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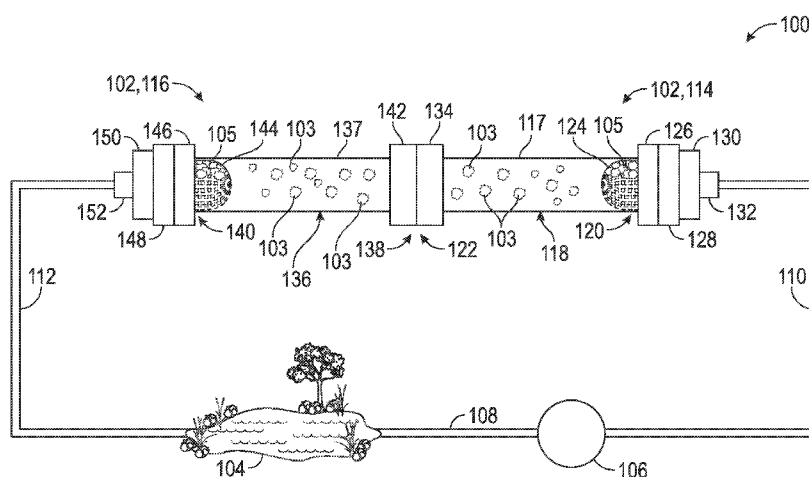


FIG. 1

(57) Abstract: The disclosure describes an algal system for improving water quality through the use of algae. In example embodiments, the algal system comprises an elongate device including algae enclosed therein and capable of reducing at least the levels of nitrates and phosphates in water directed through the device. The algae may be capable of also reducing *E. coli* bacteria, other bacteria, and viruses in the water. Preferably, the algae comprises a filamentous green algae, including without limitation, *Spirogyra grevilleana* algae. In one example embodiment, the algal system comprises an elongate device and an elongate cartridge that is pre-configured to treat certain chemical compounds, bacteria, and viruses, and certain other characteristics of water. The cartridge is delivered to the site where the water is to be treated and installed in the field, possibly replacing an existing cartridge. After use, algae may be processed into biofuel.

ALGAL SYSTEM FOR IMPROVING WATER QUALITY

CROSS REFERENCE TO RELATED APPLICATION

[0001] This application claims the benefit of U.S. Provisional Application No. 61/834,088, filed June 12, 2013, which is incorporated herein in its entirety by this reference.

FIELD OF THE INVENTION

[0002] The present invention relates, generally, to the field of systems, including apparatuses and methods, for improving water quality.

BACKGROUND OF THE INVENTION

[0003] Freshwater ecosystems have been critical to sustaining life and establishing civilizations throughout history, as evidenced by many human settlements worldwide being concentrated near freshwater ecosystems and with over half of the world's population living within 20 kilometers of a permanent river. Unfortunately, as global population has grown, increased agricultural and industrial production combined with poor sanitation practices has led to a widespread increase in water pollution with most freshwater lakes becoming contaminated to some extent by chemical and biological contaminants or pollutants such as *Escherichia coli* (*E. coli*) and dissolved nutrients in the form of nitrogen and phosphates. Such contamination has decreased the potability of the water and the ability of fish and other wildlife to survive in these freshwater lakes.

[0004] More specifically, the pollution and contamination of freshwater lakes and corresponding reduction in water quality has occurred due, at least in part, from soil erosion, nutrient mismanagement, and pesticide mismanagement. Soil erosion reduces water quality as contaminants and debris present in the soil are carried with surface water that runs off into creeks, streams, rivers and lakes. The contaminated soil then releases its nutrient load, leaching pollutants into the water and promoting growth of autotrophic and heterotrophic microorganisms, including various algae and bacteria such as *E. coli*. Nutrients also enter creeks, streams, rivers and lakes through runoff, such as storm water from rain or melting snow, which flows from roads, rooftops, farms, and lawns picking up contaminants such as wastewater, fertilizers, lawn

treatment chemicals, pesticides, and household chemicals along the way. Such polluted runoff contaminates and degrades the quality of water in creeks, streams, rivers, and lakes, and the polluted runoff can kill or damage plants, fish, and wildlife.

[0005] Excess nutrients, such as nitrogen and phosphorous, in raised concentrations increase algae productivity, especially filamentous green algae, and may contribute to out-of-control algae blooms in freshwater lakes. And, excess nitrogen and phosphorous can cause eutrophication, which is the process of increasing organic enrichment or biological productivity leading to increased algae and aquatic weed growth, oxygen shortages, and formation of carcinogens during water chlorination. The eutrophication of natural waters, caused by an increase in dissolved nutrients, is one of the most significant causes of declining water quality. Although eutrophication is a natural process of aging of lakes and water bodies, human activities can greatly accelerate eutrophication by accelerating the rate at which nutrients and organic substances enter aquatic ecosystems from their surroundings. This has occurred at Lake Victoria, the second largest lake in the world and an important water source shared by Kenya, Uganda, and Tanzania, where a surface algal mass is frequent due to increased pollution from humans and industrial waste containing phosphates and nitrates.

[0006] Similarly, in Lake Erie of the Great Lakes, phosphorus was shown to be a major nutrient contributing to and controlling phytoplankton growth. In Lake Erie, hypoxia has been directly linked to elevated in-lake total phosphorus concentrations and excessive external total phosphorus loadings. For many years, detergents having phosphates flowed into Lake Erie. Following a ban being placed on phosphates in detergents, total yearly concentrations of phosphorus and dissolved oxygen depletion rates declined significantly. As a result, algal biomass reductions were observed, especially in nuisance and eutrophic species of algae. Prior to the ban, diatom populations tended to dominate the algae species favored by nutrient rich conditions, cyanobacteria were in abundance, as well as the green algae *Cladophora* and the red algae, *Bangia*.

[0007] Excess nutrients have also been responsible for the Chesapeake Bay having massive numbers of algal blooms each spring due to fertilizer runoff from local farms and lawns. Then, as the algal blooms die off, resulting decomposition depletes life-supporting oxygen in the water and causes dead zones in the bay. According to some, such harmful algal blooms are excessive

accumulations of microscopic photosynthesizing organisms (phytoplankton) that produce biotoxins or that otherwise adversely affect humans, animals, and ecosystems. Most harmful algal blooms are cyanobacteria blooms, but others are nonbacterial blooms from algae like *Cladophora*, which is known to harbor *E. coli* populations. The presence of algal masses is, therefore, often associated with elevated *E. coli* levels.

[0008] *E. coli* are heterotrophic bacteria classified as coliform. In addition to algal blooms and other organic matter, catalysts for *E. coli* growth include swelling rainfall and other atypical events. The presence of *E. coli* is also a widely used indicator of contamination originating from domestic sewage. Although for the most part *E. coli* is not pathogenic, its presence in surface water, especially at elevated levels, can indicate fecal contamination and the likelihood that pathogens such as *Salmonella*, *Streptococci*, *Cryptosporidium*, *Giardia*, and *Enterovirus* could be present.

[0009] Scientists and engineers have attempted to remove dissolved nutrients and improve the water quality of the Chesapeake Bay and other large bodies of water through the controlled use of algae in devices commonly referred to as Algal Turf Scrubbers (“ATS”). An algal turf is a community of organisms dominated by aggregations of unicellular to branched filamentous algae and cyanobacteria (blue-green algae). Unlike the uncontrolled overgrowth of algae in bodies of water described above, algal growth in Algal Turf Scrubber systems is controlled and beneficial rather than harmful to the water quality. Algal Turf Scrubber systems work with fresh water, brackish water, and salt water, and work in a variety of waste and industrial settings such as water quality treatment plants, farm canals, rivers, and the inside of industrial smoke stacks.

[0010] Algal Turf Scrubber systems typically include a series of filtration troughs with thin screens that catch filamentous algae. Solar powered pumps add water to the open troughs from a river or other flowing body of water. The water flows through the open troughs, being filtered along the way, and then trickles back into the flowing body of water or into a stagnant body of water, such as a bay. To keep the Algal Turf Scrubber systems running smoothly and to keep fresh algae in the systems, algae are harvested every five (5) to fifteen (15) days. The harvested algae can then be converted into biofuels such as biodiesel, gasohol, methane, and butanol.

[0011] Unfortunately, Algal Turf Scrubber systems are expensive to construct and operate. Algal Turf Systems also comprise permanent installations that require massive areas on land and

on water. The average dimensions of a current land-based Algal Turf Scrubber system are 50 meters by 1,800 meters, the length of an average airport runway. To clean the Chesapeake Bay, it has been estimated that a combined 10,000 acres of land-based and floating Algal Turf Scrubber systems would be needed. Thus, the costs of construction and operation and the areas required for Algal Turf Scrubber systems render them impractical for use in reducing the levels of dissolved nutrients in smaller bodies of water. In addition, Algal Turf Scrubber systems do not attempt to directly lower the levels of *E. coli* bacteria and pathogens such as *Salmonella*, *Streptococci*, *Cryptosporidium*, *Giardia*, and *Enterovirus*.

[0012] There is, therefore, a need in the industry for an apparatus and method for reducing the levels of dissolved nutrients, reducing the levels of *E. coli* bacteria and pathogens, improving water quality, and producing potable water from freshwater creeks, streams, rivers and lakes using little land or sea area, and that resolves the difficulties, shortcomings, and problems associated with using Algal Turf Scrubber systems or other technologies with smaller bodies of water.

SUMMARY OF THE INVENTION

[0013] Broadly described, the present invention comprises an algal system, including apparatuses and methods, for improving water quality through the use of algae. In example embodiments, the algal system comprises a water treatment device or cartridge including algae enclosed therein and capable of reducing at least the levels of nitrates and phosphates from contaminated water directed therethrough. In other example embodiments, the algal system comprises a water treatment device or cartridge including algae selected and enclosed therein that is capable of killing or reducing the levels of *E. coli* bacteria, other bacteria, viruses, parasites, fungi, and pathogens in contaminated water flowing therethrough, in addition to reducing the levels of nitrates and phosphates in the contaminated water. The algae is selected based on the particular chemical compounds, bacteria, viruses, parasites, fungi, pathogens, or other contaminants in the contaminated water, the amounts or levels of which are to be reduced by the algal system. And, the use of some algae may achieve better results in certain contamination situations and locations than in others. Hence, the algal system may be configured with and utilize different types of algae, and may be configured with and utilize

different types of algae in different contamination situations and areas of the world. In still other example embodiments, the algal system comprises a water treatment device or cartridge that includes algae and other biological organisms selected and enclosed therein based on the type of contaminants to be reduced in contaminated water.

[0014] In accordance with a first example embodiment described herein and not for limitation, the algal system includes an elongate water treatment device defining a tubular chamber configured with a filamentous green algae therein. The elongate water treatment device includes a substantially hemispherical, or half dome, shaped filter therein on which the filamentous green algae grows and forms an algal turf, and through which water is directed. The algal system further comprises a pump configured to draw contaminated water at an appropriate volumetric flow rate from a body of water for treatment and to deliver the contaminated water to the elongate water treatment device. Preferably, the pump is powered with electrical energy provided by a solar photovoltaic panel or other similar device. The elongate water treatment device and substantially hemispherical shaped filter are sized and the volumetric flow rate of water is selected to cooperatively provide sufficient residence time for the contaminated water to be exposed to and come into contact with the algae, thereby enabling the algae to reduce the amounts or levels of dissolved nitrates and phosphates from the contaminated water.

[0015] According to a second example embodiment described herein and not for limitation, the algal system includes an elongate water treatment device defining a tubular chamber therein and an elongate cartridge defining a tubular chamber therein. The elongate water treatment device and elongate cartridge are configured for releasable connection therebetween such that the tubular chamber of the elongate cartridge is replaceable and configurable in fluid communication with the tubular chamber of the elongate water treatment device to enable contaminated water to flow through both tubular chambers. The elongate cartridge includes algae and, possibly, other biological organisms and even certain invertebrates therein. By pre-configuring the elongate cartridges with one or more particular types of algae and other chemicals, biological organisms, and/or other contents, elongate cartridges may be customized for subsequent delivery and use in any area of the world depending on the type and nature of contaminants, pH levels, characteristics, and/or properties in/of the contaminated water to be treated through use of the algal system. For example, cartridges may be pre-configured to reduce nitrates and phosphates

from contaminated water, while also killing or reducing the level of *E. coli* bacteria, other bacteria, viruses, parasites, and fungi.

[0016] The elongate cartridge also includes a substantially hemispherical, or half dome, shaped filter therein on which algae grows and forms an algal turf, and through which contaminated water is directed after connection of the elongate cartridge to the elongate device. Similar to the algal system of the first example embodiment, the algal system of the second example embodiment additionally includes a pump configured to draw contaminated water at an appropriate volumetric flow rate from a contaminated body of water for treatment and to deliver the contaminated water to the coupled elongate device and elongate cartridge. Also similar to the algal system of the first example embodiment, the components of the algal system of the second example embodiment are sized and the volumetric flow rate of contaminated water is selected to cooperatively provide sufficient residence time for the contaminated water to be exposed to and come into contact with the algae and other contents of the cartridge, thereby enabling treatment of the contaminated water.

[0017] Advantageously, the algal system improves the water quality of smaller contaminated bodies of water than those typically treated by Algal Turf Scrubber systems and does so in a cost effective, economical manner. The algal system is easy to set up and operate, making the algal system usable by non-scientifically trained persons. Additionally, the algal system may be used in urbanized areas or in remote areas where electricity may or may not be available. In addition, after using the algal system for a period of time, the algal turf may be removed and sent off for conversion into biofuel such as, but not limited to, biodiesel, gasohol, methane, and butanol.

[0018] Other uses and benefits of the present invention may become apparent upon reading and understanding the present specification when taken in conjunction with the appended drawings.

BRIEF DESCRIPTION OF THE DRAWING

[0019] Fig. 1 displays a schematic view of an algal system for improving water quality and environment therefor in accordance with a first example embodiment of the present invention.

[0020] Fig. 2 displays a side, perspective view of a water treatment device of the algal system of Fig. 1 in accordance with the first example embodiment of the present invention.

[0021] Fig. 3 displays an exploded view of the water treatment device of the algal system of Fig. 1 in accordance with the first example embodiment of the present invention.

[0022] Fig. 4 displays a side, perspective view of a cartridge of an algal system for improving water quality in accordance with a second example embodiment of the present invention.

[0023] Fig. 5 displays an exploded view of the cartridge of Fig. 4.

[0024] Fig. 6 displays an exploded view of the cartridge of Fig. 4 illustrating the position of the cartridge after insertion into a water treatment device of the algal system for improving water quality in accordance with the second example embodiment of the present invention.

DETAILED DESCRIPTION OF THE DRAWINGS

[0025] Referring now to the drawings in which like numerals represent like elements, Fig. 1 displays a schematic view of an algal system for improving water quality 100 and environment therefor in accordance with a first example embodiment of the present invention. The algal system for improving water quality 100 (also sometimes referred to herein as the “system for improving water quality 100” or “system 100”) substantially reduces nitrate and phosphate levels, reduces bacteria (including, without limitation, *Escherichia coli*), virus, parasite, and fungi levels, increases dissolved oxygen levels, and maintains pH levels found in the contaminated water of bodies of water 104. Generally, as used herein, the terms “body of water” or “bodies of water” includes, but is not limited to, freshwater creeks, streams, ponds, and lakes.

[0026] The system 100, as illustrated in Fig. 1, comprises a water treatment device 102 configured with algae 103 therein to improve the water quality of contaminated water present in a body of water 104. Contaminants in the contaminated water may include, but not be limited to, (i) nitrogen, nitrates, and various nitrogen compounds, (ii) phosphorous, phosphates, and various phosphorous compounds, (iii) bacteria (including, without limitation, *Escherichia coli*), (iv) viruses, (v) parasites, and (vi) fungi. The system 100 also comprises a pump 106 and a suction hose 108 extending between the body of water 104 and pump 106 such that the body of water 104 and pump 106 are in fluid communication. Additionally, the system 100 comprises an intake hose 110 extending between the pump 106 and water treatment device 102 and placing the pump 106 and water treatment device 102 in fluid communication. In addition, the system 100

comprises a discharge hose 112 extending between the water treatment device 102 and the body of water 104 so that the water treatment device 102 and body of water 104 are in fluid communication.

[0027] The algae 103 comprises one or more type(s) of algae 103 selected based at least in part on the type of contaminants present in the contaminated water to be treated by the algal system 100. According to the first example embodiment and to remove nitrates and phosphates dissolved in the contaminated water as contaminants, the algae 103 includes filamentous green algae. One form of filamentous green algae, *Spirogyra grevilleana* (*S. grevilleana*) algae, has been found to be particularly successful in significantly decreasing levels of dissolved nutrients, including nitrates and phosphates dissolved in contaminated water. *Spirogyra* algae are a genus of the order Chlorophyta (green filamentous algae). *Spirogyra* algae grow in long many-celled, hair-like strands and are commonly found in freshwater areas with relatively clean eutrophic water. In addition to efficiently and quickly absorbing nutrients, *Spirogyra spp.* secrete unique and powerful excrements known as secondary metabolites. The secondary metabolites have various antibiotic effects ranging from antibacterial (gram positive and gram negative) to antiviral to antifungal. *Spirogyra spp.* reduce bacterial levels of *E. coli* and other aquatic bacteria (such as *Giardia spp.*), not only by reducing nutrient levels needed to sustain bacterial populations, but also by secreting their antibacterial compounds into the water. It should be appreciated and understood, however, that other types or forms of algae 103 having similar properties and characteristics may be acceptable in other embodiments, and that other types or forms of algae 103 may perform better in different areas of the world.

[0028] As illustrated in Figs. 1-3, the water treatment device 102 (also sometimes referred to herein as the “device 102”) of the first example embodiment comprises a modular structure designed for ease of transportation, setup, use and maintenance. The device’s structure includes a first segment 114 and a second segment 116 that are coupled together during setup and use, and that are separated from one another for transportation of the device 102 between locations. The second segment 116 is substantially a mirror image of the first segment 114 with the exception that the first segment 114 includes a threaded male coupling 134 and the second segment 116 includes a threaded female coupling 142 for releasably joining the first and second segments 114, 116 together. When connected together, the first and second segments 114, 116 are in fluid

communication, permitting contaminated water being treated to flow from the first segment 114 into the second segment 116 through couplings 134, 142.

