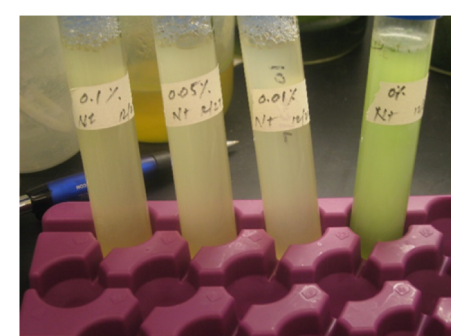


Polyhexamethylene-Guanidine Hydrochloride; a Low Dose, Safe and Effective Water-Soluble Facility and Equipment Friendly Antimicrobial Compound

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ABSTRACT

The dynamics of infection spreading throughout a population necessitates new and safe disinfectants as resistance develop and effectiveness attenuate. The biocidal properties of guanidine compounds have long been recognized and Polyhexamethylene biguanide hydrochloride (PHMB) is well established widely accepted as biodegradable, non-corrosive and nontoxic disinfectant when used as directed. Another guanidine compound, Polyhexamethylene-Guanidine Hydrochloride (PHMGH) has been demonstrated to have biocidal properties comparable or more effective than PHMB. PHMGH studies have shown that in water solution it has fungicidal as well as bactericidal activity against both gram-positive and gram-negative bacteria including effectively destroying Methicillin-resistant *Staphylococcus aureus* (MRSA), *Escherichia coli*, spore forming bacteria such as *Clostridium difficile* at biocide concentrations well below what is required for other disinfectants.

Our work at Pure Global Solutions, LLC (PGS) and Arizona State University (ASU) is focused on demonstrating the biocidal efficacy of PHMGH in a variety of applications covering diverse scenarios including controlling algal blooms in surface water, algae and bacteria in ornamental fountains, mold and mildew, mussel on submerged boat surfaces in fresh water, as well as incorporation of our proprietary formulation of PHMGH into a full range of sanitizer, disinfectant, healthcare, personal hygiene and other products as we demonstrate a broader range of effectiveness.

PHMGH as a biocide is effective at concentrations as low as 0.002%, in water, with sufficient contact time. Concentrations of 0.05% to 0.1%, in water, shortened required contact time to seconds as would be required for wiping down surfaces and including hand sanitizers. Without additives, PHMGH solutions in water are mild and safe for all surfaces that can be dampened with water. These properties make it potentially quite useful for routine or emergency disinfection of large spaces, both interior and exterior, include but not limited to hospitals, animal husbandry facilities, food processing facilities; quarantined areas (personnel and equipment); food animal transport trailers and boats; surface water contaminated by storm runoff or intentional releases.

INTRODUCTION

Algae is a major player in global ecosystem. And it produces more than half of Earth's oxygen. However, unfettered growth of algae can have serious environmental and ecological consequences. Algal blooms form under the environmental conditions that promote excessive growth of algae in surface waters. Fresh water algae consist broadly defined group of organisms (single celled or multicellular eukaryotes / prokaryotes). Typically, algal bloom consist of one or two type of algae and high density of pigmented cell result discoloration of water. Generally, in freshwaters, algae can be considered blooming when the concentration reaches thousands of cells per milliliter; however, there is no officially recognized threshold level associate with bloom. The threshold numbers can vary depending on the causative species and may be in the range of millions of cells per milliliter.

2014: Toledo, Ohio - toxic algae bloom leaves 500,000 without drinking water for three days.

2016: algal bloom stretching from central California to Alaska

2017 Sept: Lake Erie Algal Bloom – up to Canada

- New York Pilot Study in 2015 looked at 16 of 62 counties.
 - 32 reported human cases; 17 required hospitalization
 - 3 dog cases; 2 required hospitalization
- Kansas 2010 Study
 - 7 confirmed human cases; 2 required hospitalization
 - 7 dog cases; 5 fatalities; 1 hospitalization

New Jersey American Water at Canoe Brook serves 126,000 customers in 25 municipalities. They state that “Copper had lost its effectiveness for us.”. “The first treatment of the year would go great, but the rest would have no effect whatsoever,”

MATERIALS AND METHODS

Algae

Synechocystis PCC 6803 and *Chlorella* cultures were maintained in a glass reactor in the Environmental Microbiology Laboratory at Arizona State University. Culture was grown using BG-11 medium.

