efficiency rate was not measurable (Table 1). The increase in relative *E. coli* removal efficiency during Phase 2 may have been due to higher con-

centrations of secondary metabolites produced by higher mass of *S. grevilleana*. These could result in an instantaneous, rapid reduction of *E. coli*. The relatively thin layer algal biomass utilized in Phase 1 may have required additional time to build up critical levels of secondary metabolites lethal to most *E. coli*.

The additional algae added to the experimental devices in Phase 2 also increased the effectiveness of the devices for removing nitrate and phosphates from the water. In Phase 1, the experimental group had nitrate levels increased by 23.2% and phosphate levels reduced by 13.5%. The control group had nitrate levels reduced by 2.6% and phosphate levels increased by 159.1%. In Phase 2, the experimental sample had nitrate levels reduced by 23.3% and phosphate levels reduced by 29.8%. The Phase 2 control group had nitrate levels increased by 23.4% and phosphate levels increased by 185.2%.

Dissolved oxygen levels decreased in all experimental and control treatments. During Phase 1, dissolved oxygen levels decreased by 20% in treatment buckets and 14.7% in controls. Dissolved oxygen levels in Phase 2 treatment buckets decreased by 10.9% and controls decreased by 6.7%. The pH levels decreased slightly for both the experimental and control groups in Phase 1 (-0.8%) in Phase 1. During Phase 2, pH increased by 19.6% in treatment groups and 24.0% for the control group, however, dissolved oxygen (6 - 10 mg·L⁻¹) and pH (6 - 9) remained at safe levels throughout both trials.

This experiment successfully utilized *S. grevilleana* to reduce *E. coli* and excess nitrates and phosphates from the test water. The U.S. EPA sets safety standards for recreational use of the surface water (fishing and swimming), and potable water standards for human consumption. Surface water standards are based on thresholds of different parameters, including general water quality levels, bacterial concentrations, and nutrient levels. Potable water standards have stricter threshold values and include additional parameters (*i.e.* dissolved metals) relative to surface water regulations. Levels of tested parameters, *E. coli*, nitrates and phosphates, are critical components of surface and potable water [27].

The initial *E. coli* count overall for Phase 1 was too numerous to count (TNTC). Following algal filtration the *E. coli* count was reduced to EPA *E. coli* standards for surface water, and 16.7% of the samples met the EPA *E. coli* standards for potable water. The initial *E. coli* count for Phase 2 was also high but, following algal filtration the overall *E. coli* count met EPA *E. coli* standards for surface water, and 22.3% of the samples met the EPA *E. coli* standards for potable water. Rather than promoting *E. coli* growth, *S. grevilleana* decreased their population numbers and reduced the concentrations of nitrogen and phosphorous available for their growth. Algal reduction in bacterial levels is mediated through various metabolic processes, including the excretion of antibacterial secondary metabolites [28] [29].

According to the results from these experiments, there is strong evidence to suggest that *S. grevilleana* deployed in this algal filtration device has the potential to filter lake water to provide a source of potable water to EPA certified standards. In both Phase 1 and Phase 2, *E. coli* and nitrate levels were reduced to

Table 2. Comparison of the Algal Filtration final water results to EPA potable water standards.

Parameter	EPA Threshold	This experiment (averages unless stated otherwise)	
		Phase 1	Phase 2
pH (Secondary Standard)	6.5 - 8.5	8.150	8.967 ⁺
Nitrates (Primary Standard)	<10 mg-L ⁻¹	3.183 mg-L ⁻¹	2.633 mg-L ⁻¹
<i>E. coli</i> /Coliform (Primary Standard)	<5% positive plates	0% [*]	0% [*]

⁺High but pH is an optional (secondary) standard; ^{*}Based on a three plate sample from one of the water samples.

ranges that fell within the EPA threshold range for potable water. pH, which is an optional secondary standard for potable water, was also in range for Phase 1. In Phase 2, the pH falls only slightly out of range for EPA standards, but a small deviation in pH would not hinder potability of the water (Table 2). Further experimentation and testing of additional parameters, such as metals, would be needed prior to implementation.