[0029] The device's first segment 114 comprises a tubular elongate member 118 having a generally cylindrically-shaped wall 117 extending between a first end 120 and a distant second end 122. The elongate member 118 defines a first opening 119 at the first end 120, a second opening 121 at the second end 122, and an elongate chamber 123 extending between the first and second ends 120, 122 and between the first and second openings 119, 121. The first and second ends 120, 122, first and second openings 119, 121, and elongate chamber 123 are in fluid communication such that when contaminated water is being treated by the device 102, the contaminated water flows within the elongate chamber 123 from the first end 120 toward the second end 122. The elongate member 118 is manufactured from a durable, relatively shock resistant material capable of transmitting therethrough all, or substantially all, of the wavelengths of light incident upon the water treatment device 102 during use of the system 100 that are suitable for photosynthesis and growing of the algae 103. A material, acceptable in accordance with the first example embodiment, includes clear polyvinyl chloride (PVC), but other materials having a high light transmissivity may alternatively be used.

[0030] Also, the device's first segment 114 includes an algae filter 124 positioned within the segment's elongate chamber 123 near the segment's first end 120. The algae filter 124 is removably secured in position by a fitting 126 attached to the elongate member 118 proximate the segment's first end 120. The algae filter 124 is removably secured by fitting 126 to enable removal of the algae filter 124 for cleaning and maintenance. According to the first example embodiment, the algae filter 124 has a generally hemispherical, or dome, shape (similar to that of a tea filter of a tea pot) and formed from a wire mesh or plastic mesh that are both cooperatively selected and sized to cause the appropriate buildup thereon of an algal cake 105 (or algal turf 105) during operation of the system 100. The hemispherical shape of the algae filter 124 was selected because such a hemispherical shape causes the algae filter 124 to provide substantial surface area for the buildup of an algal turf 105 thereon relative to the overall size of the algae filter 124. It should be appreciated and understood that, in other embodiments, the algae filter 124 may have the same shape or a different shape that provides substantial surface area for the buildup of an algal turf 105 thereon.

[0031] Another fitting 128 of the first segment 114 is releasably secured to fitting 126 and receives a rubber washer 129 (to aid in preventing water leakage from the device 102) and a plug 130 therein. An appropriately-sized barbed adapter 132 is mounted to and extends from the plug 130 for connection to the system's intake hose 110 during setup and operation of the system 100. The barbed adapter 132 and a hole 133 in the outboard end of the barbed adapter 132 form an intake port 135 of the water treatment device 102. The algae filter 124, fittings 126, 128, plug 130, and barbed adapter 132 are in fluid communication such that during the system's operation, contaminated water being treated flows from the intake hose 110 and through the hole 133 in the barbed adapter 132, the barbed adapter 132, plug 130, fittings 126, 128, and algae filter 124 into the segment's elongate chamber 123. A threaded male coupling 134 is secured to elongate member 118 at the segment's second end 122 to enable the water treatment device's first segment 114 to be releasably connected to the device's second segment 116.

[0032] In substantially a mirror image to the first segment 114, the device's second segment 116 comprises a tubular elongate member 136 having a generally cylindrically-shaped wall 137 extending between a first end 138 and a distant second end 140. The elongate member 136 defines a first opening (not visible) at the first end 138, a second opening 139 at the second end 140, and an elongate chamber 141 extending between the first and second ends 138, 140 and between the first opening and second opening 139. The first and second ends 138, 140, first opening, second opening 139, and elongate chamber 141 are in fluid communication such that when contaminated water is being treated by the device 102, the contaminated water flows within the elongate chamber 141 from the first end 138 toward the second end 140. The elongate member 136 is manufactured from a durable, relatively shock resistant material capable of transmitting therethrough all, or substantially all, of the wavelengths of light incident upon the water treatment device 102 during use of the system 100 that are suitable for or contributed to photosynthesis and growth of the algae 103. A material, acceptable in accordance with the example embodiment, includes clear polyvinyl chloride (PVC), but other materials having a high light transmissivity may alternatively be used.

[0033] The second segment 116 includes a threaded female coupling 142 secured to elongate member 136 at the segment's first end 138 to enable the water treatment device's second segment 116 to be releasably connected to the device's first segment 114. When so connected,

the first segment's elongate chamber 123 and the second segment's elongate chamber 141 are in fluid communication and define a water treatment chamber 158 where contaminated water being treated is exposed to algae 103. The second segment 116 also includes an algae filter 144 positioned within the segment's elongate chamber 141 near the segment's second end 140. The algae filter 144 is removably secured in position by a fitting 146 attached to the elongate member 136 proximate the segment's second end 140. The algae filter 144 is removably secured by fitting 146 to enable removal of the algae filter 144 for cleaning and maintenance. According to the first example embodiment, the algae filter 144 has a generally hemispherical, or dome, shape (similar to that of algae filter 124) and formed from a wire mesh (or plastic mesh) that are both cooperatively selected and sized to cause the appropriate buildup thereon of an algal cake 105 (or algal turf 105) during operation of the system 100. The hemispherical shape of the algae filter 144 was selected because a hemispherical shape causes the algae filter 144 to provide substantial surface area for the buildup of an algal turf 105 thereon relative to the overall size of the algae filter 144. It should be appreciated and understood that, in other embodiments, the algae filter 144 may have the same shape or a different shape that provides substantial surface area for the buildup of an algal turf 105 thereon.

[0034] Another fitting 148 of the second segment 116 is releasably secured to fitting 146 and receives a rubber washer 149 (to aid in preventing water leakage from the device 102) and a plug 150 therein. An appropriately-sized barbed adapter 152 is mounted to the plug 150 for connection to the system's discharge hose 112 during setup and operation of the system 100. The barbed adapter 152 and a hole 154 in the outboard end of the barbed adapter 152 form a discharge port 156 of the water treatment device 102. The algae filter 144, fittings 146, 148, plug 150, barbed adapter 152, and hole 154 of the barbed adapter 152 are in fluid communication such that during the system's operation, contaminated water being treated flows from the segment's elongate chamber 141 and through the algae filter 144. After flowing through fittings 146, 148, plug 150, and adapter 152, the now treated and less contaminated water flows into the discharge hose 112 and back into the body of water 104.

[0035] It should be appreciated and understood that couplings 134, 142, fittings 126, 128, 146, 148 and plugs 130, 150 are also generally manufactured from a durable, relatively shock resistant material. If possible, such components should be manufactured from a material that

permits transmission therethrough of all, or substantially all, of the light incident upon the water treatment device 102 during use, and especially those wavelengths of light that contribute to photosynthesis and growth of algae 103. A material for such components, acceptable according to the example embodiment, includes clear polyvinyl chloride (PVC). Other clear plastic or acrylic materials may also be used. However, in alternate embodiments, such components may be manufactured from an opaque material such as opaque polyvinyl chloride (PVC) or other opaque plastic or acrylic materials, and without substantial degradation in the performance of the system 100 in treating contaminated water.

[0036] It also should be appreciated and understood that the pump 106, suction hose 108, intake hose 110, and discharge hose 112 are generally sized relative to the water treatment device 102 to permit a desired flow rate of contaminated water from the body of water 104 to be treated by the system 100 in a particular implementation. Thus, for example, in systems 100 capable of treating small flow rates of contaminated water from the body of water 104, the pump 106 may have a small impeller driven by a fractional horsepower electric motor at a relatively low rotational speed and the suction hose 108, intake hose 110 and discharge hose 112 may comprise rubber tubing. Alternatively, for example, in systems 100 capable of treating larger flow rates of contaminated water from the body of water 104, the pump 106 may have a larger impeller driven at a faster rotational speed by a multi-horsepower electric motor and the suction hose 108, intake hose 110 and discharge hose 112 may comprise rubber hoses or plastic (or metal) pipes having diameters of multiple inches. It should also be appreciated and understood that electrical power for operating the pump 106 may be supplied by electrical connection of the pump's motor to a utility electrical power grid or to an appropriately sized and configured photovoltaic solar power system that may include batteries to store and supply electrical power to the pump's motor at night or at other times when sufficient sunlight is unavailable.

[0037] The system 100 is configured for operation by inserting algae 103 into one of the elongate members 118, 136, placing pipe sealing tape around the threads of coupling 134, and threadably engaging couplings 134, 142 until the couplings 134, 142 are fully tightened together. When so configured, the algae 103 and contaminated water being treated may spread and/or flow throughout the elongate water treatment chamber 158 formed by the elongate chambers 123, 141

of the elongate members 118, 136. However, the algae 103 cannot pass through algae filters 124, 144 and is, hence, trapped within the elongate water treatment chamber 158.

[0038] After insertion of the algae 103, one end of the suction hose 108 is placed into the body of water 104 from which contaminated water is to be drawn for treatment. The other end of the suction hose 108 is connected to the intake port of the pump 106. Then, one end of the intake hose 110 is connected to the discharge port of the pump 106. The other end of the intake hose 110 is connected to barbed adapter 132 and the device's intake port 135. Next, one end of the discharge hose 112 is connected to barbed adapter 152 and the device's discharge port 156. The other end of the discharge hose 112 is placed into the body of water 104 so that treated water is returned to the body of water 104.

[0039] Once the hoses are connected, electrical power is supplied to the pump 106, causing the pump 106 to pull contaminated water in from the body of water 104 and direct such contaminated water to the water treatment device 102. As the contaminated water flows into and through the device's water treatment chamber 158 and algae filters 124, 144, the contaminated water passes through algae filter 124 coming into contact with algae 103 and an algal turf 105 of algae 103 that builds up on the algae filter 124. The algae 103 reduces the levels of nutrients in the forms of nitrogen and phosphorous from the contaminated water as "food" and, hence, reduces the levels of such nutrients in the contaminated body of water 104, causing algae 103 in the body of water 104 to starve and die off. Additionally, by virtue of the walls of the elongate members 118, 136 allowing light having wavelengths suitable for photosynthesis to pass therethrough, the algae performs photosynthesis and grows within the water treatment chamber 158 and on the algae filters 124, 144 to form an algal turf 105 thereon. Because the contaminated water must pass through the algae filters 124, 144 and algal turfs 105 thereof, the time duration and level of exposure of the contaminated water to the algae 103 is enhanced, thereby improving the amount of contaminants that are removed from the contaminated water.

[0040] The system 100 may remain in place at a particular contaminated body of water 104 to continually provide treatment thereof. From time-to-time, it may be desirable to clean the water treatment device 102 and the algae filters 124, 144 thereof to lessen the thickness of the algal turfs 105 built up thereon. The removed algae 103 may be sent to a processing facility and converted into a biofuel such as biodiesel, gasohol, methane, and butanol. After cleaning is

complete, new algae 103 is inserted into the water treatment device 102 as described above and the device 102 is reassembled to again operate to treat contaminated water of the body of water 104.

[0041] According to a second example embodiment, the algal system 100' is substantially similar to the algal system 100 of the first example embodiment and includes the components thereof with the exception that the algal system 100' further comprises one or more cartridges 180' and a water treatment device 102' differently configured to replaceably receive a cartridge 180' therein. Each cartridge 180' is independently configured and customizable with algae 103' of one or more particular types and, possibly, with other biological organisms that are selected and enclosed therein based at least in part on the types of contaminants to be reduced in contaminated water at a particular location. Cartridges 180' may be configured and delivered to the site of a contaminated body of water for insertion into a water treatment device 102' already present at the site. Once delivered, an existing no longer useable cartridge 180' is removed from the water treatment device 102' and replaced with the newly delivered cartridge 180'. When connected together, the cartridge 180' and water treatment device 102' are in fluid communication, permitting contaminated water being treated to flow through the cartridge 180' and water treatment device 102'.

[0042] Similar to the first example embodiment and if the contaminants in the contaminated water include dissolved nitrogen and phosphorous, the algae 103' includes a form of filamentous green algae such as, but not limited to, *Spirogyra grevilleana* (*S. grevilleana*) algae. As described above, in addition to efficiently and quickly absorbing nutrients such as nitrates and phosphates, *Spirogyra spp.* secrete unique and powerful excrements known as secondary metabolites. The secondary metabolites have various anti-pathogenic effects ranging from antibacterial (gram positive and gram negative) to antiviral to antifungal. *Spirogyra spp.* reduce bacterial levels of *E. coli* and other aquatic bacteria (such as *Giardia spp.*), not only by reducing nutrient levels needed to sustain bacterial populations, but also by secreting their antibacterial compounds into the water. *Spirogyra spp.* may also reduce levels of viruses, parasites and fungi present in contaminated water.

[0043] Figs. 4 and 5 respectively display side, perspective and exploded views of a cartridge 180' of the algal system 100' of the second example embodiment. The cartridge 180' comprises

a tubular elongate member 182' having a first end 184 and a distant second end 186'. The elongate member 182' has a generally cylindrically-shaped wall 188' that defines a first opening 190' at the first end 184', a second opening 192' at the second end 186', and an elongate chamber 194' extending between the first and second ends 184', 186' and between the first and second openings 190', 192'. One or more types of algae 103' and, possibly, other biological organisms reside within the elongate chamber 194'. The algae 103' and biological organisms are selected and included within the elongate chamber 194' based at least in part on the particular contaminants present in the contaminated water to be treated by the algal system 100' and, possibly, on the location of the site at which the algal system 100' will be used. The first and second ends 184', 186', first and second openings 190', 192', and elongate chamber 194' are in fluid communication such that when contaminated water is being treated by the system 100', the contaminated water flows within the elongate chamber 194' from the first end 184' toward the second end 186'. The elongate member 182' and wall 188' thereof is manufactured from a durable, relatively shock resistant material capable of transmitting therethrough all, or substantially all, of the wavelengths of light incident upon the water treatment device 102' during use of the system 100' that are suitable for photosynthesis and growing of algae 103'. A material, acceptable in accordance with the second example embodiment, includes clear polyvinyl chloride (PVC), but other materials having a high light transmissivity may alternatively be used.

[0044] The cartridge 180' includes first and second male threaded couplings 196', 198' secured to the elongate member 182', respectively, at the elongate member's first and second ends 184', 186'. The cartridge 180' also includes first and second algae filters 200', 202' having rims 205', 207' that abut, respectively, against the first and second male threaded couplings 196', 198' near the elongate member's first and second ends 184', 186'. The algae filters 200', 202' respectively protrude into the elongate chamber 194' from the elongate member's first and second ends 184', 186' and receive light passing through the cartridge's wall 188'. According to the second example embodiment, the algae filters 200', 202' have a generally hemispherical, or dome, shape (similar to that of a tea filter of a tea pot) and are formed from a wire mesh (or plastic mesh) that are both cooperatively selected and sized to cause the appropriate buildup thereon of an algal cake 105' (or algal turf 105') during operation of the algal system 100'. The

hemispherical shape of the algae filters 200', 202' was selected because such a hemispherical shape causes the algae filters 200', 202' to provide substantial surface area for the buildup of an algal turf 105' thereon relative to the overall size of the algae filters 200', 202'. It should be appreciated and understood that, in other embodiments, one or both of the algae filters 200', 202' may have a different shape that provides substantial surface area for the buildup of an algal turf 105' thereon.

[0045] The cartridge 180' also includes rubber washers 204', 206' that abut respective rims 205', 207' of the algae filters 200', 202'. The rubber washers 204', 206' and algae filters 200', 202' are releasably held in position by respective end caps 208', 210'. The end caps 208', 210' have internal female threads that are operative to receive the male threads of the first and second male threaded couplings 196', 198'. Together, the rims 205', 207', rubber washers 204', 206', and end caps 208', 210' aid in preventing water and/or algae 103' leakage from the cartridge 180' during delivery or storage of the cartridge 180'.

[0046] Fig. 6 displays an exploded view of the cartridge 180' after insertion into a water treatment device 102' of the algal system 100' in accordance with the second example embodiment. The water treatment device 102' (also sometimes referred to herein as "device 102' ") comprises a generally elongate modular structure operable with a cartridge 180' and designed for ease of transportation, setup, use and maintenance. The water treatment device 102' has a first end 212' and distant second end 214', and includes a first portion 216' and a second portion 218' between which a cartridge 180' (absent end caps 208', 210') is inserted and coupled during use. When connected together, the first and second portions 216', 218' and cartridge 180' are in fluid communication, permitting contaminated water being treated to flow from the first portion 216', through the cartridge 180', and into the second portion 218' before being discharged from the water treatment device 102' as treated water.

[0047] The first portion 216' of the water treatment device 102' comprises a female coupling 220' having internal threads. The female coupling 220' receives a plug 222' having male threads therein at the first end 212' of the water treatment device 102'. An appropriately-sized barbed adapter 224' (partially visible) is mounted to and extends from the plug 222' for connection to the system's intake hose 110' during setup and operation of the system 100'. The barbed adapter 224' and a hole (not visible) in the outboard end of the barbed adapter 224' form an intake port

226' of the water treatment device 102'. The female coupling 220' also receives a portion of the cartridge's first male threaded coupling 196' therein such that the female coupling 220' and first male threaded coupling 196' are threadably engaged. When so engaged, rubber washer 204' and algae filter 200' are releasably held in position by cooperation between the female coupling 220' and the first male threaded coupling 196'.

[0048] The second portion 218' of the water treatment device 102' comprises a tubular elongate member 228' having a first end 230' and a distant second end 232'. The elongate member 228' has a generally cylindrically shaped wall 230' that defines a first opening (not visible) at the first end 232', a second opening (not visible) at the second end 234', and an elongate chamber 236' extending between the first and second ends 232', 234' and between the first opening and second opening. The first and second ends 232', 234', first and second openings, and elongate chamber 236' are in fluid communication such that when contaminated water is being treated by the system 100' and treated water is received from the cartridge 180', the treated water flows within the elongate chamber 236' from the first end 232' toward the second end 234'.

[0049] The second portion 218' also includes a first threaded female coupling 238' secured to elongate member 228' at the member's first end 232' to enable the water treatment device's second portion 218' to be releasably connected to the cartridge 180'. The first female coupling 238' receives a portion of the cartridge's second male threaded coupling 198' therein such that the first female coupling 238' and second male threaded coupling 198' are threadably engaged. When so engaged, rubber washer 206' and algae filter 202' are releasably held in position by cooperation between the first female coupling 238' and the second male threaded coupling 198'. Additionally, when so engaged, the cartridge's elongate chamber 194' and the second portion's elongate chamber 236' are in fluid communication.