PHMGH Dilution

The PHMGH formulation (50%) provided by the Pure Global Solution Inc. was diluted in Nanopure water to make 10% working solution. The working solution was used to obtain 0.01%, 0.05% and 0.1% active ingredient in 50 mL volume in separate reactors.

Experimental Plan Algaecidal Efficacy

In a 200 mL glass containers, 50 mL algal culture (150,000 cell/mL) were added. Culture was allowed to stand for 10 minutes and then PHMGH was added to achieve the specified concentration and incubated at room temperature. Each reactor was sampled at 6 hrs, 12 hrs, 48 hrs and 72 hrs. Each sample was analyzed to determine the concentration of viable algal cells.

Experimental Plan Residual Effect

In a 200 mL glass containers, 50 mL algal culture (150,000 cell/mL) were added. Culture allowed to stand for 10 minutes and then PHMGH was added to achieve specific concentration. The spiked culture was incubated at room temperature. To each reactor new algal culture (150,000 cells) was added on a weekly basis, and prior to that a culture aliquot was collected to determine the concentration of viable cells. Study continued for 3 weeks.

Experimental Setup Longitudinal Effect

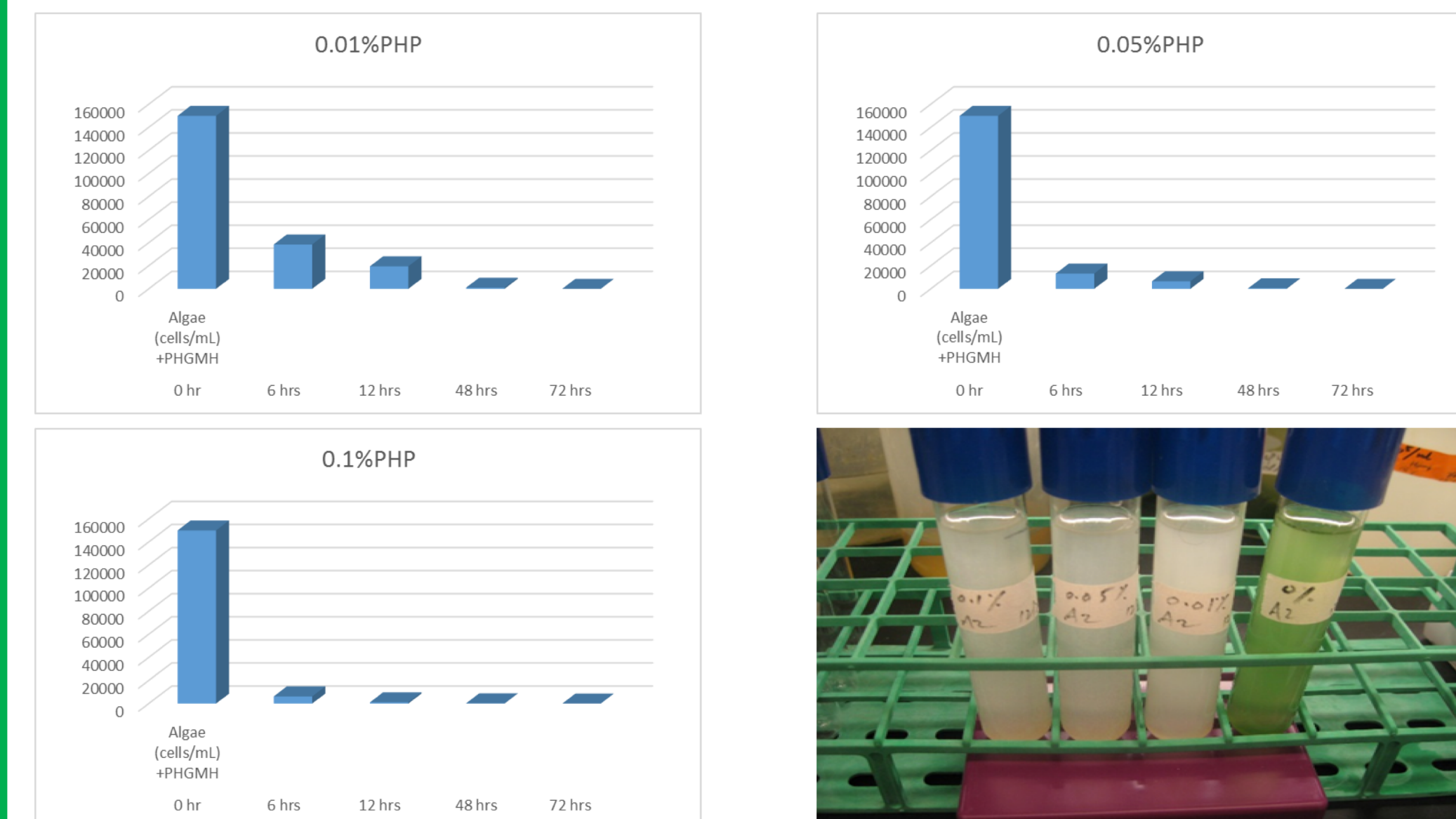
Experiments were conducted in glass containers to study the long-term biocidal effect of PHMGH on the cyanobacteria.