The U.S. EPA posts surface water regulations to monitor bodies of water for level of recreational use. To ascertain the potential correlation of the results to EPA water standards, adherence to parameter thresholds was considered. According to EPA standards, the filtered water from both Phase 1 and Phase 2 would have been considered safe for swimming.

The algal filtration device tested in this experiment differs from currently utilized filtration devices, such as ATS technology. The algal filtration device requires only a small footprint and is portable and inexpensive. Unlike stationary, expensive ATS systems, the AFD described here can be used in multiple types of freshwater areas from small neighborhood ponds to larger lakes. The device also requires specific algae to be inserted and locked into a clear, closed device with an interior filter rather than populating the filter with algae from the incoming water in an open system such as ATS.

If this algal filtration method were utilized in a lake, multiple devices could be deployed simultaneously to decrease the time needed to filter all of the water through the device(s) one time. To determine the time and number of devices needed for filtration, a pair of relationships must be established. In the equation below, t is time (day), V is volume (m³), and F is the flow rate integrated from time 0 to time t (m³).

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

The smallest ATS available covers 1012 m² of land (roughly 2.5 acres) versus one unit of the algal filtration system, which covers 4.3×10^{-2} m² of land (roughly 1.1×10^{-3} acres). Thus the three devices needed to clean the 100,000 m³ volume of lake water would only occupy 1.3×10^{-1} m² of land (roughly 3.3×10^{-3}

acres). Even though more experimental devices would be needed to achieve the same flow as the ATS, the algal filtration system would require much less space to achieve the same filtration rate under ideal conditions. One algal filtration device costs roughly \$350 to build, including the PVC pipe and connectors, fittings, tubing, filters, recirculating pump, and algae. The three devices needed to clean the 100,000-m³ lake would cost approximately \$1050. Even if multiple devices are needed for a larger lake, the device's small size and utilization of commonly available construction materials offer an economical and attainable solution.

Overall, the *S. grevilleana* paired with the algal filtration device reduced *E. coli* levels and lowered nitrate and phosphate levels, effectively improving the water quality in the freshwater lake water samples and showing the feasibility of effective utilization of the algal filtration unit for use in the field improving the water quality of freshwater lakes. Future testing of the efficacy of the algal filtration device should include various water source additional algal species and a range of algal biomass levels. The circulating pump could be solar powered to make the device self-sufficient, portable and sustainable. Experimentation could then take place in the field setting at a freshwater lake to investigate the potential for generating potable water using this algal filtration method.

The algal filtration device is sustainable, economical, and portable, which makes the device suitable to improve water quality in freshwater lakes of different sizes and in multiple areas. *S. grevilleana* was selected as it is native to Georgia lakes. *S. grevilleana* has also been shown to increase dissolved oxygen concentrations [30] [31]. *S. grevilleana* is found in freshwater lakes worldwide; however, different algae could also be tested to determine the most efficient algal species for algal filtration under region specific conditions.

Nutrient mismanagement, including excess fertilizer use, is a major threat to water quality and chemical removal of *E. coli* and excess nutrients from water is expensive. In addition to spiking *E. coli* levels, fertilizer pollution from runoff can damage lake ecosystems and food chains, and impairs water quality. Utilizing algal biological conversion to reduce *E. coli* and pollution from runoff could provide a sustainable method for improving our increasingly scarce and impaired water resources.

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 Adopt-a-Stream. Support was also provided by the science department at Johns Creek High School in Johns Creek, Georgia, USA, and by Dr. Alan P. Covich of the University of Georgia's Odum School of Ecology and Dr. Todd C. Rasmussen of the University of Georgia's Warnell School of Forestry and Natural Resources.

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