[0050] Additionally, the second portion 218' of the water treatment device 102' includes a second threaded female coupling 240' secured to elongate member 228' at the member's second end 234'. The second threaded female coupling 240' receives a plug 242' having male threads therein at the second end 214' of the water treatment device 102'. An appropriately-sized barbed adapter 244' is mounted to and extends from the plug 242' for connection to the system's discharge hose 112' during setup and operation of the system 100'. The barbed adapter 244' and

a hole 246' in the outboard end of the barbed adapter 244' form a discharge port 248' of the water treatment device 102'. The second threaded female coupling 240', plug 242', barbed adapter 244', and hole 246' of the barbed adapter 244' are in fluid communication such that during the system's operation, treated water flows from the second portion's elongate chamber 236' and through the second threaded female coupling 240', plug 242', barbed adapter 244', and hole 246', the now treated and less contaminated water flows into the discharge hose 112' and back into the body of water 104'.

[0051] It should be appreciated and understood that elongate member 228', couplings 220', 238', 240' and plugs 222', 242' are also generally manufactured from a durable, relatively shock resistant material. If possible, such components should be manufactured from a material that permits transmission therethrough of all, or substantially all, of the light incident upon the water treatment device 102' during use, and especially those wavelengths of light that contribute to photosynthesis and growth of algae 103'. A material for such components, acceptable according the example embodiment, includes clear polyvinyl chloride (PVC). Other clear plastic or acrylic materials may also be used. However, in alternate embodiments, such components may be manufactured from an opaque material such as opaque polyvinyl chloride (PVC) or other opaque plastic or acrylic materials, and without substantial degradation in the performance of the algal system 100' in treating contaminated water.

[0052] The algal system 100' of the second example embodiment is configured for operation by inserting a cartridge 180' pre-filled with algae 103' and, possibly, other biological organisms into the water treatment device 102'. To do so, the end caps 208', 210' are removed from the cartridge 180' and a portion of the cartridge's first male threaded coupling 196' is inserted into and threadably engaged with the female coupling 220' of the device's first portion 216'. The rubber washer 204' and algae filter 200' are held in position by the female coupling 220' and the cartridge's first male threaded coupling 196'. Then, a portion of the cartridge's second male threaded coupling 198' is inserted into and threadably engaged with the first female coupling 238' of the device's second portion 218'. Such engagement holds rubber washer 206' and algae filter 202' in position and results in the cartridge's elongate chamber 194' and the second portion's elongate chamber 236' being in fluid communication. However, even though the cartridge's elongate chamber 194' and the second portion's elongate chamber 236' are in fluid

communication, the algae 103' remains trapped within the cartridge's elongate chamber 194' between algae filters 200', 202'.

[0053] After insertion of the cartridge 180', one end of the suction hose 108' is placed into the body of water 104' from which contaminated water is to be drawn for treatment. The other end of the suction hose 108' is connected to the intake port of the pump 106'. Then, one end of the intake hose 110' is connected to the discharge port of the pump 106'. The other end of the intake hose 110' is connected to barbed adapter 224' and the device's intake port 226'. Next, one end of the discharge hose 112' is connected to barbed adapter 244' and the device's discharge port 248'. The other end of the discharge hose 112' is placed into the body of water 104' so that treated water is returned to the body of water 104'.

[0054] Once the hoses 110', 112' are connected, electrical power is supplied to the pump 106', causing the pump 106' to pull contaminated water in from the body of water 104' and direct such contaminated water to the water treatment device 102'. As the contaminated water flows into and through the cartridge's elongate chamber 194' and algae filters 200', 202', the contaminated water comes into contact with algae 103' and algal turfs 105' of algae 103' that build up on the algae filters 200', 202'. The algae 103' reduces the levels of nutrients in the forms of nitrogen and phosphorous from the contaminated water as "food" and, hence, reduces the levels of such nutrients in the contaminated body of water 104', causing algae in the body of water 104' to starve and die off. Additionally, by virtue of the wall 188' of the cartridge's elongate member 180' allowing light having wavelengths suitable for photosynthesis to pass therethrough, the algae 103' performs photosynthesis and grows within the cartridge's elongate chamber 194' and on the algae filters 200', 202' to form algal turfs 105' thereon. Because the contaminated water must pass through the algae filters 200', 202' and algal turfs 105' thereof, the time duration and level of exposure of the contaminated water to the algae 103' is enhanced, thereby improving the amount of contaminants that are removed from the contaminated water.

[0055] The non-cartridge components of the algal system 100' of the second example embodiment generally remain at a site where contaminated water of a body of water 104' is being treated. However, after a period of operation, the cartridge 180' of the algal system 100' may require maintenance or be no longer providing desired water treatment performance. In either event, the cartridge 180' is removed from the water treatment device 102' by reversing the

steps used to insert the cartridge 180' described above. A new cartridge 180' is then inserted into the water treatment device 102' and water treatment continued. The removed cartridge 180' is sent to a processing facility where the algae 103' and algal turfs 105' are removed and converted into a biofuel such as biodiesel, gasohol, methane, and butanol. Once the algae 103' and algal turfs 105' have been removed and converted, the removed cartridge 180' may be re-configured for subsequent use by loading the cartridge 180' with algae 103' and, possibly, other biological organisms appropriate for the body of water 104' with which the cartridge 180' will be used. Then, the re-configured cartridge 180' is delivered to the site of the body of water 104' to be treated and, ultimately, inserted into a water treatment device 102' at such site.

[0056] Further information pertaining to the algal system 100 and the operation thereof is included in the technical papers of Appendices A and B that follow.

[0057] Whereas the present invention has been described in detail above with respect to example embodiments thereof, it should be appreciated that variations and modifications might be effected within the spirit and scope of the present invention.

APPENDIX A

Entry to the Stockholm Junior Water Prize (2014)

**Utilization of *Spirogyra grevilleana* as a
Method of Algal Filtration for Reduction of
Limnotic *Escherichia coli* Levels**

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Abstract

This study evaluated the impact of *Spirogyra grevilleana* when utilized in an algal filtration system on improving water quality in terms of reducing levels of *Escherichia coli*, nitrates and phosphates. Initially, the algal filtration device prototypes were designed and constructed. Water was collected from a freshwater lake and run continuously through the filtration devices in a lab, with an increased quantity of *S. grevilleana* added prior to experimentation in Phase 2. Samples were tested over time for *E. coli*, nitrates, phosphates, dissolved oxygen, and pH levels. In both phases the algal filtration devices utilizing *S. grevilleana* reduced *E. coli* by almost 100%, were more efficient at *E. coli* reduction than the control devices without *S. grevilleana*, and reduced nitrate and phosphate levels while maintaining dissolved oxygen and pH levels. Utilizing *S. grevilleana* in the 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 the algal filtration device is potentially capable of creating potable water.

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Key Words

Spirogyra grevilleana; *Escherichia coli*; Algal Filtration; Bioremediation; Algae; Freshwater Lakes

Abbreviations and Acronyms

ATS – Algal Turf Scrubbers; *E. coli* – *Escherichia coli*; EPA – Environmental Protection Agency; HAB – Harmful Algal Blooms; MeOH – Metal hydroxides; PVC – Polyvinyl Chloride; *S. grevilleana* – *Spirogyra grevilleana*; spp. – species; TNTC – too numerous to count; UGA – University of Georgia; U.S. – United States

Introduction

Freshwater ecosystems have been critical to sustaining life and establishing civilizations throughout history. Many human settlements worldwide are concentrated near freshwater ecosystems, with “over half the world’s populations [living] within 20 km of a permanent river” [17]. One of the most widespread global issues of the 21st century is scarcity of clean water. As global populations grow, increased agricultural and industrial production, combined with poor sanitation practices, leads to a widespread increase in water pollution. Most freshwater lakes are becoming contaminated by chemical and biological pollutants, such as *Escherichia coli* (*E. coli*), thus decreasing the potability of the water.

This study focuses on utilizing *Spirogyra grevilleana* (*S. grevilleana*), a filamentous green algae, as a potential algal filter to naturally decrease levels of *E. coli* and dissolved nutrients in freshwater lakes. Potential positive effects of algae on water quality have been established through prior experimentation and research. This experiment utilized *S. grevilleana* in a novel way as an algal filter in an enclosed device to improve water quality in terms of *E. coli*, nitrates, and phosphates while maintaining dissolved oxygen and pH. If shown to be effective, this device could be used in freshwater lakes to decrease the negative effects of *E. coli*, as well as nutrient pollution and subsequent eutrophication.

Algae are photosynthetic protists that, according to Gibson [16], are “estimated [to] produce 73-84% of the net global production of oxygen.” They are usually unicellular and mobile [32]. The algae utilized in this experiment are *Spirogyra*, a genus of the order Chlorophyta (green filamentous algae) [20]. The origin of the name “*Spirogyra*” comes from the Greek “*Spiera*” (meaning coil) and “*gyros*” (meaning twisted) since the cells contain spiral chloroplasts [30]. According to ABC’s of nature [33], “*Spirogyra* grows in long many-celled, hair-like strands.” *Spirogyra* are commonly found in freshwater areas with relatively clean eutrophic water [20]. *Spirogyra* shows antimicrobial activity against a multitude of bacteria and fungi, including extracellular chemicals from algal extracts, like n-butanol, which have been shown to be effective on both gram-positive and gram-negative bacteria [24].

In addition to efficiently and quickly absorbing nutrients from their aqueous environment, *Spirogyra spp.* have unique and powerful excrements called secondary metabolites. These secondary metabolites have various antibiotic effects ranging from antibacterial (gram positive

and gram negative) to antiviral to antifungal. Many early civilizations, including ancient Egyptians, used natural compounds such as secondary metabolites extracted from samples of *Spirogyra spp.* as primitive antibiotics. *Spirogyra spp.* reduce bacterial levels of *E. coli* and other aquatic bacteria (such as *Giardia spp.*), not only by reducing nutrient levels needed to sustain bacterial populations but also by secreting antibacterial compounds into the water [24]. *Spirogyra spp.* are easily grown in culture and available for global distribution.

There are over 400 species under the genus *Spirogyra* worldwide, living in almost every freshwater body on the planet [20] [30]. *S. grevilleana* was initially chosen for experimentation as this species of *Spirogyra* is commonly found in freshwater lakes in Georgia, including Lake Lanier, which is the main source of drinking water for multiple southern U.S. states. *S. grevilleana* was subsequently shown in research to affect and maintain water quality [19].

According to Rosen [36], the 1994 National Water Quality Inventory listed bacteria as the leading cause of impairment for rivers and streams. *E. coli* are heterotrophic bacteria classified as coliform. In addition to organic matter, catalysts for *E. coli* growth include algal blooms, swelling, rainfall, and other atypical events [9]. *E. coli* are also a widely used indicator of contamination originating from domestic sewage [41]. Although for the most part *E. coli* is not pathogenic, its presence in surface water, especially at elevated levels, can indicate fecal contamination and the likelihood that pathogens such as *Salmonella*, *Streptococci*, *Cryptosporidium*, *Giardia*, Enterovirus, etc. could be present [12]. Lakes and rivers allow direct exposure of people to water that may contain pathogens [34].

Algae are known to have antimicrobial and antioxidant activity [3]. According to Prakash, et al. [30], “the use of algae for therapeutic purposes has a long history.” Commercial development of algal antibiotic compounds began in 1950. Algae produce antibiotic substances, including fatty acids, acrylic acid, halogenated aliphatic compounds, terpenes, sulfur-containing heterocyclic compounds, carbohydrates, and phenols, that are effective against gram-positive and gram-negative bacteria [31]. Algae also produce metal hydroxides (MeOH) and quercetin, which both have antibacterial activity [1]. Anti-fungal compounds can be found in extracts of green algae, Diatoms, and Dinoflagellates [31].

Secondary Metabolites created by algae are chemicals that include carotenoids, phenolic compounds, phycobiliprotein pigments, polysaccharides, and unsaturated fatty acids. Some

effects of Secondary Metabolites have antioxidant, anticancer, antimicrobial, antibacterial, antiviral, antialgal, and antifungal activity, as well as bioremediation potentials. Macroalgae have bioactive compounds that have a positive impact on human health, including antiviral, antifungal, antibacterial, antioxidant, anti-inflammatory, hypercholesterolemia, hypolipidemic, and antineoplastic properties [35].

Water quality is threatened by soil erosion, nutrient mismanagement, and pesticide mismanagement [7]. Soil erosion, also known as surface water runoff, damages water quality as debris from erosion enters surface water [43]. Contaminated soil will release its nutrient load, leaching pollutants into water and promoting growth of autotrophic and heterotrophic microorganisms, including various algae and bacteria such as *E. coli*. Nutrients can also enter the water through runoff, such as storm water from rain or melting snow, which flows over areas and picks up contaminants, such as fertilizers. According to the Mid-American Regional Council [39], “polluted storm water contaminates streams, rivers, and lakes. [The polluted water] can kill or damage plants, fish, and wildlife, while degrading the quality of the water.” Urban sources of water pollution include runoff from rooftops, roads, and lawns, as well as wastewater, lawn treatment and chemicals, and household chemicals [8].

Excess nutrients, such as nitrogen and phosphorous from fertilizers, are major contributors to pollution in freshwater lakes. Nitrogen and phosphorous in raised concentrations increase algae productivity, especially filamentous green algae, and may contribute to out-of-control algae blooms [13] [37]. According to Baird [6], “excess nitrogen and phosphorous in surface waters and nitrogen in ground water cause eutrophication,” which is the process of increasing organic enrichment or biological productivity, leading to increased algae and aquatic weed growth, oxygen shortages, and formation of carcinogens during water chlorination [36]. Today, eutrophication of natural waters, caused by an increase in dissolved nutrients, is one of the most significant causes of declines in water quality [29]. According to Buhvestova, et al [10], “Eutrophication is the most serious environmental issue in most lakes in low lying areas.” Although eutrophication is a natural process of aging of lakes and water bodies, human activities can greatly accelerate eutrophication by accelerating the rate at which nutrients and organic substances enter aquatic ecosystems from their surroundings [11].

World populations will continue to grow, reaching an estimated 9.2 billion by 2050, increasing pressures on productive capacity of agriculture and industry, leading to increased nutrient pollution and fertilizer use, reducing water quality in freshwater lakes critical for many communities, and causing eutrophication and excessive *E. coli* levels in freshwater lakes worldwide. Lake Tai, a large freshwater lake in China providing drinking water for 30 million people, is highly polluted from increased urbanization and population growth of surrounding areas. Rapid growth and industrialization have led to polluted freshwater lakes all over China. Lake Victoria, the second largest lake in the world and an important water source shared by Kenya, Uganda, and Tanzania, frequently has a surface algal mass due to increased pollution from humans and industrial waste containing phosphates and nitrates [38].

Phosphorus has been shown to be a major 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 loadings. Phosphates in detergents were limited by state laws as an effort to reduce these effects. Following the ban, target loads for total phosphorus yearly concentrations of total phosphorus and dissolved oxygen depletion rates declined significantly. Algal biomass reductions were observed, especially in nuisance and eutrophic species [14]. Prior to the ban, diatom populations shifted towards the dominance of species favored by nutrient rich conditions, cyanobacteria were in abundance, as well as the green algae *Cladophora* and the red alga, *Bangia* [21] [28]. *Cladophora* can harbor high levels of fecal indicator bacteria and bacterial pathogens [28].

Chesapeake Bay has massive algal blooms each spring caused by increased nitrogen and phosphorus due to fertilizer runoff from local lawns and farms. As algal blooms dies off, resulting decomposition depletes life-supporting oxygen in the water, causing dead zones in the bay [38]. According to Hilborn, et al. [23], “harmful algal blooms (HABs) are excessive accumulations of microscopic photosynthesizing organisms (phytoplankton) that produce biotoxins or otherwise adversely affect humans, animals, and ecosystems.” Most HABs are cyanobacteria blooms, but others are nonbacterial blooms from algae like *Cladophora*, which is known to harbor *E. coli* populations [23] [42].

E. coli is commonly harbored on masses of filamentous algae in lakes, including the Great Lakes of USA and Canada [27]. The presence of algal masses is therefore often associated with

elevated *E. coli* levels. While algae may seem to harbor *E. coli*, they can actually reduce it [5]. According to Ansa, et al. [5], algae raise dissolved oxygen levels, and high dissolved oxygen levels can be attributed to *E. coli* death. Fluctuations in pH, also attributed to algal growth, are linked to *E. coli* death as well [5].

Algae are currently utilized to improve water quality on a large scale through a method called “Algal Turf Scrubbers,” abbreviated ATS [2]. Adey and Bannon [2] 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 at the Smithsonian Museum of Natural History [22] [40]. Currently, University of Maryland researcher Patrick Kangas is using ATS systems to clean bodies of water such as the Chesapeake Bay. Unlike the uncontrolled overgrowth of algae in bodies of water, algal growth in ATS systems is controlled and beneficial rather than harmful to the water quality [18]. ATS systems work with fresh water, brackish water, and salt water, as well as with a variety of waste and industrial settings, such as water quality treatment plants, farm canals, rivers, and the inside of industrial smoke stacks [4] [36].

ATS are a series of filtration troughs with thin screens that catch filamentous algae [17] [25]. Solar powered pumps add water to open troughs from a river or other flowing body of water. The water flows through the trough, being filtered along the way, then trickling back into the flowing body of water or into a stagnant body of water, such as a bay. To keep the ATS system running smoothly and to keep fresh algae in the system, algae are harvested every five to fifteen days [4]. Harvested algae can then be converted into biofuels, such as biodiesel, gasohol, methane, and butanol [4] [40].

Negative aspects of ATS include size and cost. ATS are permanent installations that are expensive to install and run, and take up massive amounts of area on land and on water [40]. Wheeler [40] states that approximately a combined 10,000 acres of land-based and floating ATS systems would be needed to clean bodies of water such as the Chesapeake Bay, and that the average dimensions of a current ATS land-based system are 50 m by 1800 m, the length of an average airport runway.

