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Harmful Algal Bloom Mitigating using Recycled Concrete Aggregate coated with Fixed-Quat.

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HARMFUL ALGAL BLOOM MITIGATION USING RECYCLE
CONCRETE AGGREGATE COATED WITH FIXED-QUAT

by

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B.S. Federal University of Technology Owerri, 2014

A thesis submitted in partial fulfillment of the requirements
for the degree of Master of Science
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ABSTRACT

Human activities generate surplus nutrients which may lead to algal bloom events in water resources along with serious ecological problems and thus substantial economic losses. Particularly, harmful algal blooms (HABs) represent toxic cyanobacterial blooms which produce cyanotoxins. The primary concerns of HABs are the exposures to a wide variety of cyanotoxins via ingestion of contaminated drinking water, inhalation during recreational activities, and consumption of contaminated fish and shellfish. However, conventional physical and chemical methods are not always possible to efficiently handle these HABs events. It is urgent to develop viable and rapid solutions to control HABs in field and mitigate the effects of HABs in fresh water, particularly in those that serve as sources of drinking water supply.

Quaternary ammonium compounds (Quats) represent a wide range of cationic compounds with different formulation that constitutes products for agriculture, domestic and medical and industry. As organic antimicrobial compounds, Quats can be used as alternatives to existing chemical-based technique for HABs control due to its less toxicity and its affinity to variety of surface. In this study, recycled concrete aggregate (RCA) from a regional construction and demolition (C&D) waste recycling facility was used as a sustainable and environmentally friendly substrate and coated with a composite of silica-quaternary ammonium compounds (Fixed-Quat). Then, the algistatic capabilities of imparting antimicrobial properties of Quats to the RCA surface, which involve the covalent attachment of the biocides to the surfaces (sol-gel technique), were evaluated with HABs-causing algal species, *Microcystis aeruginosa*. Chlorophyll-*a* was measured to determine the efficiency of HABs mitigation using Fixed-Quat coated RCA in terms of photosynthetic inactivation of the selected algae. OD₆₆₀ and pH were measured as key parameters to monitor algal cell growth and cement hydration. Notably, a 61% reduction of

chlorophyll-*a* within 6 hours and complete removal of chlorophyll-*a* within 8 hours were achieved, indicating that Fixed-Quat coated RCA would be efficient in growth inhibition of *Microcystis aeruginosa*. Overall, with an appropriate design for field application and further evaluations like lifetime of Quat coating and potential recovery of treated algae, the Fixed-Quat antimicrobial coated RCA would be a promising and sustainable alternative to conventional HABs mitigation methods in various aquatic systems.

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LIST OF ACRONYMS

AHs – Aromatic Hydrocarbons

BAC – Benzalkonium chloride

BBM – Bold's Basal Medium

° C – Degrees Celsius

CAPB – Cocamidopropyl betaine

CO₂ – Carbon dioxide

CeO₂ – Cerium oxide

Chl-*a* – Chlorophyll-*a*

C&D – Construction and Demolition

CPCC – Canadian Phycological Culture Centre

CTAB – Cetrimonium bromide

CTAC – Cetrimonium chloride

DDAC – Didecyldimethylammonium chloride

DEAB – Diethylaminobenzaldehyde

DI – deionized water

E. coli – Escherichia coli

EDS – Energy-dispersive X-ray Spectroscopy

Fixed-Quat – Silica-quaternary ammonium compound

kg/m³ – kilogram per cubic meter

kHz – kilohertz

kV – kilovolt

L – Liter

nm – nanometer

MC – microcystin

mg/l – milligram per liter

mg/m³ – milligram per cubic meter

MIB – Methylisoborneol

MIC – Minimum Inhibitory Concentration

OD₆₆₀ – optical density at wavelength of 600 nm

Quats – Quaternary ammonium compound

HAB(s) – Harmful Algal Bloom(s)