The reaction volume was 50 mL with 12500 cell/mL

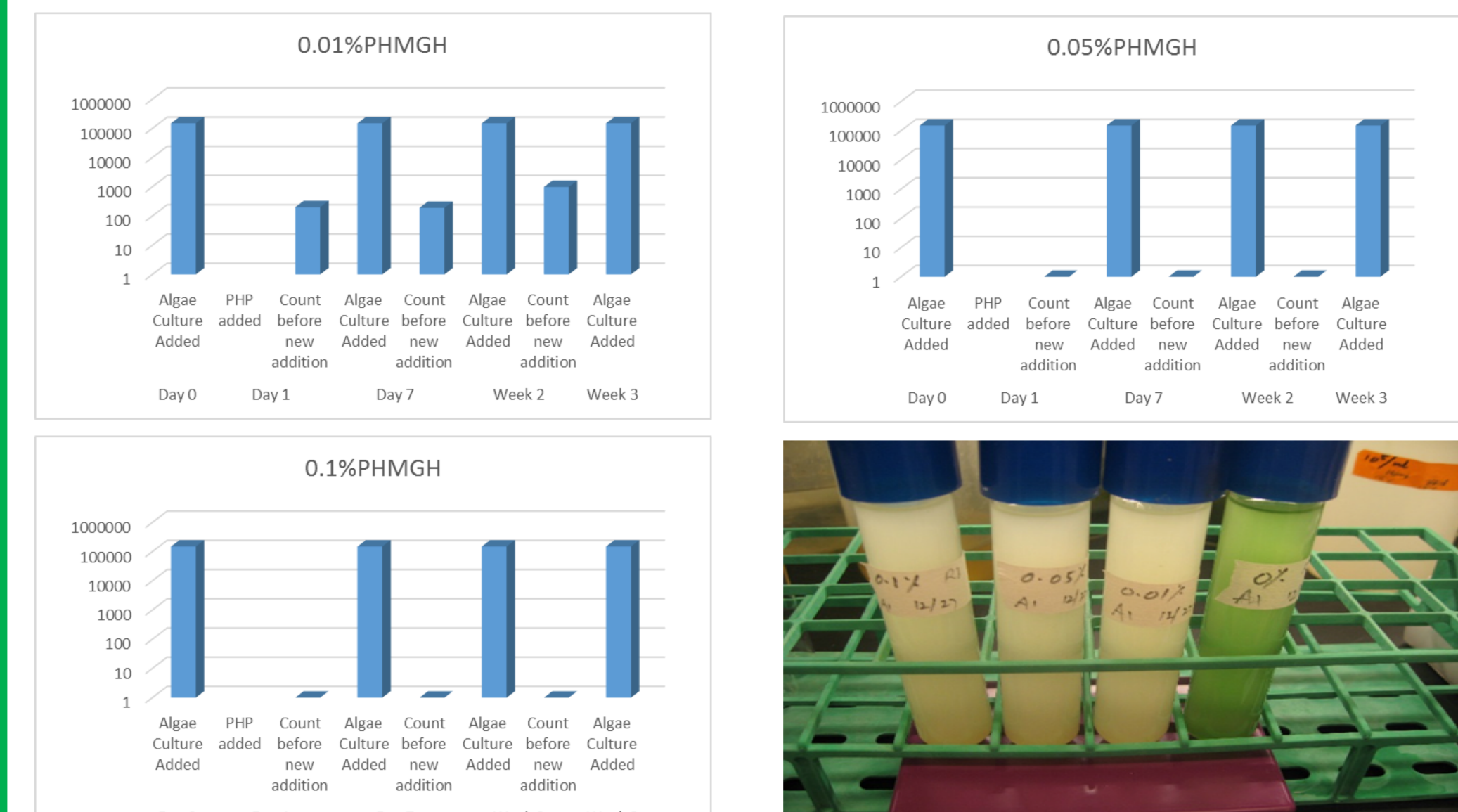
Biocidal effect of each concentration (0.1%, 0.05% and 0.01%) of PHMGH was studied in a separate reactor. Each reactor was sampled at 12hr, 24hr, 48hr, 72hr, 1 week, 2 week, 3 week, and 4 week. Samples were analyzed to count the viable algal cells.