Water quality monitoring is essential for human recreation and consumption. The U.S. EPA sets standards for surface water and potable water for safety of the public. Surface water

standards for recreational use refer to safety of the water for fishing and swimming, whereas potable water standards refer to safety for human consumption. Surface water standards are based on thresholds of different parameters, ranging from general water quality levels to bacterial concentrations to nutrient levels. Potable water standards are stricter threshold regulations of parameters measured for surface water designations, including additional parameters such as dissolved metals. Levels of these parameters, such as *E. coli*, nitrates and phosphates, are critical components of surface and potable water; therefore, monitoring these levels is tantamount [15].

Flexible, low-cost, and sustainable methods of bacterial and nutrient reduction in freshwater lakes are a critical need worldwide. This experiment aimed to biologically reduce *E. coli*, nitrate and phosphate levels utilizing *S. grevilleana* as an algal filtration method. A long term goal of this experiment is to produce potable water from freshwater lakes using algal filtration devices.

Materials and Methods

The experiment was conducted in two phases, each utilizing a different amount of algae in

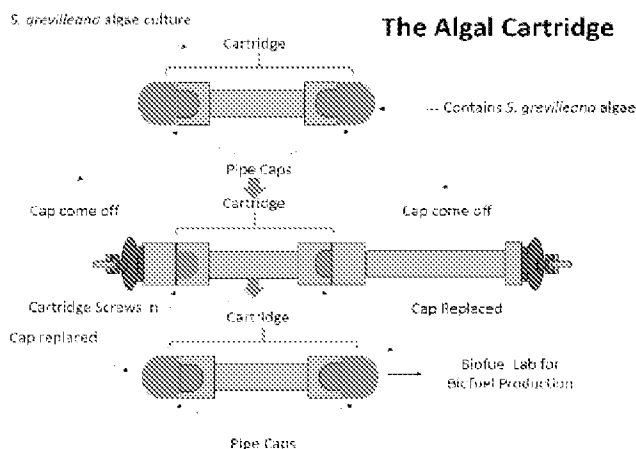


Diagram 1: Schematic diagram of the cartridge system

A cartridge with domed mesh inserts fitted inside on both pipe ends and clear connectors was constructed to screw into the algal filtration device. Bushing and barbed fittings were added on the other end of the pipe to provide a seal

the algal cartridge. Initially twelve prototypes of the Algal Filtration Device were constructed using the exact same materials, measurements, and procedures. 7.62 cm PVC pipes were fitted together to specification with two ends, the algal cartridge and center connector for a total length of 60.96 cm. Clear PVC pipe and connectors were used to expose algae to sunlight for photosynthesis.

Pumping Water Through the Algal Filtration Device

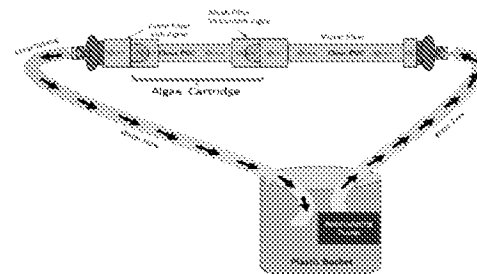


Diagram 2: Diagram of the flow of water through the algal filtration system

between the pipe and tubing. The male and female connectors were screwed together and tubing secured on each end (see Diagram 1). The devices were tested for function and seal with distilled water, then sanitized with bleach solution. For Phase 1, twelve 19L sample buckets were filled with 16L of water collected from a freshwater lake at 33°59'29.04''N, 84°15'26.64''W. Sample buckets were sealed and transported to the lab. Six samples were labeled as "Control" and six as "Experimental." The sample buckets had lids removed and were left overnight.

The baseline levels of nitrates, phosphates, dissolved oxygen, and pH were then established using Hach company methods. Each sample bucket was outfitted with a circulating pump and an algal filtration device (see Diagram 2). Each of the experimental devices was unscrewed in the

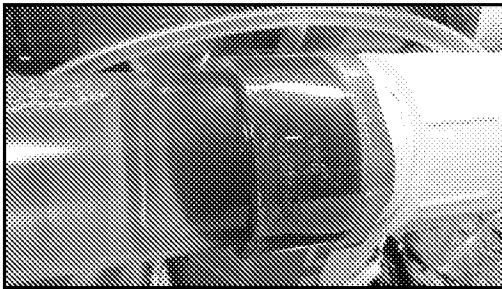


Photo 1: Experimental Device with *S. grevilleana*

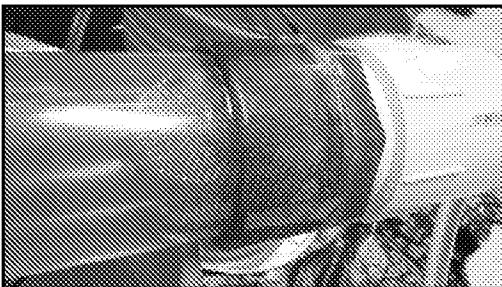


Photo 2: Control Device with no *S. grevilleana*

center of the device on the cartridge end and 10 test tubes of *S. grevilleana* were added to each cartridge (see Photo 1). No algae were added to the Control cartridges (see Photo 2). 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, then connected to the closest barbed fittings on the experimental device using clear 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 testing was conducted every Monday and Wednesday following proper safety precautions. The incubator was turned on at the beginning of the experiment and set for 35°C. There were three sample coliform plates from each sample bucket, plus a control plate for a negative control. Using a sterile glass 2 mL pipette, 1 mL of distilled water was pipetted onto each 3M *E. coli* plate. After all of the samples were plated, the plates were stacked in threes by sample bucket and placed in the incubator. The plates were incubated for 48 hours plus or minus

one hour at 35°C plus or minus 1°C. The plates were read every Wednesday and Friday after being incubated for 48 hours.

On Tuesdays and Thursdays, nutrient testing was conducted following proper safety precautions. Dissolved oxygen tests were conducted. The PASCO PASSPORT dissolved oxygen probe was connected to the SPARK system and inserted between 5 cm and 10 cm below the water surface of each sample and dissolved oxygen level was read. The probe tip of the Oakton pH Eco Tester 2 meter was inserted 3 cm below the surface of each sample bucket and pH levels were read. Nitrate and phosphate levels were read using a DR890 by utilizing programs 57 and 79 respectively.

At the end of Phase 1 testing the algae were properly bleached and disposed. The devices were then sanitized with bleach water.

For Phase 2, the six sanitized control devices that did not utilize algae in the algal filtration cartridges during Phase 1 were set up for experimentation. Six 19L sample buckets were filled with 16L of water collected at the same location as Phase 1. Sample buckets were sealed and transported to the lab. Three samples were labeled as “Control” and three as “Experimental.” Sample buckets had lids removed and were left overnight.

Initial baseline testing was completed using the same procedures as in Phase 1. Each of the experimental device cartridges was unscrewed and 30 test tubes of *S. grevilleana* were added to each cartridge. No algae were added to the Control cartridges. The cartridges were added to the filtration devices and the experiment was conducted using the same procedures as Phase 1.

At the end of Phase 2 testing algae were properly bleached and disposed and devices sanitized.

Results

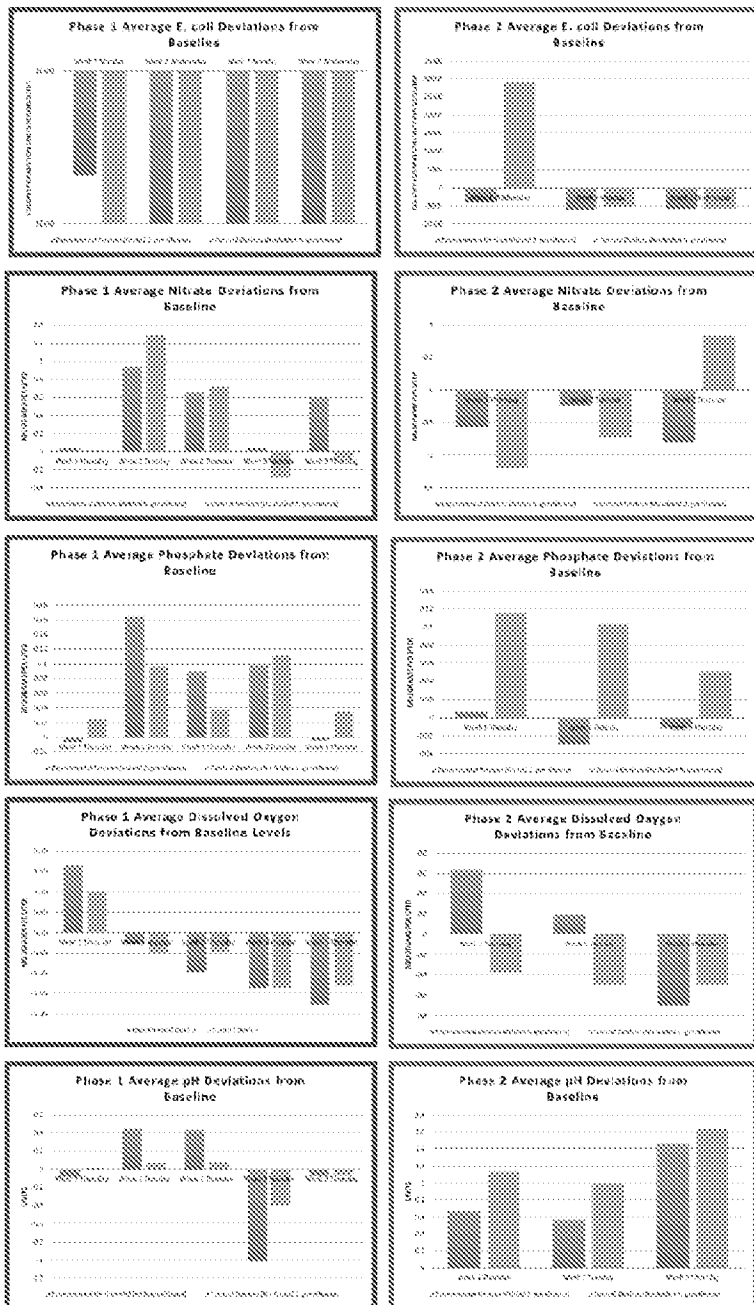
Percent Change from Baseline by Parameter

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

+ approximate deviation has a factor of 10^{-8} , so efficiency was used to differentiate testing groups

The results were separated into Phase 1 and Phase 2. Change was measured from baseline levels.

Phase 1 differed from Phase 2 in that three times the amount of algae was added to experimental algal cartridges in Phase 2 compared to Phase 1. *E. coli* was analyzed using relative efficiency due to minor differences between testing groups. All levels were significant except for pH in both phases.



In both Phase 1 and Phase 2, *E. coli* levels decreased almost 100% compared to baseline for the experimental group. In both phases *E. coli* levels for the control also decreased almost 100% compared to baseline but at an overall slower rate. *E. coli* levels during Phase 1 were reduced from 1.000×10^{10} to 1.940×10^2 for the experimental group and from 1.000×10^{10} to 2.220×10^2 for the control group. *E. coli* levels during Phase 2 were reduced from 6.178×10^3 to 3.333×10^1 for the experimental group and from 6.011×10^3 to 2.200×10^1 for the control group.

During Phase 1 nitrates had a slight increase for the experimental group of 23.2% and an eventual negative fluctuation for the control group of 2.6%. During Phase 2 nitrates had a negative fluctuation for all post-baseline levels and an overall

reduction of 23.3% for the experimental group, while the control group had an initial drop followed by an overall increase of 23.4%. Phase 1 phosphates oscillated for the experimental

group, resulting in an overall decrease of 13.5%, while the control group had positive fluctuations for all post-baseline levels and increased by 159.1%. Phase 2 phosphates were characterized by a slight increase and then decrease for the experimental group, for an overall reduction of 29.8%. The control group showed a positive increase of 185.2% compared to baseline. During Phase 1, dissolved oxygen had similar initial increases, then decreases starting Week 2 for both groups, leading to an overall decrease of 20.0% for the experimental group and an overall decrease of 14.7% for the control. During Phase 2 dissolved oxygen increased and then decreased for an overall decrease of 10.9% for the experimental group, while the control group showed negative fluctuations for all post-baseline levels, decreasing 6.7% overall. Phase 1 pH had oscillations for both testing groups with greater magnitudes of change compared to baseline for the experimental group and an overall decrease for the experimental and control devices of 0.80%. Phase 2 pH showed continuously increasing pH for both testing groups, with an overall 19.6% increase for the experimental group and 24.0% increase for the control group.

Following the 1.5IQR rule for determining statistical outliers, there were several outliers in Phase 1. For Week 1 Thursday, both dissolved oxygen levels were outliers. Also, Week 3 Tuesday had both pH levels as outliers. Phase 2 did not have any outliers according to the 1.5IQR definition. Phase 2 had greater fluctuations in all levels compared to Phase 1.

E. coli levels were analyzed using a relative efficiency formula

$$E = \left(\frac{dy_E}{dt} \div \frac{dy_C}{dt} \right) - 1 = \frac{\frac{dy_E}{dt} \cdot \frac{dy_C}{dt}}{\frac{dy_C}{dt} \cdot \frac{dy_C}{dt}} \text{ The equation is a ratio of instantaneous rates of the } E. coli$$

regressions with a correction constant to center the graph over y=0 rather than y=1. This formula indicates the experimental group is more efficient than the control group when $E > 0$. For Phase 1,

$$E = \frac{(1.599x^2 + 6.048x + 1.656)(10^{0.555x^2 - 0.024x^2 + 0.456x + 10.110}) - (1.473x^2 + 6.048x + 1.656)(10^{0.491x^2 - 0.024x^2 + 1.015x + 10.185})}{(1.473x^2 + 6.048x + 1.656)(10^{0.491x^2 - 0.024x^2 + 1.015x + 10.185})}$$

and $E > 0$ for $t \geq 1$, meaning that after 1 day of filtration, the experimental group became more efficient relative to the control group for the remainder of the duration of the

$$\text{experimentation. For Phase 2, } E = \frac{-0.576 \times 10^{-0.576x + 0.291} - (1.156x - 0.823)(10^{0.575x^2 + 0.825x + 3.907})}{(1.156x - 0.823)(10^{0.575x^2 + 0.825x + 3.907})} \text{ and}$$

$E > 0$ for $0 < t < 1$, meaning that during the first day, the rate of *E. coli* reduction was greater for the experimental devices than the control devices. Therefore, at times very close to the initial filtration in the experiment, the algae was more efficient relative to the control. This concurs with the data as Phase 2 was characterized by a rapid decrease in *E. coli* levels that occurred during the time period that $E > 0$.

Discussion

This experiment utilized devices that were designed to contain *S. grevilleana* and form an algal filter for filtering lake water. The devices used for the experimental and control groups in both phases were built identically. The original idea for this device was based on a tea bag with live algae inside to place in water to filter nutrients. Expanding on the concept of tea bags, convex metal mesh tea filters were placed on both ends inside of a removable cartridge designed to contain algae. The cartridge and experimental devices were designed with transparent PVC pipe parts so that algae would have exposure to the sun for photosynthesis. The cartridge containing algae was opened on both ends and inserted into the experimental device, the device was closed, and the pump was turned on. The lake water was continuously circulated through the PVC pipe through clear tubing attached to a recirculating fountain pump in the sample bucket so water would circulate through the filters and algae. As water started circulating through the device, the algae formed an algal turf on the mesh filter inside the pipe. The convex shape of the filter allowed for more surface space for the algae to form a larger algal turf in the pipe than a flat mesh filter.

All of the experimental and control devices for both phases were built utilizing the same specifications. The only difference between the two testing groups was the addition of *S. grevilleana* to the algal cartridge in the experimental devices in both phases. Therefore, the differences in the results were due to the addition of *S. grevilleana*, not the algal filtration device itself.

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, so the amount of algae was increased in Phase 2 to create a thicker

algal mat to completely cover the mesh filter. The efficiency of varying amounts of algae merits investigation.

E. coli was reduced by almost 100% for experimental and control groups in both phases. The experimental groups in both phases had a higher rate of efficiency than the control groups during the peak periods of *E. coli* reduction. Since relative efficiency is a comparison of the rates of reduction, it can be observed from the efficiency that the periods of greatest reduction correlate with the intervals in which the experimental group had a larger magnitude of slope in the regression of the reduction. Furthermore, at time intervals after the initial reduction (maximum rate), the effect of efficiency is negligible as there is a relatively minute amount of *E. coli* still in the water, thus the efficiency is comparing relatively small rates. This means that in two comparable lakes running devices, the algal filtration device with *S. grevilleana* would more efficiently reduce *E. coli* levels until those levels were reduced to such a small magnitude that efficiency rate could not be compared.

One proposed reason for the patterns in the relative efficiency is that large amounts of *S. grevilleana* in Phase 2 produced enough secondary metabolites to cause an almost instantaneous, rapid reduction of *E. coli*, while the relatively small amount of algae in Phase 1 may have needed time to build up the levels of secondary metabolites needed to kill off the majority of the *E. coli* population.

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 control group had nitrate levels increased by 23.4% and phosphate levels increased by 185.2%. The additional algae added to the experimental devices in Phase 2 increased the effectiveness of the devices. By removing nitrate and phosphates from the water, *S. grevilleana* proved to be an effective algal filter.

The experimental group had dissolved oxygen levels decreased by 20% in Phase 1, and decreased by 10.9% in Phase 2. The control group had dissolved oxygen levels decreased by 14.7% in Phase 1, and decreased by 6.7% in Phase 2. Both the experimental and control groups had pH levels decreased by 0.8% in Phase 1. For Phase 2, pH for the experimental group was

increased by 19.6% and increased by 24.0% for the control group. Changes in pH for both phases were negligible. For both phases the dissolved oxygen and pH remained at safe levels.

Water quality is a critical issue as clean water, both potable and nonpotable, is in scarcity in the world today. Many people are without access to clean water due to bacterial contamination and nutrient overload. The same pollution is responsible for a decline in the biota in Earth's fresh, brackish, and salt waters. All living things need water, and with declining clean water sources and current increases in global pollution it is critical to find new ways to improve water quality throughout the world. Lake water quality is especially important in Georgia as Lake Lanier is a major source of drinking water. An algal genus commonly found in Georgia lakes, *S. grevilleana* utilized in an algal filtration device could potentially improve the water quality of Lake Lanier and subsequent drinking water.