PAC – Polyaluminum chloride

RCA – Recycled Concrete Aggregate

ROS – Reactive oxygen species

SEM – Scanning Electron Microscope

TiO₂ – Titanium dioxide

TN – Total Nitrogen

TP – Total Phosphorus

USEPA – United States Environmental Protection Agency

μL – microliter

μg/L – microgram per liter

UV – Ultraviolet

W – Watt

XRD – X-ray diffractometer

at. % – atomic percentage

wt. % – weight percentage

CHAPTER ONE: INTRODUCTION

Urbanization, atmospheric deposition and anthropogenic activities such as sewage and animal wastes, groundwater inflow, agricultural and fertilizer runoff introduce large number of organic and inorganic substances in water and lead to increased nutrient levels in aquatic systems [1, 2]. Nitrogen (N) and phosphorus (P) are the nutrients globally cycled by these activities and their abundance varies with marine and freshwater habitats [2]. Along with nutrients, sufficient sunlight and carbon dioxide can stimulate algae formation and proliferation in water bodies [3]. Algae are the most abundant and prolific microorganisms and the prime organic sources supporting food webs in freshwater systems. Owing to their unicellular or simple multicellular structure, they can grow rapidly in water systems, live in harsh condition [4], deteriorate water quality, and subsequently cause eutrophication [5]. Eutrophication or algal blooms (Figure 1) is the rapid increase of phytoplankton that lead to visible discoloration of water (mostly green, yellow or brown) which occurs at the surface or at specific depths in water column depending on the light and nutrient levels [6].

Algae are sensitive indicators of change in the environmental and thus are principal in the stability of aquatic ecosystems, and also the first trophic level for the production of organics and oxygen [7]. Harmful algal blooms (HABs) is a phenomenon of excessive accumulations of algae in water systems. HABs could be toxic or nontoxic; Non-toxic HABs imparts damage to ecosystem and its components via accumulated algae which supersede indigenous species, causes alteration, reduction in light penetration and depletion of oxygen at the bottom of the water column [8]. Oxygen consumption also occur with decays of these accumulated biomass leading to mortalities of plants and animals in the affected system [8, 9]. Toxic HABs transfer of toxins through the food

web or release of toxic compounds lethal to marine life leading to death in aquatic organisms, severe illness and in most cases death to humans [9].

One of the largest sub-groups of gram-negative photosynthetic prokaryotes classified as algae are the cyanobacteria or blue-green algae [6]. Cyanobacteria are single-celled, colonial or filamentous phytoplankton [10] that have persisted through geochemical and climatic changes by morphological, physiological and ecological modifications thus exist in the widest range of ecological habitats [6, 11]. Cyanobacteria have superior photosynthetic capabilities and are a good source of bioenergy production. Like microalgae, cyanobacteria can provide significantly more biodiesel than oilseed crops by harvesting solar energy in an economically effective and environmentally sustainable manner and at high enough rates to replace a substantial fraction of limited petroleum-based fuel supplies [12-14]. However, cyanobacteria cause noxious blooms in nutrient-enriched (mostly phosphorous enrichment) freshwater and brackish ecosystems [6]. In the event of HABs, cyanobacteria release compounds such as geosmin and 2-methylisoborneol (MIB) that impart undesirable tastes and odors to surface water [15] as well as alter food web by producing bioactive compounds (Table 1) or creating anoxic conditions that causes mortality to livestock and wildlife [16].