RESULTS

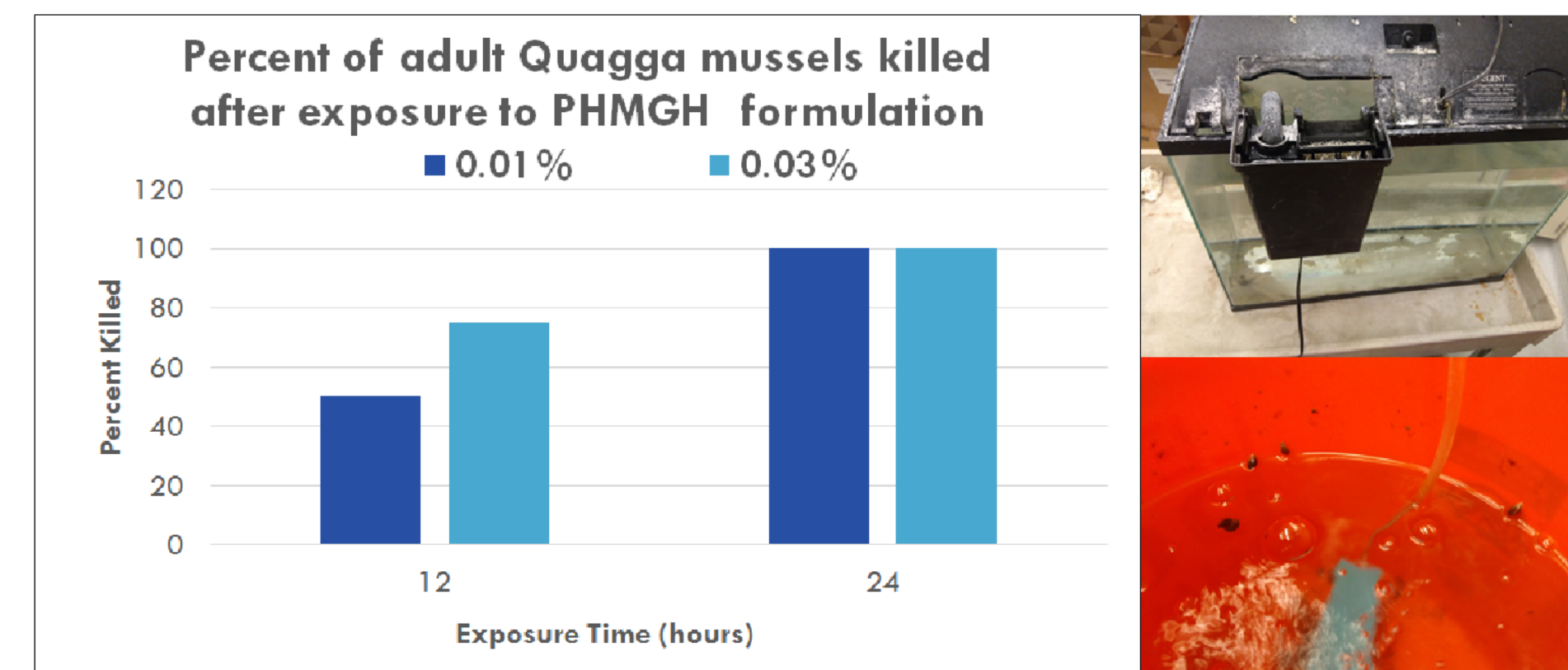
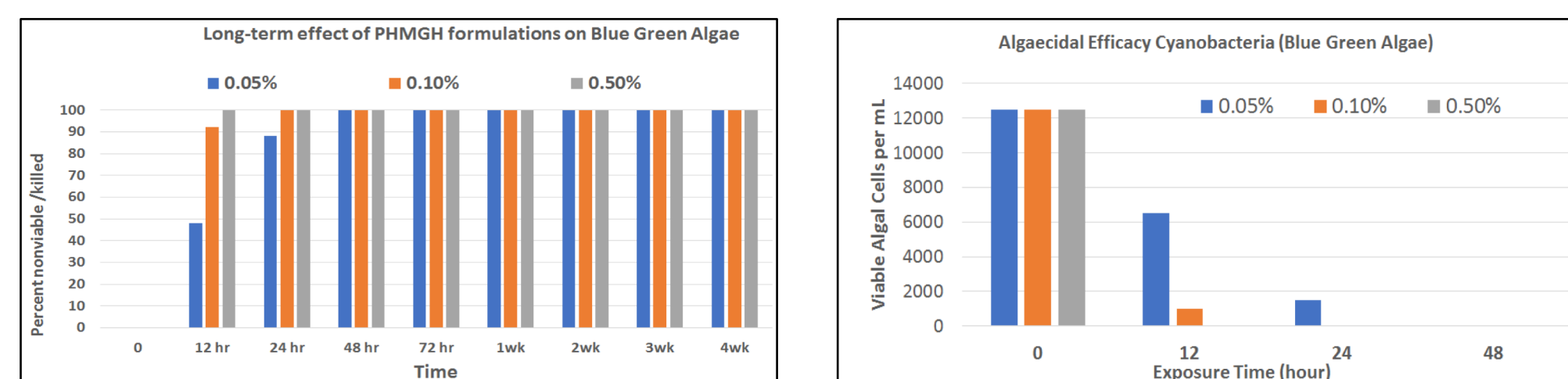
Longitudinal Biocidal Effect of PHMGH on Green Algae