Fertilizer runoff is also a major environmental issue in freshwater lakes. In addition to spiking *E. coli* levels, fertilizer pollution from runoff can damage lake ecosystems and food chains, and hurt water quality. According to background research, nutrient mismanagement, including fertilizer pollution, is a major threat to water quality. Adding chemicals to remove *E. coli* and excess nutrients from water is not a sustainable solution. Utilizing algae to reduce *E. coli* and pollution from runoff can be an environmentally friendly method of biological cleaning power.

This experiment successfully utilized *S. grevilleana* to reduce *E. coli* and excess nitrates and phosphates from the water. Research indicates that *S. grevilleana* decreases *E. coli* levels. Rather than harboring *E. coli*, *S. grevilleana* decreased nitrogen and phosphate available as nutrients for *E. coli* growth. The results comply with background research as sources indicate that algae is an effective water quality improver. Algae propagate reduction in bacterial levels through various metabolic processes, including the excretion of antibacterial secondary metabolites. Future experiments could potentially refine the prototypes so that the algal filtration unit can be used to improve the water quality in fresh water lakes of different sizes and in multiple locations.

The algal filtration device tested in this experiment is different in many aspects from currently utilized filtration devices, such as those used in ATS technology. The algal filtration device is different from ATS in that the body of water being filtered lacks flow, is closed from the elements, and requires only a small footprint. In addition, the device is portable, inexpensive,

and viable in multiple locations, unlike stationary, expensive ATS systems. Rather than being designed for industrial applications, the device tested in the experiment 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 the experiment were to be redone, water from different lakes would be tested to further verify the findings. Different types and amounts of algae would be tested with the removable cartridges. The circulating pump could be solar powered to make the device self-sufficient, portable and sustainable. Experimentation would take place in the field at a freshwater lake. The cartridge from the algal filtration device could be sent to a biofuel lab for potential biofuel production from the residual algae. Further experimentation would investigate the potential to create potable water from this algal filtration method.

The reduction of *E. coli* overall was of a magnitude of 10. The initial *E. coli* count overall for Phase 1 was 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 overall was high levels of *E. coli*. 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.

Comparison of EPA Surface Water Standards

Parameter	EPA Standard	This experiment (averages unless state otherwise)	
		Phase 1	Phase 2
Dissolved Oxygen	5-6 mg L ⁻¹ , up to 14.6 mg L ⁻¹	8.150	8.967
pH	6-8, lowest 3	7.083	5.700
<i>E. coli</i> /Coliform (Water Designated for Swimming)	<235 CFU dL ⁻¹	193.4 CFU dL ⁻¹	33.3 CFU dL ⁻¹

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 most likely have been considered safe for swimming. Confirmation would require additional testing.

According to the results from the experiment and the EPA standards for potable water, there is strong evidence to suggest that *S. grevilleana* paired with the algal filtration device has

Comparison of the 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 ⁺
E. Coli/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

the potential to filter lake water to provide a source of potable water. A comparison of *E. coli* and nitrate and pH levels to EPA

potable water standards was conducted. In both Phase 1 and Phase 2, *E. coli* and nitrate levels were reduced to ranges that fell within the EPA threshold range for potable water. pH, which is an optional secondary standard for potable water, was 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. Further experimentation and testing of additional parameters, such as metals, will be conducted to confirm this postulate.

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 relationship must be established. The expression $n = \frac{\Gamma Q}{V}$ represents the volume flow rate (Q), the undefined flow efficiency constant (Γ), the number of devices (n) which is a constant, and the volume of water in a given lake (V). Using substitution of: $Q = \frac{dV}{dt}$ and rearranging the equation to form a differential equation: $n = \frac{\Gamma}{V} \frac{dV}{dt} \rightarrow n dt = \frac{\Gamma}{V} dV$ This equation allows the expression to be integrated: $\int \frac{\Gamma}{V} dV = n \int dt \rightarrow \Gamma \ln|V| = nt + C$ Another undefined constant, the flow intercept constant (Λ) defines C, leaving the final equation: $\Gamma \ln V = nt + \Lambda$ which shows the mathematical model relating the number of devices to the time needed to filter a lake of a certain volume one time. For example, a lake of 100,000 m³ can be filtered once completely by 3 devices in approximately 5 weeks.

As a comparison to an ATS system, the smallest ATS available covers 1,012 m² of land (roughly 2.5 acres) versus one unit of the algal filtration system, which covers 4.258x10⁻² m² of land (roughly 0.0011 acres). Thus the three devices needed to clean the 100,000 m³ lake would

only occupy $1.277 \times 10^{-1} \text{ m}^2$ of land (roughly 0.0033 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 \$350.00 to build, including the PVC pipe and connectors, fittings, tubing, filters, recirculating pump, and algae. The three devices needed to clean the $100,000 \text{ m}^3$ lake would cost approximately \$1,050. Even if multiple devices are needed for a larger lake, the device's small size and construction of common materials offer an economical and attainable solution.

S. grevilleana was chosen for this project based on prior years' experimentation demonstrating these algae to be effective in improving water quality in terms of dissolved oxygen, as well as a previous experiment showing the algal filtration device to be a promising solution to lake fertilizer pollution. 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.

Conclusions

1. The functionality of the concept of the utilization of a *S. grevilleana* algal filter to reduce *E. coli* in freshwater lakes has been accepted by experimentation.
2. The *E. coli* levels in the lake water were reduced by almost 100%, with the experimental group containing *S. grevilleana* exhibiting greater efficiency than the Control in both trials in terms of reducing *E. coli* levels.
3. Nitrate and phosphate levels were reduced, while dissolved oxygen and pH levels were maintained.
4. Since all of the algal filtration devices were identical, the difference in results demonstrated that the addition of *S. grevilleana*, not the algal filtration device itself, improved the effectiveness of the freshwater filtration and subsequent reduction of *E. coli*.

5. 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.
6. *S. grevilleana* was selected as it is native to Georgia lakes where the scientist lives, and based on prior experimentation proving *S. grevilleana* to be effective in improving water quality in terms of dissolved oxygen. *S. grevilleana* is found in freshwater lakes worldwide. Different algae could also be tested to determine the most efficient algal species for algal filtration under different conditions and in specific regions of the world.
7. Further design modifications could enhance the ease of use and increased sustainability through a self-sufficient power source.
8. The conclusion of the experiment is that *S. grevilleana* is a potentially effective algae for use in an algal filtration device to reduce *E. coli* levels in freshwater.
9. These algal filtration devices could be utilized in small private lakes or in larger public lakes both locally and potentially in freshwater lakes worldwide.
10. The ability to produce potable water is also a potential utilization of algal filtration, and should be explored.

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APPENDIX B

Entry to the Stockholm Junior Water Prize (2013)

Using an Algal Filter to Improve Water Quality

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Abstract

This study evaluated the use of *Spirogyra grevilleana* as a means of algal filtration to improve water quality and potentially mitigate nutrient pollution in freshwater lakes. The testing was split into two phases simulating oligotrophic and eutrophic conditions. Initially the algal filtration device prototypes were designed and constructed. Water was collected from a freshwater lake and run continuously through the filtration devices in a lab, with fertilizer added prior to experimentation in Phase 2. Samples were tested for nitrates, phosphates, dissolved oxygen, and pH levels for two weeks. In both phases, samples utilizing *S. grevilleana* in the algal filtration device had decreased nitrate levels by as much as 60%, decreased phosphate levels by as much as 75%, increased levels for dissolved oxygen, and negligible changes in pH. The control saw no decrease in nitrate or phosphate levels, varied levels for dissolved oxygen, and negligible changes in pH. Utilizing *S. grevilleana* in the algal filtration device could potentially provide a sustainable, flexible, and low cost solution to nutrient pollution in freshwater lakes worldwide.

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Key Words

Algal Filter; *Spirogyra grevilleana*; Nutrient Pollution; Water Quality; Algal Turf Scrubbers; Eutrophication

Abbreviations and Acronyms

ATS – Algal Turf Scrubbers; PVC – Polyvinyl Carbonate; S. – Spirogyra; ppm – parts per million;

lpd – liters per day; UGA – University of Georgia

Introduction

Freshwater ecosystems have been critical to sustaining life and establishing civilizations throughout history. As a result of this dependence, many human settlements worldwide are concentrated near freshwater ecosystems, with “over half the world’s populations [living] within 20 km of a permanent river” [12]. One of the most widespread global issues of the 21st century is scarcity of drinking water in many regions of the world. As global populations grow, increased agricultural and industrial production lead to a widespread increase in nutrient pollution. Most freshwater bodies the world over are becoming polluted, thus decreasing potability of the water. Today, eutrophication of natural waters, caused by an increase in dissolved nutrients, is one of the most significant causes of a decline in water quality [18].

This study focuses on utilizing *S. grevilleana*, a filamentous green algae, as a potential algal filter to naturally decrease the effect of dissolved nutrients in freshwater lakes. Based on prior experimentation and research, the potential positive effects of algae on water quality have been established. This experiment utilized *S. grevilleana* in a novel way as an algal filter in an enclosed device to improve water quality in terms of nitrates, phosphates, dissolved oxygen, and pH. If shown to be effective, this device could be used in freshwater lakes to decrease the effects of nutrient pollution and subsequent eutrophication.

The three main threats to water quality are soil erosion, nutrient mismanagement, and pesticide mismanagement [4]. Soil erosion, also known as surface water runoff, damages water quality as the debris from erosion enters surface water. Soil erosion affects water quality physically, chemically, and biologically. Sources of soil erosion include agricultural fields, woodlands, highway road banks, construction sites, and mining operations [7].

Nutrients can also enter the water through runoff, such as storm water from rain or melting snow. This water flows over areas and picks up contaminants, such as fertilizers. According to the Mid-American Regional Council [25], “polluted storm water contaminates streams, rivers, and lakes. [The polluted water] can kill or damage plants, fish, and wildlife,

while degrading the quality of the water.” Urban sources of water pollution include runoff from rooftops, roads, and lawns, as well as wastewater, lawn treatment and chemicals, and household chemicals [5].

Fertilizers are a major contributor to environmental pollution. As primary nutrients in fertilizer, nitrogen and phosphorous in raised concentrations increase algae productivity, especially filamentous green algae [8]. High levels of phosphorus and nitrogen may contribute to out-of-control algae blooms, blocked sunlight, depleted O₂ at night, and reduced biodiversity [23].

Nutrient pollution from fertilizer is an enormous problem in freshwater lakes. According to Baird [3], “excess nitrogen and phosphorous in surface waters ... and nitrogen in ground water cause eutrophication ...” Eutrophication is the process of increasing organic enrichment or biological productivity and is generally equated with increased phosphorous, leading to increased algae and aquatic weed growth, oxygen shortages, and formation of carcinogens during water chlorination [22]. According to Buhvestova, et al [6], “Eutrophication is the most serious environmental issue in most lakes in low lying areas.” Although eutrophication is a natural process of aging of lakes and water bodies, human activities can greatly accelerate eutrophication by accelerating the rate at which nutrients and organic substances enter aquatic ecosystems from their surroundings [8]. World population will continue to grow, reaching an estimated 9.2 billion by 2050, which will increase pressures on the productive capacity of agriculture and industry, leading to increases in nutrient pollution. Noting that nutrients in runoff can cause extra algal blooms, Wierderholt and Johnson [27] state that one extra pound of phosphates in water equates to hundreds of pounds of extra algae and aquatic plant mass, which lowers dissolved oxygen levels. Eutrophication limits water usage for drinking, fisheries, recreation, and industrial use.

Nutrient pollution caused by runoff from fertilizers, primarily nitrogen and phosphorus, is causing eutrophication in freshwater lakes worldwide from Shahpura Lake in Bhopal India, to Tai Lake in China, to Lake Erie in the U.S. One significant example of the effects of nutrient pollution is Lake Victoria, the second largest freshwater lake in the world and an important water source shared by Kenya, Uganda, and Tanzania. The lake is threatened by pollution and has an algal mass on the surface due to increased pollution from human and industrial waste containing phosphates and nitrates [24]. Another important example of the impact of nutrient pollution is

Lake Erie. Phosphorus has been shown to be a major nutrient controlling phytoplankton growth in the Great Lakes [15]. Cultural eutrophication that causes excess algae growth can be linked to various degraded water quality conditions. In Lake Erie, hypoxia has been directly linked to elevated in-lake total phosphorus concentrations and excessive external total phosphorus loadings. Phosphates in detergents were limited by state law as an effort to reduce these effects. Following the ban, target loads for total phosphorus yearly concentrations of total phosphorus and dissolved oxygen depletion rates declined significantly. Reductions in algal biomass were also observed, especially in nuisance and eutrophic species [9]. Prior to the ban, the diatom populations shifted towards the dominance of species favored by nutrient rich conditions, cyanobacteria were in abundance, as well as the green algae *Cladophora* and the red alga, *Bangia* [15].

Algae can pose problems for water quality under certain conditions. For example, the Chesapeake Bay has massive algal blooms each spring caused mostly by fertilizer runoff from lawns and farms that is deposited into the bay by multiple rivers that feed water into the bay. When the algal bloom dies off, the resulting decomposition depletes life-supporting oxygen from the water, causing dead zones in the bay [26].

Conversely, algae can also be helpful to water quality. One invention that improves water quality by using algae and then harvesting the algae for biodiesel is called “Algal Turf Scrubbers,” abbreviated ATS [1]. Adey and Bannon [1] defined algal turfs as “communities of organisms dominated by aggregations of unicellular to branched filamentous algae and cyanobacteria (blue-green algae). The algae are attached to hard substrates, rock, wood, and plant stems.”

The original use of ATS, as invented by Walter Adey, was to clean aquariums at the Smithsonian Museum of Natural History [26]. Currently, University of Maryland researcher Patrick Kangas is using ATS systems to clean bodies of water such as in the Chesapeake Bay. Unlike the uncontrolled overgrowth of algae in bodies of water, like the Chesapeake Bay, algal growth in ATS systems is controlled and therefore beneficial rather than harmful to the water quality [10]. ATS systems work with fresh water, brackish water, and salt water, as well as with a variety of waste and industrial settings, such as water quality treatment plants, farm canals,

rivers, and the inside of industrial smoke stacks [2] [17]. Currently, commercial rights for the ATS systems are held by HydroMentia [16].

There are several key components to the functionality of ATS systems. The design of ATS devices is a series of troughs with thin screens that catch algae. The algae involved in the filtration process are filamentous algae [12]. Solar powered pumps add water to the open troughs from a river or other flowing body of water. The water flows through the trough, being filtered along the way. Finally, the water trickles back into the flowing body of water or into a stagnant body of water, such as a bay. The troughs are designed to maximize algal growth by emulating the conditions near coral reefs, including mimicking the pulsation of waves [2]. To keep the ATS system running smoothly and to keep fresh algae in the system, the algae are harvested every five to fifteen days [2]. The harvested algae can then be converted into biofuels, such as biodiesel, gasohol, methane, and butanol, an alcohol that can be used in lieu of gasoline [2] [26].

ATS are highly productive and efficient systems. Algal turfs in ATS environments remove large quantities of nutrients and CO₂ from the water and, as a result, the algae increase dissolved and airborne O₂ in addition to elevating the pH of the water [2]. Wheeler [26] states that, in terms of nutrient absorption, one acre of ATS is equivalent to approximately fifty acres of farmland populated with cover crops; cover crops are the most efficient land-based remover of aqueous nitrogen and phosphorous.

Negative aspects of ATS systems include size and cost. In addition, ATS systems take up massive amounts of area on land (for land-based ATS systems) and on water (for floating ATS systems) [26]. Wheeler [26] states that approximately a combined 10,000 acres of land-based and floating ATS systems would be needed to clean bodies of water such as the Chesapeake Bay, and that the average dimensions of a current ATS land-based system is a width of 50 meters by a length of 1800 meters, the length of an average airport runway.

Algae are photosynthetic protists that, according to Gibson [11], are "... estimated [to] produce 73-84% of the net global production of oxygen." They are usually unicellular and mobile. Algae can be good indicators of the trophic status of bodies of water. Green algae live mostly in oligotrophic water, while cyanobacteria live mostly in eutrophic water. Oligotrophic water has poor nutrient content, and eutrophic water is rich in nutrients [21].

The algae utilized in this experiment are *Spirogyra*, a genus of freshwater green filamentous algae. The origin of the name “*Spirogyra*” comes from the Greek “*Spiera*” (meaning coil) and “*gyros*” (meaning twisted) since the cells contain spiral chloroplasts [19]. According to ABC’s of nature [21], “*Spirogyra* grows in long many-celled, hair-like strands.” There are more than 400 species of *Spirogyra* [19]. *S. grevilleana* was initially chosen for experimentation as this species of algae is commonly found in freshwater lakes in the scientist’s home state of Georgia, and was subsequently shown in research to affect and maintain water quality [14].

S. grevilleana was chosen for this project based on prior year’s experimentation that proved these algae to be effective in improving water quality in terms of dissolved oxygen. Previous work also indicated the harmful effects of slow release fertilizer on water quality, leading to this study investigating the impact of an algal filter utilizing *S. grevilleana* to improve water quality and mitigate the effect of increased nutrients in freshwater lakes due to nutrient pollution. The study also aimed to build a cost effective and portable device utilizing *S. grevilleana* as an effective algal filter to provide a sustainable solution to improve water quality of freshwater lakes in a variety of sizes and locations.

Methods

The experiment was conducted in two phases, each with slightly different procedures. Phase 1 was designed to simulate oligotrophic conditions by utilizing lake water without additional nutrients added in the lab. Phase 2 was designed to simulate eutrophic conditions by adding fertilizer to the lake water in the lab prior to experimentation.

Phase 1: Initially two prototypes of the Phase 1 Experimental Device were constructed using the exact same materials, measurements, and procedures. Two white 5.08 cm PVC pipes, with a length of 30.48 cm each, were both fitted with a clear PVC connector, one male and one female, on one end of the pipe with a plastic strainer placed between the pipe and the connector (see Diagram 1). Clear PVC was used for the connectors to allow sunlight to reach the algae inside. 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 the tubing secured on each end. Then the devices were tested for function and seal with distilled water.