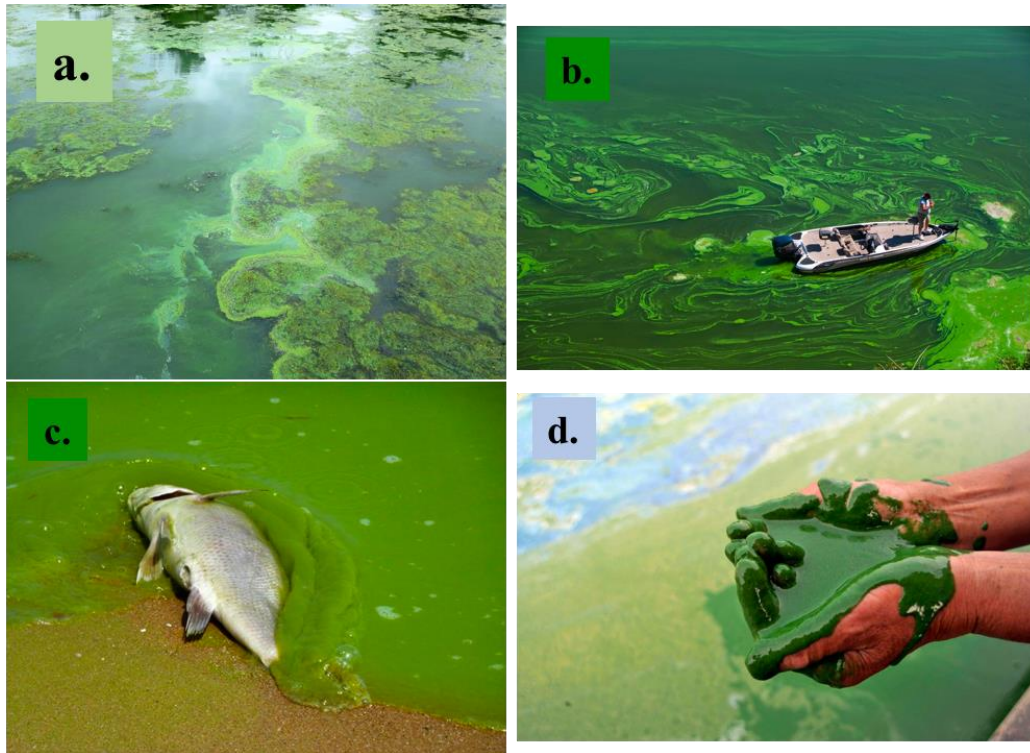


Figure 1. Eutrophication in affected water systems. a. Blue-green algal bloom Washington Lake in southwest Minnesota. b. Toxic algae bloom in California's Klamath River. c. Massive algae bloom in Lake Erie in 2011 led to fish suffocation. d. Algal bloom from China's Chaohu Lake in 2009. (Source: National Geographic sites [17]).

Table 1. Toxins produced by cyanobacteria and health advisory levels by U.S. EPA [18, 19]

Toxins	Target mammalian organ	Cyanobacteria	USEPA* health advisory levels
Microcystin	Liver	<i>Anabaena</i> , <i>Oscillatoria</i> , <i>Microcystis</i>	0.3 µg/L for children; 1.6 µg/L for adults
Anatoxins	Nerve synapse	<i>Anabaena</i> , <i>Aphanizomenon</i> , <i>Oscillatoria</i> , <i>Cylindrospermum</i>	Varies with states
Cylindrospermopsin	Liver	<i>Cylindrospermopsis</i>	0.7 µg/L for children; 3 µg/L for adults
Saitoxin	Nerve axons	<i>Anabaena</i> , <i>Planktothrix</i>	-
Cytotoxin	-	Fresh and marine cyanobacteria	-

*United States Environmental Protection Agency (USEPA)

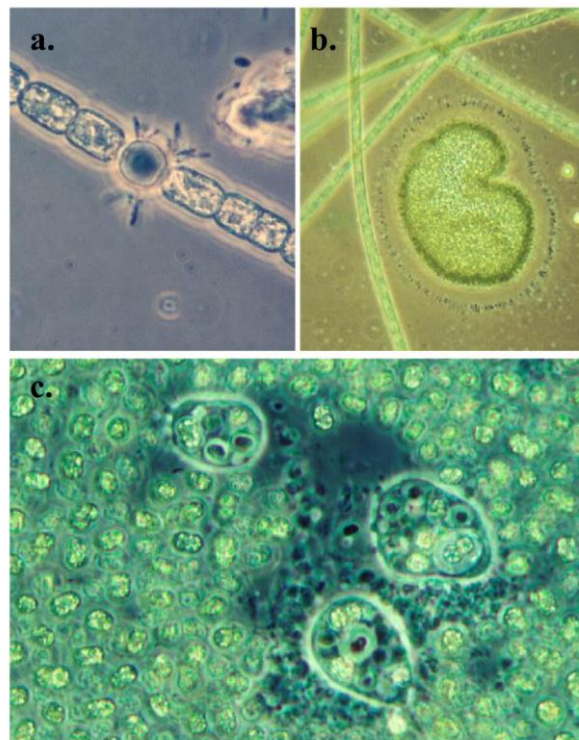


Figure 2. Images of bloom forming cyanobacteria. a. *Anabaena oscillarioides* b. Bacterial halo around host cyanobacteria *Gomphosphaeria*. c. *Microcystis aeruginosa* [6].