Residual Biocidal Effect of PMGH on Green Algae



RESULTS



CONCLUSIONS

- Based on the residual and longitudinal studies, the product has a clear biocidal effect. After 12 hour exposure to 0.05% PHMGH, only 4.4% of green algae and 52% of cyanobacteria were viable. Whereas after 48 hour exposure 99.4% green algae and 100% Cyanobacteria are killed.
- The biocidal effect is long lasting under the laboratory conditions. The PHMGH spiked at 0.05% concentration continued to fully inactivate new population of green algae entering the reactor for a minimum of 3 weeks.
- The product has the applicability for controlling algal growth.
- The product has shown potential as surface disinfectant, which needs further investigation of its efficacy under different application scenarios.

Pure Global Solution Future Research Plan

- Evaluate reported efficacy of PHMGH against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella choleraesuis*, methicillin-resistant *S. aureus* (MRSA) and *Escherichia coli* vs. reported results of less than 0.04 % (w/v), within 1.5 min. - Journal of Med Microbiol, 2008 Dec; 57(Pt 12):1523-8. doi: 10.1099/jmm.0.2008/003350-0.
- Verify reported concentration required to kill *B. subtilis* spores on stainless steel or glass surfaces as 0.52 % (w/v) for 90 s of contact and 0.36 % (w/v) for 3 min. - Journal of Medical Microbiology 61: 1421-1427, doi: 10.1099/jmm.0.047514-0.
- Establish efficacy of PHMG in market driven sectors including agriculture fungicide, fresh food spoilage reduction, decontamination of animal husbandry facilities, veterinary medicine, personal care products, storm water retention pond management, prevention of mold and mildew in buildings in damp climates, fountain and pool management, etc.