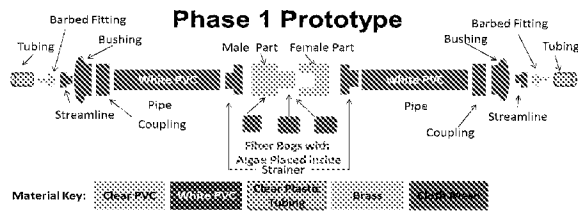


Diagram 1: Expanded view of Phase 1 experimental device

A lab was then set up at the high school. Two 19L buckets were filled with 16L of water collected from a freshwater lake at 33°59'29.04''N, 84°15'26.64''W. The buckets were then transported to the lab by car. One bucket was labeled "Control" and the other "Spirogyra." The buckets had their lids removed, and were left to sit overnight.

The baseline levels of nitrates, phosphates, dissolved oxygen, and pH were then established using LaMotte test kits for both samples following proper lab safety precautions. After the baseline testing was completed, three test tubes of *S. grevilleana* were poured one at a time into

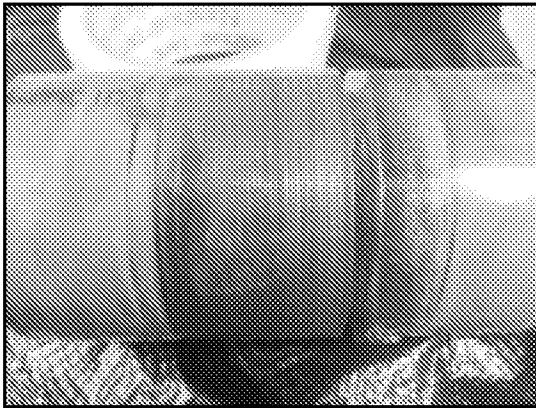


Photo 1: *S. grevilleana* in mesh bags inserted in clear center section of the Phase 1 Device

three separate mesh bags that were held over the waste bucket. Then, the bags were inserted into the clear male piece of the Phase 1 experimental device. After the algae bags were inserted, the device was closed by twisting the male piece into the female piece. Three empty mesh bags (without algae) were then inserted into the clear male piece of the Phase 1 Control device. After the empty bags were inserted, the Control device was closed by twisting the male piece into the female piece.

Fountain pumps were utilized to recirculate the water. One pump was affixed to the bottom of the Spirogyra sample bucket using suction cups on the bottom of the pump, then connected to the closest barbed fittings on the Spirogyra experimental device using clear rubber tubing. An additional tube was attached to the far end of the Spirogyra experimental device and fed back into the sample bucket. Tubing was connected for the control sample bucket pump using the

same method. The pumps were turned on until the testing was completed on the last day of the trial.

The testing of nitrates, phosphates, dissolved oxygen, and pH was conducted every school day for two weeks following proper safety precautions. At the end of Phase 1 testing the algae was properly bleached and disposed.

Phase 2: After completing Phase 1, improvements were made to the overall design. A source for clear PVC pipe was found to allow more sunlight to reach the algae. A convex mesh teapot filter was also incorporated into the new design to allow the algae to move freely within the tube and to provide a greater surface area for the *S. grevilleana* to form an algal filter within the tube. Two prototypes of the Phase 2 Experimental Device were then constructed, each using the exact same materials, measurements, and procedures.

Two clear 5.08 cm PVC pipes, with a length of 30.48 cm each, were each fitted with a clear PVC connector, one male and one female, on one end of the pipe (see Diagram 2). A concave

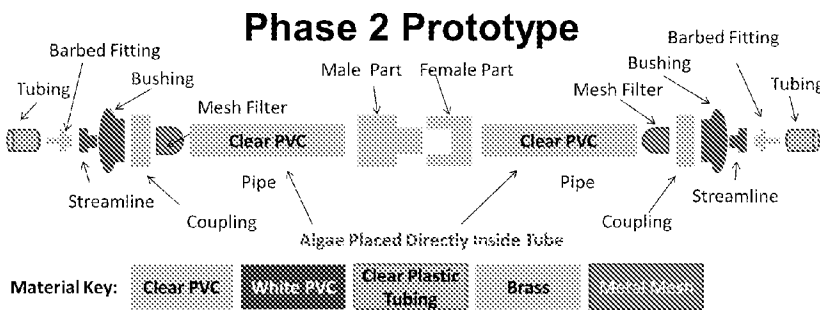


Diagram 2: Expanded view of Phase 2 experimental device

mesh filter was placed on the end of the pipe, then bushing and barbed fittings were added on the end of the pipe to provide a seal between the pipe and the tubing. The male and female connectors were screwed together and tubing secured on each end. Then the

devices were tested for function and sealed with distilled water.

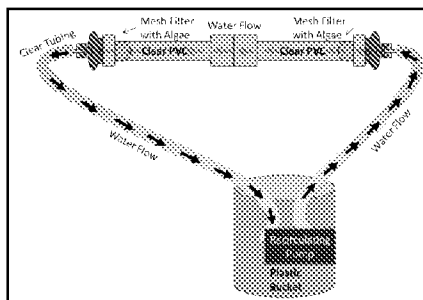
Two 19L buckets were filled with 16L of water was collected from a freshwater lake at: 33°59'29.04''N, 84°15'26.64''W. The buckets were then transported to the lab by car. One bucket was labeled "Control" and the other labeled "Spirogyra." The bucket lids were removed.

Fertilizer was made by measuring 4g of NH_4NO_3 , 2g of NaH_2PO_4 , 3g of KNO_3 , and 2g of $\text{Na}_2\text{SO}_4 \cdot 10\text{H}_2\text{O}$ with a triple-beam balance. The procedures for the fertilizer were repeated to create a second sample of fertilizer. Preliminary nitrate and phosphate levels were taken using

LaMotte test kits. One container of fertilizer was added to each sample bucket and stirred, then the sample buckets were allowed to sit overnight before further testing.

Baseline levels of nitrates, phosphates, dissolved oxygen, and pH were established using LaMotte test kits. Ten test tubes of *S. grevilleana* were poured into one side of the clear PVC pipe over the waste bucket into the Phase 2 Spirogyra experimental device. The device was closed by screwing the male part into the female part and coating the male part with sealing tape. The Phase 2 control device was closed without any algae by the same method.

Diagram 3: Process diagram of water circulation



One of the recirculating pumps was affixed to the bottom of the Spirogyra sample bucket using the suction cups on the bottom of the pump, then connected to the closest barbed fittings on the Spirogyra experimental device using clear rubber tubing (see Diagram 3). An additional tube was attached to the far end of the Spirogyra Experimental device and fed back into the sample bucket. Tubing was connected for the control bucket pump using the same method. The pumps were turned on until after testing was completed.



Photo : Phase 2 Devices.

Bottom device: Spirogyra with *S. grevilleana* forming an algal turf inside; Top device: Control

The testing of nitrate, phosphate, dissolved oxygen, and pH levels was conducted using LaMotte test kits every school day for ten weekdays. At the end of testing the algae was properly bleached and disposed.

Results

The results were separated as Phase 1, simulating oligotrophic conditions, and Phase 2, simulating eutrophic conditions through the addition of fertilizer prior to experimentation. Changes were measured from baseline levels. The percent change from baseline for nitrates was a 100% decrease for *Spirogyra* for Phase 1 and a 60% decrease for Phase 2. There was no change from baseline for control in either Phase 1 or Phase 2. The fluctuations for nitrate levels for *Spirogyra* followed a similar pattern for both Phase 1 and Phase 2 (see Figures 1 and 2).

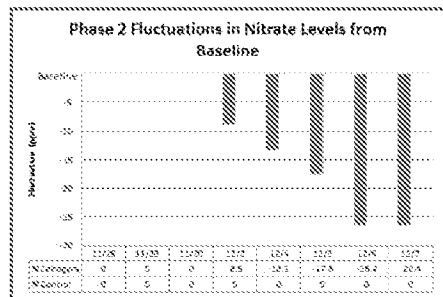


Figure 1

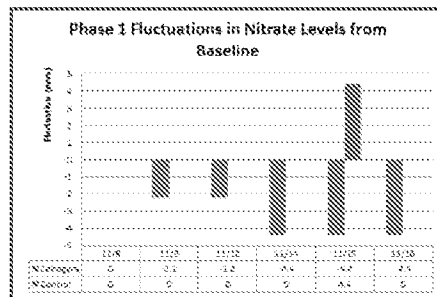


Figure 2

The percent change from baseline for phosphates for *Spirogyra* was a 100% decrease for Phase 1 and a 75% decrease for Phase 2. There was relatively no change for phosphates for control in either Phase 1 or 2. The fluctuation pattern was varied for *Spirogyra* for Phase 1 and consistently negative for Phase 2 (see Figures 3 and 4).

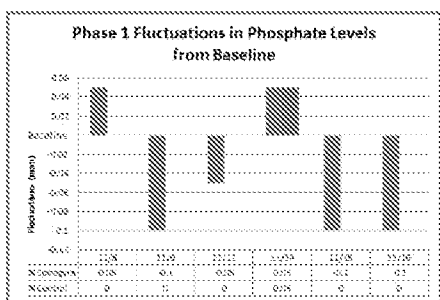


Figure 3

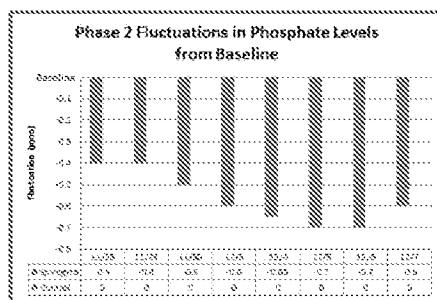


Figure 4

For Phase 1, the percent change from baseline for dissolved oxygen was a 2.4% increase for Spirogyra and a 4% increase for the control. For Phase 2, the percent change from baseline for dissolved oxygen was a 4.6% increase for Spirogyra and a 9.8% decrease for control. The dissolved oxygen fluctuations for Spirogyra followed a somewhat similar pattern of positive and negative fluctuations for both Phase 1 and Phase 2 (see Figures 5 and 6).

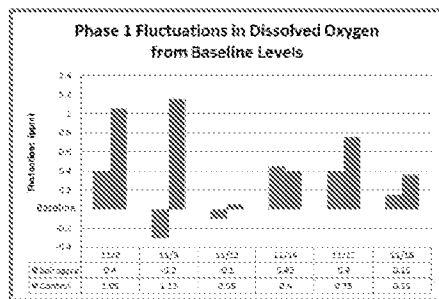


Figure 5

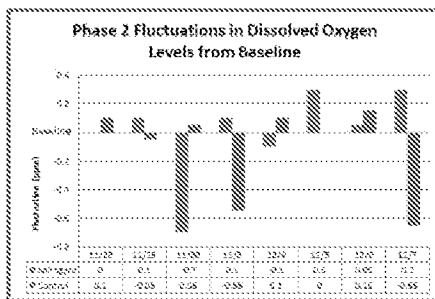


Figure 6

The percent change from baseline for pH for Phase 1 for both Spirogyra and control was no change, compared to an 8.3% increase for Spirogyra and a 4% increase for control in Phase 2. The pH levels overall were consistently higher for Phase 2 than Phase 1 (see Figures 7/8).

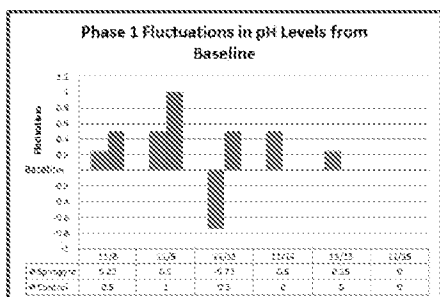


Figure 7

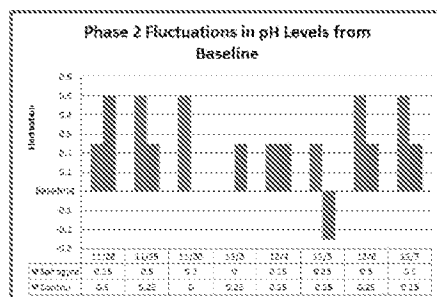


Figure 8

Percentage Change from Baseline

The percentage changes from baseline for the control samples overall showed little to no change for Phase 1 and Phase 2. With the exception of pH for Phase 1, the Spirogyra samples showed

fluctuations throughout the experimentation period, especially for nitrates and phosphates, which were dramatically reduced for both oligotrophic and eutrophic conditions (see Table 1).

Discussion

The experiment was conducted in two phases. The *Spirogyra* sample in both phases, which utilized an algal filtration device with *S. grevilleana* added, had reduced nitrate and phosphate levels, increased dissolved oxygen levels, and maintained pH levels. The control in both phases, which also utilized an algal filtration device but with no algae added, did not decrease nitrate or phosphate levels and maintained a constant pH. The control had an increase in dissolved oxygen in Phase 1 and a decrease in Phase 2.

	Spirogyra	Control
Phase 1 – Oligotrophic Conditions		
Dissolved Oxygen	2.4% Increase	5.6% Increase
pH	No Change	No Change
Nitrates	100% Decrease	No Change
Phosphates	100% Decrease	No Change
Phase 2 – Eutrophic conditions		
Dissolved Oxygen	4.6% Increase	9.8% Decrease
pH	8.3% Increase	4% Increase
Nitrates	60% Decrease	No Change
Phosphates	75% Decrease	No Change

Table 1: Percent Change from Baseline for samples utilizing the algal filtration unit

Both phases utilized devices designed to contain *S. grevilleana* and form an algal filter inside the pipe for the lake water to be pumped through. Both devices were designed with transparent PVC pipe parts so that the algae would have exposure to the sun for photosynthesis. They both have different methods for containing the algae. The two devices used for control and *Spirogyra* in Phase 1 were designed and built with identical features. The device utilized in Phase 1 had transparent male and female connectors in the center section of the device between white PVC pipes. The second prototype had clear PVC pipe and connectors to increase access to light for photosynthesis. Both the control and *Spirogyra* Phase 2 experimental devices were designed and built with the same modifications.

The original idea for this device was based on a tea bag with live algae inside to put into the water. In Phase 1, the algae were placed in mesh filter bags and inserted into the transparent center section, the device was closed, and the pump turned on so that the lake water was

continuously circulated through the PVC pipe through clear tubing attached to a recirculating fountain pump in the sample bucket. The device utilized in Phase 2 had both transparent PVC pipe and transparent male and female connectors in the center sections to increase the amount of sunlight exposure. Expanding on the idea of tea bags, convex metal mesh inserts designed to hold loose tea inside a teapot were substituted in the design rather than mesh bags. The algae were put directly inside the pipe and held inside by dome shaped mesh filters sealed on either end of the pipe. Concurrent with Phase 1, the device was closed, and the pump turned on so that the lake water was continuously circulated through the PVC pipe through clear tubing attached to a fountain pump. The algae in Phase 2 formed an algal turf inside the pipe on the mesh filter. The convex shape of the filter allowed for more surface space for the algae to form a larger algal turf in the small diameter of the pipe than a flat mesh filter.

The filtration worked in both oligotrophic (Phase 1) and eutrophic (Phase 2) conditions, both times following similar trends. In Phase 1, simulating oligotrophic conditions with a low level of nutrients, the lake water used did not have fertilizer added to either sample bucket. In Phase 2, simulating eutrophic conditions with a high level of nutrients, the lake water had fertilizer added in the lab to both buckets. Although Phase 2 had a greater range of values and fluctuation from baseline, both Phase 1 and Phase 2 *Spirogyra* samples had decreased nitrate and phosphate levels, increased dissolved oxygen levels, and maintained pH levels. Therefore, the filtration device was effective in both high and low nutrient situations, making it more versatile and more beneficial to the environment.

In Phase 1, the *Spirogyra* sample had nitrate and phosphate levels that were reduced to undetectable levels while the control had negligible changes in nitrate and phosphate levels. The *Spirogyra* and control samples both had raised dissolved oxygen levels and mainly unchanged pH levels. In Phase 2, the eutrophic condition simulation, the *Spirogyra* sample had nitrate levels reduced by 60% and phosphate levels reduced by 75%, while the Control bucket had no measurable changes in nitrates and phosphates during the entire experiment. Phase 1 had raised dissolved oxygen levels while the control bucket had decreased dissolved oxygen levels. Both the *Spirogyra* and control buckets had mainly unchanged pH levels. By removing such a large percentage of the nitrates and phosphates from the lake water, the *S. grevilleana* proved to be an effective algal filter.

In both phases, there were large differences in the nitrate levels of the two testing groups. The Spirogyra sample absorbed nearly 100% of nitrates compared to baseline in Phase 1, and 60% of nitrates compared to baseline in Phase 2. The Control sample had only one nitrate level that was different than baseline during the entire experiment, which was a nearly 100% increase compared to the Phase 1 baseline on 11/15/2012. The nitrate level returned to baseline for the rest of Phase 1 testing period. The control nitrate level remained constant with the baseline level throughout Phase 2 testing.

In both phases, there were large differences in the phosphate levels of the two testing groups. The Spirogyra sample absorbed 100% of the phosphates compared to baseline in Phase 1, and 75% of the phosphates compared to baseline in Phase 2. The control sample had only one phosphate level that was different than baseline, which was a 50% increase compared to the Phase 1 phosphate baseline for 11/14/2012. The phosphate level returned to baseline for the rest of the Phase 1 testing period. The control phosphate level remained constant compared to the baseline level throughout the entirety of Phase 2 testing.

In Phase 1, the Spirogyra and control samples both had an increase in dissolved oxygen levels. Spirogyra had an increase of 2.4% and control had an increase of 5.6%. In Phase 2, Spirogyra had an increase of 4.6%, and control had a decrease of 9.8%. Therefore, Spirogyra had an increase in dissolved oxygen compared to baseline while control had a decrease in dissolved oxygen compared to baseline. That means that the filtration devices themselves did not increase the dissolved oxygen in Phase 1. Further testing could provide be conducted concerning the effect on dissolved oxygen levels based on flow rate and other variables.

In both phases, changes in the pH levels of both testing groups were negligible. Both Phase 1 and Phase 2 were slightly acidic overall, with Phase 1 hovering around 7 (baseline level) to 7.5, and Phase 2, hovering around the levels of 6 to 6.5 (both of which were baseline levels).

In Phase 1, the control had an elevated level of 8.8 ppm of nitrates on 11/15/2012. Based on the elevation in dissolved oxygen and the oily residue on the surface of the sample bucket, it is probable that the cause was a small die off of algae occurring in the bucket. This could be correlated to a 0.4 ppm drop between 11/15/2012 and 11/16/2012.