Some examples of these harmful cyanobacteria are shown in Figure 2. They include the surface bloom formers: *Anabaena*, *Aphanizomenon*, *Nodularia*, *Microcystis*; and subsurface bloom formers: *Cylindrospermopsis*, *Oscillatoria* [6, 20]. Typical water temperature and pH which promote formation of cyanobacterial blooms are in the range of 15–30 °C [21], a pH > 6 [22], vertical mixing during warmer temperatures, elevated nutrient levels (P concentrations results to high growth rates *Microcystis* cells), sufficient sunlight and carbon dioxide [23].

Appropriate methods to control these rapid and fast thriving organisms are necessary to parallel immediate improvement of human living [24]. A previous study highlight the high surface area and high porosity of recycle concrete aggregate (RCA) as an option for French drain [25]. Another previous study showed successful microbial inactivation against *E. Coli* using an antimicrobial Quat coated RCA [26], indicating RCA as a novel substrate for water filtration and disinfection. ***The objective of this study is to develop an algistatic surface by modifying RCA with quaternary ammonium compounds (Quats) via sol-gel technique for sustainable water treatment, particularly HABs mitigation.*** To prevent secondary environmental problems from leaching out of chemicals, a composite of silica-quaternary ammonium compounds (Fixed-Quat) containing didecyldimethylammonium chloride (DDAC) was prepared as a coating material on RCA using a sol-gel technique. With an expectation that the Fixed-Quat coated RCA would be a promising alternative for the control and/or mitigation harmful cyanobacteria growth in aquatic systems, Fixed-Quat coated RCA was investigated for its algistatic activity against freshwater cyanobacterial harmful algal bloom causing species, *Microcystis aeruginosa*, which included changes in chlorophyll-*a* concentrations and effect on algal biomass growth.

CHAPTER TWO: LITERATURE REVIEW

HABs mitigation refers to actions taken to suppress or destroy and/or inhibit algal cells in the events of algal blooms. The available techniques for HABs mitigation discussed in this literature fall into three categories in general: chemical, biological and physical control. The algaecides interact with microbial cells through a variety of mechanisms was also summarized in the subsections below.

2.1 Approaches for Controlling Algal Blooms

2.1.1 Chemical Controls

Chemical control methods are mostly regarded as one of the economical and rapid treatment methods for HAB control, although they have not been actively pursued due to the possible toxic by-products generation during treatment and unavailability of environmentally friendly chemicals. Some of these chemicals can be obtained naturally from bacteria or artificially derived. These chemicals act by suppressing algal growth, depriving algae from required nutrients or lyse algal cells by rupturing cell walls and/or disintegration of cellular membrane. Use of surfactants, or surface active agents, which attach onto surface of a system altering the free energies of the surface [27], has been investigated for algal growth inhibition.

Using biosurfactant sophorolipid produced by fermentation of *Torulopsis* species [28] the inhibition tests were studied on three common harmful algae *Alexandrium tamarense*, *Heterosigma akashiwo* and *Cochlodinium polykrikoides* [29]. This biosurfactant was treated with different portions of ethanol to increase the biocide activity and extracted with ethyl acetate. Optimum concentration of sophorolipid was 20 mg/L which was fatal to the cellular membrane of the algal cells.

Lactone-type sophorolipid exhibited a superior antimicrobial activity compared to acid-type sophorolipid in a study conducted by Kim et al. [30], and motility and growth of algal cells were inhibited at the concentration of 20 and 5 mg/L sophorolipid, with the latter considered to be an effective concentration for HABs mitigation by sophorolipid [31].