In Phase 2, the 11/30/2012 Spirogyra dissolved oxygen level of 5.5 ppm was an outlier compared to the trend of other experimental data. The probable cause, based on observations and

a drop from stable dissolved oxygen levels of 6.2 ppm and 6.3 ppm, was most likely from a die off of the lake algae in the bucket. The low level is within the 0.7 ppm range limit with a 0.2 ppm range.

In both phases, the amount of visible algal turfs in the *Spirogyra* sample was decreased to no visible algae. However, there were increased visible algal turfs in the control samples. Since no *S. grevilleana* came in contact with the control samples during experimentation, these visible algal turfs were most likely composed of algae that were in the original lake water samples. Thus, it can be hypothesized that the *S. grevilleana* algal filter in the experimental device reduced the other algae in the water that was filtered, most probably due to a biological competition for nutrients. In the closed environment, the *S. grevilleana* algal turf in the device was effective at removing the nutrients from the lake water by absorbing the nutrients for its own growth, which in turn caused the preexisting lake algae to die from lack of nutrients.

The past four years of experimentation have followed certified Georgia Adopt-A-Stream guidelines utilizing LaMotte test kits to test water quality. If this experiment were repeated, a spectrometer could be utilized to obtain a higher degree of testing accuracy.

Water quality is a major global issue. Clean water, both potable and nonpotable, is in scarcity in the world today, making innovative ways to improve water quality critical throughout the world.

Fertilizer pollution from runoff containing Nitrogen and Phosphorous can damage lake ecosystems and food chains, and hurt water quality. According to the background research, nutrient mismanagement, including fertilizer pollution, is one of the three main threats to water quality. Using algae to reduce fertilizer pollution from runoff can be a sustainable method to utilize biological cleaning power. This experiment successfully used *S. grevilleana* to remove excess nitrates and phosphates from the water. This complies with the background research as sources indicate that algae are an effective water quality improver, such as in ATS.

The algal filtration device tested in this experiment is different than ATS technology in many aspects. One major difference is that the body of water being filtered does not have a large area of flowing water like ATS. Rather than requiring acres of space to operate like ATS, the device tested in this experiment requires only a small area for setup with a length of roughly 0.85 meters. In addition, the device is portable, inexpensive to produce, made of easily obtainable

materials, and flexible for use in multiple locations, unlike the stationary, expensive ATS systems. Rather than being designed for mainly large scale applications like the ATS, the device tested in this experiment is designed for use in multiple types of fresh water areas from small neighborhood ponds to larger lakes. Based on observations, there was an oily substance on the surfaces of the water in both buckets the last three days of experimentation for both Phases, which were most likely lipids released by the algae. Since the *Spirogyra* sample had more of the oily substance than the control sample, it can be inferred that the additional algae from the algal filter were releasing more lipids. If those were actually lipids, which could be tested, the algae in the device could be harvested for biofuels, and then fresh algae could be placed in the device, a concept that is similar to ATS. If the biomass were to grow in size, then most of the algae would need to be removed and replaced to allow for more cleaning power and growth. The other possibility would be that the algae would die over time and would need to be replaced. In this case, fresh algae would need to be available in order to restock the device. Different native algae could be available for use in different locations. This could be investigated in future experimentation.

If the experiment were to be repeated, multiple algal filtration devices would be built and there would be more trials. Water from different lakes would be used to further verify the findings. Also, the method to put the algae into the device would be revised, such as utilizing a cartridge-loading type set-up, to make adding and removing the algae easier. The pump would also possibly be solar powered to make the device self-sufficient. Not needing a wired power source, the device would be more portable and sustainable. Further experimentation could also be done to investigate the potential to create potable water from this algal filtration method.

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 relationship must be established. The expression $n = \frac{\Psi Q}{V}$ represents the volume flow rate (Q), the flow efficiency constant (Ψ) which is currently not defined, the number of devices (n) which is a constant, and the volume of water in a given lake (V). Using substitution of $Q = \frac{dV}{dt}$ and forming a differential equation:

$$n = \frac{\Psi dV}{V dt} \rightarrow n dt = \frac{\Psi}{V} dV$$

This equation allows the expression to be integrated:

$$\int \frac{\Psi}{V} dV = n \int dt \rightarrow \Psi \ln|V| = nt + C$$

Another undefined constant, the volume relation constant (Φ) defines C, leaving the final equation:

$$\Psi \ln V = nt + \Phi$$

which shows the mathematical model relating the number of devices to the time needed to filter a lake of a certain volume one time.

One algal filtration device costs roughly \$250.00 to build, including the PVC pipe and connectors, fittings, tubing, recirculating pump, and algae. Even if multiple devices are needed for a larger lake, the device's small size and construction of common materials make it an economical and attainable solution.

The algal filtration system requires a much smaller footprint for operation than the ATS. The smallest ATS system available covers 1,012 m² of land (roughly 2.5 acres) and filters approximately 37.8 million liters per day. One unit of the algal filtration system covers 4.258×10⁻² m² of land and filters approximately 12,720 liters per day. To increase the filtration amount per day, additional devices can be utilized (see Table 2).

Table 2: Analysis and comparison of the experimental device and the ATS system

Device	(A) Area Needed per unit	(B) Ideal Daily Flow (lpd = liters per day)	(C) Number of Units Needed to Clean 100,000,000 lpd (n=100,000,000/B)	(D) Area needed to clean 100,000,000 lpd (A×C)
This Experiment	4.258×10 ⁻² m ²	12,720 lpd	7,862 devices	335 m ²
ATS (Source: HydroMentia.com)	1,012 m ²	37,854,117 lpd	4 devices	4,048 m ²

Table 2 compares the smallest commercial ATS system to the device tested in the experiment in terms of ideal (efficiency = 1) daily flow per device and area needed per unit in addition to comparing the devices under an equal set condition. This table therefore illustrates that, even though more devices are needed to achieve the same flow, the device from the

experiment requires much less space to operate under ideal conditions. Larger algal filtration devices could be designed to increase the ideal flow for future experimentation.

Future experiments can potentially refine the prototypes so that the algal filtration unit can be used to improve the water quality in fresh water lakes of different sizes and in multiple locations.

Conclusions

- (1) The use of *S. grevilleana* as an algal filter to improve water quality in water from a freshwater lake proved successful as nitrate and phosphate levels were decreased, dissolved oxygen levels were raised, and pH levels were maintained.
- (2) The algal filter absorbed 100% of the nitrates in the *Spirogyra* sample compared to baseline in oligotrophic conditions and 60% of the nitrates compared to baseline in eutrophic conditions. The Control samples in both phases showed no decrease in nitrates.
- (3) The algal filter absorbed 100% of the phosphates in the *Spirogyra* sample compared to baseline in oligotrophic conditions, and 75% compared to baseline in eutrophic conditions. The control samples in both phases showed no decrease in phosphates.
- (4) The algal filter raised dissolved oxygen 2.4% in the *Spirogyra* sample compared to baseline in oligotrophic conditions, and 4.6% compared to baseline in eutrophic conditions. The control sample in Phase 1 showed a 5.6% increase in dissolved oxygen and in Phase 2 showed a 9.8% decrease. Therefore, it can be concluded that the *S. grevilleana* algal filter was likely responsible for the increase in dissolved oxygen levels. The algal filter resulted in little to no change in pH levels in both phases of testing. Both *Spirogyra* and control sample pH levels were unchanged compared to baseline in oligotrophic conditions. The *Spirogyra* pH level showed an 8.3% increase and the control showed a 4% increase compared to baseline in eutrophic conditions.
- (5) In both nutrient conditions, the amount of visible algal turfs in the samples utilizing the *S. grevilleana* algal filter was decreased to no visible algae, as opposed to increased visible algal turfs in control samples. Since no *S. grevilleana* came in contact with the control sample, it can be concluded that these visible algal turfs were most likely composed of algae that were originally in the lake water prior to experimentation. Thus, the *S. grevilleana* algal filter reduced the other algae in the water that was filtered, probably due to biological competition for

nutrients, with *S. grevilleana* absorbing nutrients for its own growth, which in turn caused the preexisting lake algae to die from lack of nutrients.

(6) Fertilizer pollution from runoff containing Nitrogen and Phosphorous can damage lake ecosystems and hurt water quality. Using algae to remove nutrient pollution from runoff can be a sustainable method to remove excess nutrients from the water. This experiment successfully used *S. grevilleana* to remove excess nitrates and phosphates from the water, correlating with background research indicating algae are an effective water quality improver.

(7) The algal filter is significantly smaller, less expensive to produce, and more portable than current ATS systems. Its size and flexibility could allow use in a variety of freshwater lake sizes and locations.

(8) *S. grevilleana* was selected as it is native to Georgia lakes where the scientist lives, and based on prior experimentation proving *S. grevilleana* to be effective in improving water quality in terms of dissolved oxygen. Different algae could be tested to determine the most efficient for algal filtration under different conditions and in specific regions of the world.

(9) Further design modifications could enhance the ease of use and increased sustainability through a self-sufficient power source. Additional experimentation could determine if additional algal species could be effective. The ability to produce potable water is also a potential utilization of algal filtration, and should be explored.

(10) Based on observations, there was an oily substance on the surfaces of the water in both buckets the last three days of experimentation for both phases, which were potentially lipids released by the algae. Whether those were lipids, and their potential for biofuels, warrants further experimentation.

(11) *S. grevilleana* paired with the algal filtration device effectively improved water quality in freshwater lake water samples of both oligotrophic and eutrophic states, thus demonstrating the potential utilization of the algal filtration unit for use in the field improving water quality of fresh water lakes.

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CLAIMS

What is claimed is:

1. An apparatus for improving water quality of a body of water, said apparatus comprising:
a water treatment device comprising a wall formed from a material allowing the passage of light therethrough suitable for growing algae, said wall defining a substantially enclosed chamber therein for treating water received from a source of water to be treated, said water treatment device further comprising a structure suitable for growing an algal turf thereon and for allowing water to pass therethrough; and
algae resident within said chamber adapted for removing nitrogen and phosphorous dissolved in water.
2. The apparatus of Claim 1, wherein said structure protrudes into said chamber.
3. The apparatus of Claim 1, wherein said structure is manufactured from a wire mesh.
4. The apparatus of Claim 1, wherein said structure is manufactured from a plastic mesh.
5. The apparatus of Claim 1, wherein said structure has a substantially hemispherical shape.
6. The apparatus of Claim 1, wherein said water treatment device has an intake port for receiving water from the source of water to be treated, and wherein said structure is positioned relative to said intake port so as to require received water to pass through said structure.
7. The apparatus of Claim 6, wherein said structure protrudes into said chamber.
8. The apparatus of Claim 1, wherein said algae comprises a filamentous green algae.
9. The apparatus of Claim 8, wherein said algae comprises *Spirogyra grevilleana* algae.

10. The apparatus of Claim 1, wherein said wall is formed from a clear polyvinyl chloride material.
11. The apparatus of Claim 1, wherein said structure comprises a first structure and said water treatment device further comprises a second structure suitable for growing an algal turf thereon and for allowing water to pass therethrough.
12. The apparatus of Claim 11, wherein said water treatment device has a discharge port for discharging water from the water treatment device, and wherein said second structure is positioned relative to said discharge port so as to require discharged water to pass through said second structure.
13. The apparatus of Claim 1, wherein said algae is further adapted to secrete secondary metabolites capable of killing bacteria.
14. The apparatus of Claim 1, wherein said algae is further adapted to secrete secondary metabolites capable for killing viruses.
15. The apparatus of Claim 1, wherein the apparatus further comprises a pump in fluid communication with the water treatment device for drawing water from a source of water to be treated and for directing the water to the water treatment device.
16. A system for improving water quality of a body of water, said system comprising:
a water treatment device including an intake port for receiving water from a source of water to be treated, wherein the received water has a contaminant therein, said water treatment device further including a discharge port for discharging treated water; and
a cartridge configured for insertion in fluid communication between said intake port of said water treatment device and said discharge port of said water treatment device, said cartridge comprising a wall formed from a material allowing the passage of light therethrough suitable for growing algae, said wall defining a substantially enclosed chamber therein for

receiving water from said intake port of said water treatment device and for directing water to said discharge port of said water treatment device, said cartridge further comprising a structure suitable for growing an algal turf thereon and for allowing water to pass therethrough, said cartridge further comprising algae resident within said chamber specifically selected and suitable for reducing the amount of the contaminant in the received water.

17. The system of Claim 16, wherein the contaminant comprises dissolved nitrogen.

18. The system of Claim 16, wherein the contaminant comprises dissolved phosphorous.

19. The system of Claim 16, wherein the contaminant comprises a bacterium.

20. The system of Claim 19, wherein the algae is capable of producing secondary metabolites that cause a reduction in the amount of the contaminant in the received water.

21. The system of Claim 19, wherein the bacterium comprises *Escherichia coli*.

22. The system of Claim 16, wherein the contaminant comprises a virus.

23. The system of Claim 22, wherein said algae is capable of producing secondary metabolites that cause a reduction in the amount of the contaminant in the received water.

24. The system of Claim 16, wherein said cartridge comprises a first cartridge and is further configured for removal and replacement by a second cartridge.

25. The system of Claim 24, wherein said second cartridge is substantially similar to said first cartridge, and wherein said second cartridge comprises algae resident within said chamber of said second cartridge specifically selected and suitable for reducing the amount of the contaminant in the received water.

26. The system of Claim 24, wherein the contaminant is a first contaminant and the received water has a second contaminant, wherein said second cartridge is substantially similar to said first cartridge, and wherein said second cartridge comprises algae resident within said chamber of said second cartridge specifically selected and suitable for reducing the amount of the second contaminant in the received water.

27. The system of Claim 16, wherein said algae comprises a filamentous green algae.

28. The system of Claim 27, wherein said algae comprises *Spirogyra grevilleana* algae.

29. The system of Claim 16, wherein said structure protrudes into said chamber.

30. The system of Claim 29, wherein said structure has a substantially hemispherical shape.

31. A method for improving water quality of a body of water, the method comprising the steps of:

directing contaminated water from a body of contaminated water into a substantially enclosed chamber of a device configured to remove a contaminant from the contaminated water;

exposing the contaminated water within the chamber to algae capable of at least partially eliminating the contaminant from the contaminated water; and

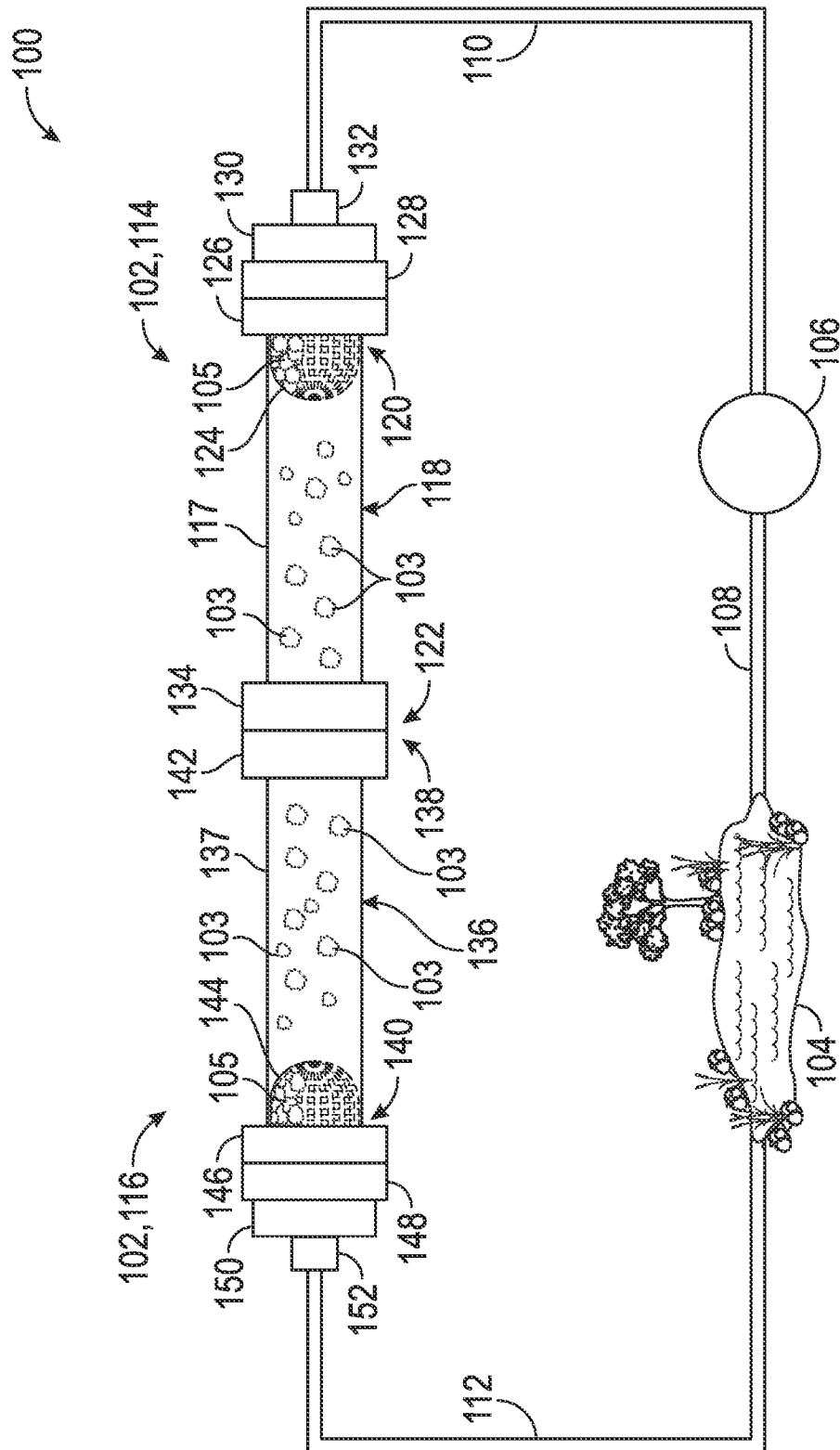
returning the contaminated water after at least partial removal of the contaminant to the body of contaminated water.