To find favorable synthetic surfactants for HABs mitigation, eleven surfactants (one cationic, two anionic, one amphipathic, six non-ionic surfactants and one biosurfactant) were tested on species *Cochlodinium polykrikoides* and *Alexandrium tamarens* [32]. 20 mg/L of the biosurfactant (sophorolipid) was considered as a control for effective HABs mitigation. Four of the surfactants; Polyoxiethylene nonylpenylether, alpha oleffinsulfornate, laurylamine, cocamidopropyl betaine showed good inactivation compared to the control, sophorolipid, and were tested for biodegradation using fresh natural seawater to validate its practical application. Cocamidopropyl betaine (CAPB) showed high inhibition efficiency via cell lysis and with the increase of CAPB concentrations from 10mg/l to 50mg/l, the contact time required for motility inhibition of cells reduced significantly from 24 hours to 5 minutes respectively.

Cerium oxide (CeO_2) nanoparticles was also used to carry out a luminescence inhibition of cyanobacteria *Anabaena* and green alga *Pseudokirchneriella subcapitata* [33]. Using different micro-sized particle ranging from 12-176 nm (Brunauer-Emmet-Teller, nm), at higher concentration, nanoparticle aggregates cells causing membrane rupture, cytoplasm leakage, and intracellular damage in both algal species.

Photocatalytic activities of Titanium dioxide (TiO_2) by suspending TiO_2 coated low cost and light weighted materials, a Nickel foam and a non-woven fabric, in an algal growth culture of *Anabaena sp.* was also studied [34]. Photocatalytic activities of these materials were evaluated by the degradation of methylene blue (representing organics) under ultraviolet (UV) irradiation with

the nickel substrate, resulting in better decolorization rate compared to fabric substrate. However, the fabric substrate supported with a cocatalyst, palladium (Pd) nanoparticles, showed high inhibition activity for algal growth which indicated that use of a cocatalyst or high surface area is required for TiO₂ coating.

Use of clay and flocculants are also considered as chemical controls. Use of phosphatic clay to a seawater culture was effective in removing cells and toxins of *Karenia brevis*. After ultrasonication, chemical flocculant, polyaluminum chloride (PAC), was added and aided the complete removal of extracellular toxins [35]. Furthermore, a test was conducted over a 14-day period to assess the viability of the clay on a long-term basis and results showed brevetoxins escaped clay floc over time.

A soil modified with chitosan [36], a polymer with high cationic charge density similar to coagulants and flocculants, removed 99% of algal cells from a water column within a day. However, an increase in dissolved microcystin (MC-RR and MC-LR) was detected, indicating that flocculation alone removes algal cells, not toxins. Further treatment using a microorganism-modified soil capping (*Pseudomonas sp.* An18) showed a 90% reduction in microcystins, as a promising technology for both flocculation and MC-degradation.

2.1.2 Biological Controls

Algicidal microorganisms have been tested and a variety of others can theoretically be used to control HABs. These include algicidal bacteria, viruses and plankton grazers [37, 38]. A bacterial strain, A27, belonging to the genus *Exiguobacterium* was tested on thirteen different toxic algae and cyanobacteria species [39] to target a bloom forming species, *Microcystis aeruginosa* in the study area Lake Taihu. The bacterial strain, A27, showed an effective algicidal activity inhibiting cell growth of *Microcystis aeruginosa*, but had no effect on two other algal strains tested. Interestingly, A27 facilitated the growth of *Microcystis wesenbergii* which is phylogenetically alike to the targeted specie *Microcystis aeruginosa*.

Actinomycete strain, O4-6, belonging to the genus *Streptomyces* was also tested and had a substantial algicidal effect inhibiting the growth of HAB causing specie *Phaeocytis globose* [40] with minimum inhibitory concentration (MIC) at 0.5 µg/l . During the characterization, O4-6 strain was figured to be vulnerable to heat but could operate in a wide range of pH and possesses salinity tolerance.