32. The method of Claim 31, wherein the step of exposing the contaminated water comprises a step of causing the contaminated water to flow through a structure on which the algae has formed an algal turf.

33. The method of Claim 32, wherein the structure provides substantial surface area relative to the dimensions of the structure for formation of the algal turf.

- 34. The method of Claim 32, wherein the structure has a substantially hemispherical shape.
- 35. The method of Claim 31, wherein the algae comprises a filamentous green algae.
- 36. The method of Claim 35, wherein the algae comprises *Spirogyra grevilleana* algae.
- 37. The method of Claim 31, wherein the chamber comprises a wall through which light having one or more wavelengths suitable for growth of the algae passes.
- 38. The method of Claim 31, wherein the contaminant comprises dissolved nitrogen.
- 39. The method of Claim 31, wherein the contaminant comprises dissolved phosphorous.
- 40. The method of Claim 31, wherein the contaminant comprises a bacterium.
- 41. The method of Claim 40, wherein the bacterium comprises *Escherichia coli*.
- 42. The method of Claim 31, wherein the contaminant comprises a virus.

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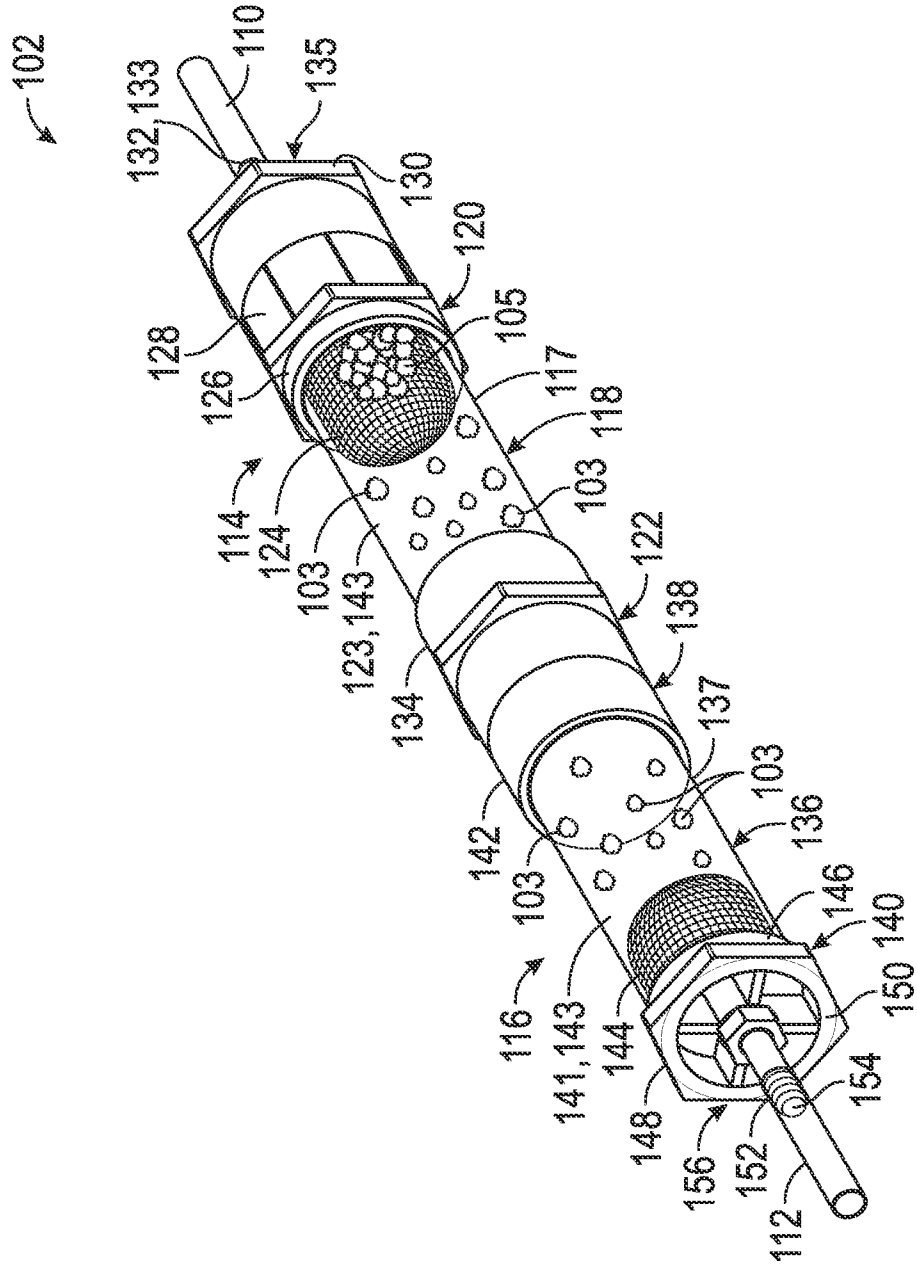


FIG. 2

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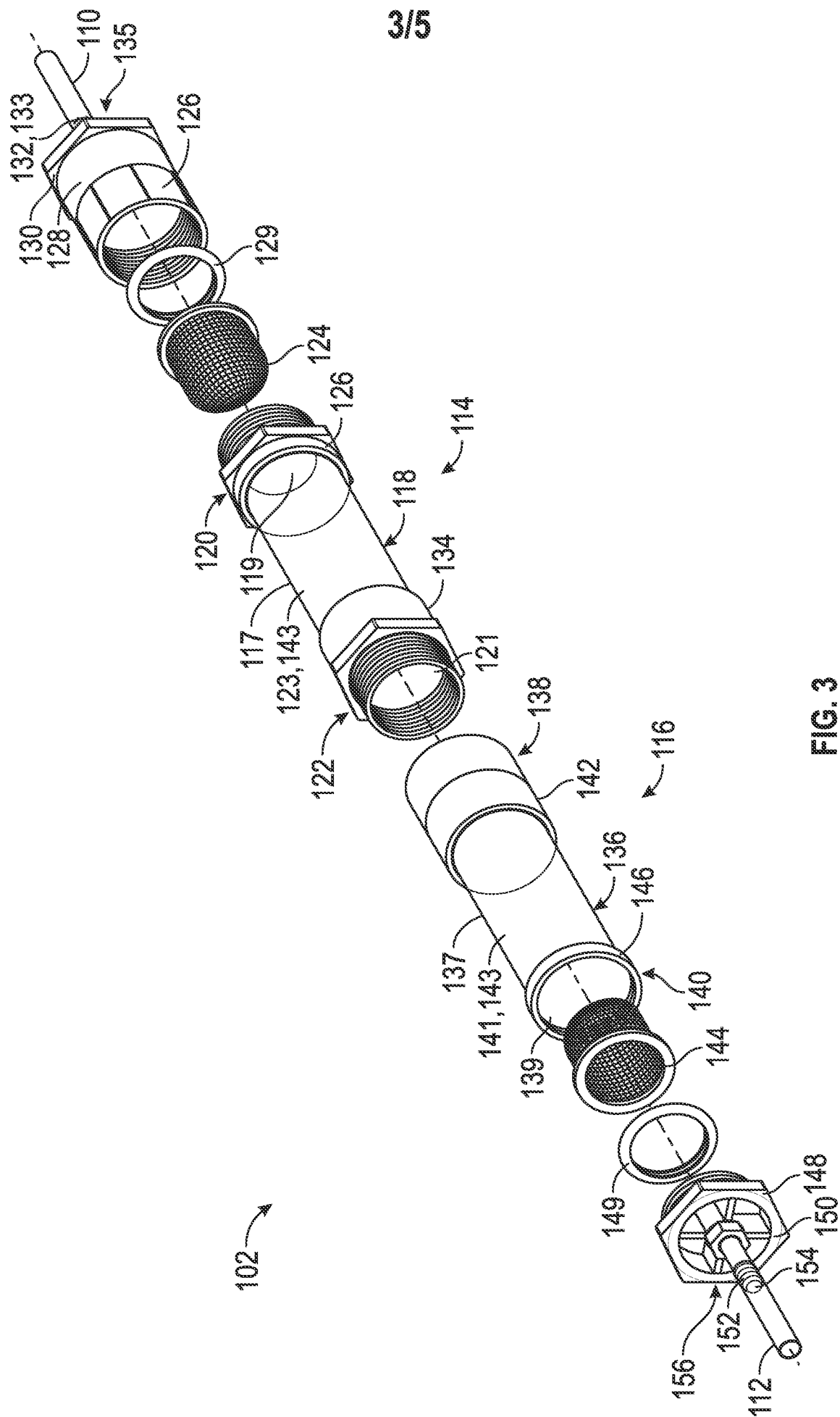
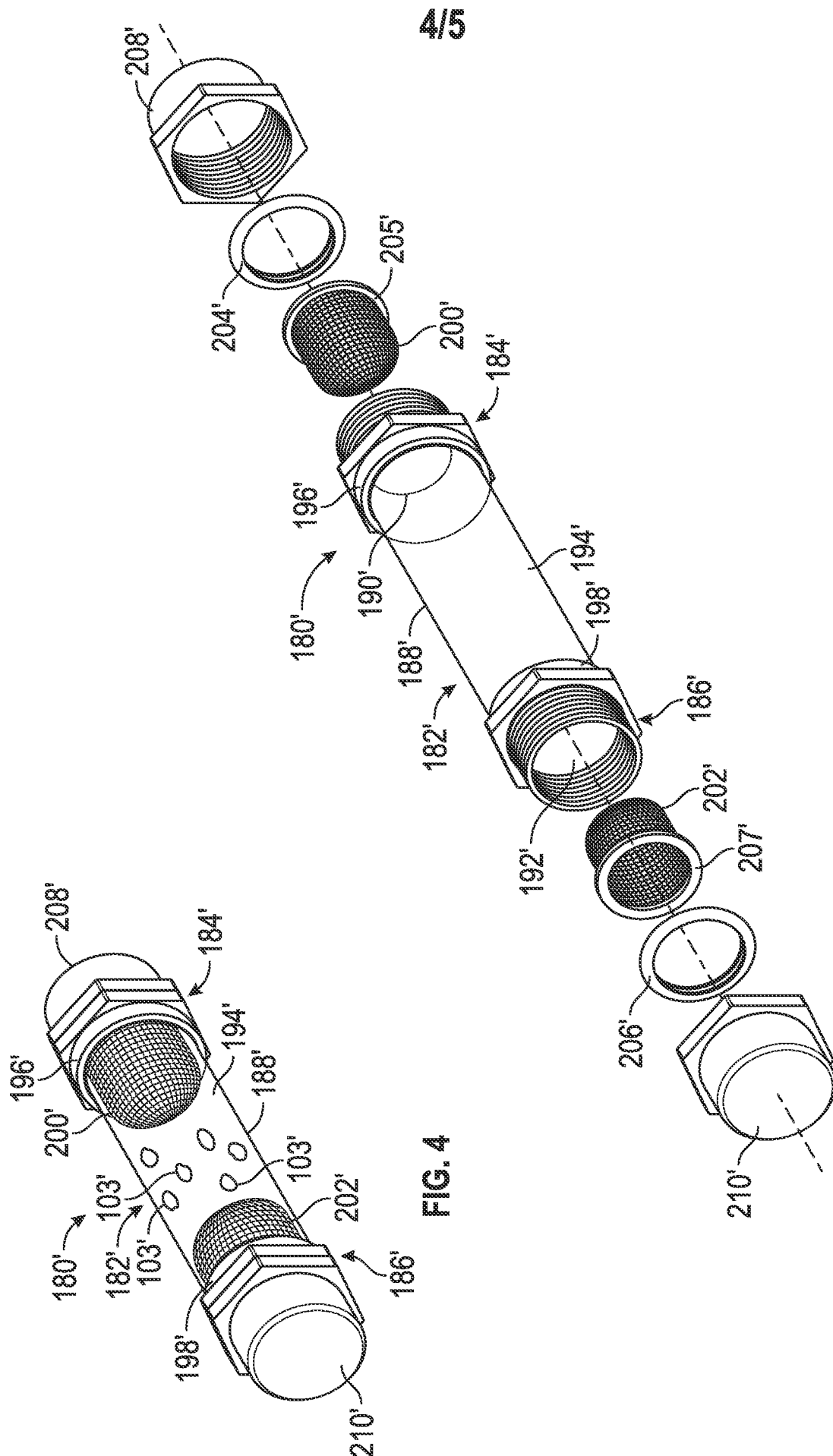
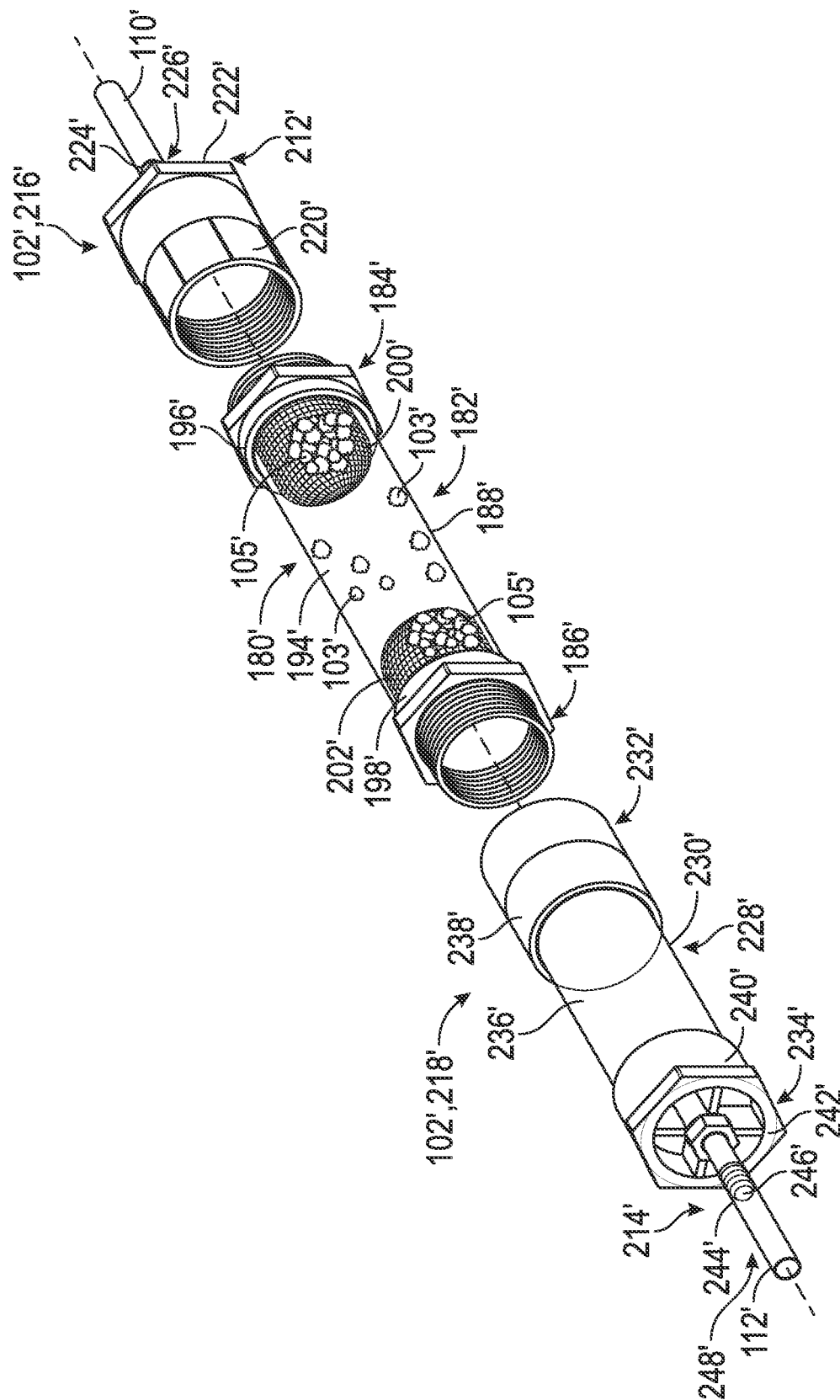


FIG. 3





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INTERNATIONAL SEARCH REPORT

PCT/US 14/42193

International application No.

PCT/US 14/42193

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A01N 65/00 (2014.01)

CPC - A61K 36/185; A61K 8/97

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC(8) - A01N 65/00 (2014.01)

CPC - A61K 36/185; A61K 8/97

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

USPC - 424/725; 210/764

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Minnesoft Patbase; Google Scholar, keywords: (filamentous w5 alg*) and PA = (Barnard w2 Malcolm), nitrogen, NH4*, NO3*, PO4*, phosphorus, phosphate, nitrate, water w2 (quality or purity or purification or amelioration), Spyrogira, Spirogyra, Spirogira, mesh, wire, (algal w2 turf) and (bacteria and viruses; (net or mesh) w15 (semispherical or hemisph

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2011/0266215 A1 (Robinson et al.) 03 November 2011 (03.11.2011) para [0003], [0013], [0014], [0027], [0036], [0037], [0038], Fig 2A	31, 37, 38, 39
Y		1-30, 32-36, 40-42
Y	US 5,851,398 A (Adey) 22 December 1998 (22.12.1998)(title, abstract; Fig 1, col 4, lines 25-30; col 4, lines 60-65; Fig 1, col 8, lines 45-50:	1-30, 32-36
Y	US 3,598,726 A (Welch) 10 August 1971 (10.08.1971); Figures 1-4, abstract; col 2, lines 55-72; col 3, lines 35-40.	2, 7, 24, 25, 26, 29, 30
Y	KR 2005/024728 A (Hwang et al.) 11 March 2005 (11.03.2005) (Abstract)	3
Y	CN 202680233 U (Wang et al.) 23 January 2013 (23.01.2013) (Abstract)	5, 30, 34
Y	US 6,346,252 B1 (Moigne) 12 February 2002 (12.02.2002) col 1, lines 25-35	13, 19-21, 26, 40, 41
Y	US 2007/0224216 A1 (Teas) 27 September 2007 (27.09.2007) para [0029], [0030]	14, 22, 23, 42
Y	US 4,966,096 A (Adey) 30 October 1990 (30.10.1990) col 1, lines 30-35	19-21, 40-42

☐ Further documents are listed in the continuation of Box C.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

08 September 2014 (08.09.2014)

Date of mailing of the international search report

03 OCT 2014

Name and mailing address of the ISA/US

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