White-rot fungus, *Phanerochaete chrysoprium*, is also globally recognized for its ability to hinder the formation of algal blooms and thus the algicidal efficiency and mechanism were studied for three HABs species: *Cryptomonas obovata*, *Oscillatoria sp.*, and *Scenedesmus quadric* [41]. The analysis after co-culture with the three species for 48 hours showed the suppression of algal growth by a decrease in chlorophyll-*a* content after 48 hours of treatment, as well as in dehydrogenase activity and soluble protein content while an increase in malodialdehyde content (i.e., algal cells were ruptured by membrane lipid peroxidation). Fungal species, *Trichoderma citrinoviride*, inhibited the growth of *M. aeruginosa* degrading its microcystin by using as a carbon source for their growth [42]. Fungi can also interact with algae using branching hypha to form

ellipsoidal fungus-algal pellets during the process of palletization which removes algae from water bodies [43].

Macroalgae can also inhibit growth of HABs using their vegetative tissue of known as thalli. HABs inhibiting effects observed in live thalli were likely a consequence of a low but sustained level of release of the allelochemicals. Green macroalgae, *Ulva lactuca*, restricted the growth of microalgae species that form HABs globally [44, 45]. The experiment by Zheng et al, 2013 ([40]) was conducted to prove growth inhibiting mechanism resulted from allelopathic effects or release of toxins secreted by macroalgae as opposed to nutrient assimilation/competition (i.e., preventing neighboring species from accessing required nutrients).

Barley straw works in treatment of filamentous algae but is only effective for prevention. Barley straw an algistat that prevents the growth of algae through its decay and release of chemicals that inhibit alga growth [46].

2.1.3 Physical Controls

Physical methods for HABs control involves the removal of the harmful algae cells from the water column using physical means using is a suite of devices that enables mixing of the water column thus limiting the spatial extent of a bloom [38]

Ultrasound radiation is the most considered physical methods mostly suitable for surface bloom formers mainly composed of cyanobacteria. High intensity (power at 630W and a frequency of 22kHz), ultrasound disrupts gas vesicles in cells, hindering photosynthesis or compromises cell membranes [47] by producing free radicals and subsequently lipid peroxidation. Ultrasound radiation studies were conducted on *Microcystin aeruginosa* [48] which showed the effects of ultrasound radiation on algal cell density and chlorophyll-*a* concentration after three days. When

ultrasonication was halted, cyanobacteria growth resurfaced, indicating that the growth was only temporarily restrained.

2.2 Problems of Current Technologies

Although some of previously described algae control methods can promise relatively low cost with high algicidal efficiency, it is important that certain limitations regarding their implementation be considered. Physical processes such as ultrasonication, sedimentation and flocculation may exhibit a negative impact as algal cells may tear up releasing toxins or possibly transport retained toxins via sedimentation through the water column. Turbidity problems from resuspension of settled sediments is possible, thus, physical processes should be combined with biological controls or other processes that include the generation of reactive oxygen species (ROS) effective for removal of algal toxins to improve HABs mitigation (e.g., photocatalysis or hydrogen peroxide).

Chemical methods with mechanism of algae removal through cell lysis may release toxins. Chemicals used for controlling algae are mostly toxic and non-environmentally friendly, causing deleterious effect on other aquatic organisms and/or producing by-products in water systems. Although the lifetime of certain chemicals (e.g., hydrogen peroxide) is relatively short (i.e., half-life of 24 hours when exposed to air), others have the potential of bioaccumulation and the use nutrient-altering chemicals may lead to deterioration of water quality. Possible measures that controls the release and effect of chemicals on other organism would apply the chemicals as coating/wetting agents on suitable substrates of fixed (non-fluidized) media.

Biological methods have used bacterial strains, genetically engineered organisms, barley straw and macroalgae which often produce allelochemicals. However, barley straw releases additional organic materials to ponds through its decay and carries the risk of a fish kill. Other

biological methods need more validation before practical applications as it can develop predator species which may cause misbalance in food web dynamics of aquatic habitat. There is also a scare of using biological methods which introduce non-native species into the environment. Thus, for any biological control approaches to be implemented, a considerable research including field tests must be conducted to elucidate the effect on other species coexisting with the target harmful algal species along with the ecological benefits from the interactions.

Overall, to develop and apply an ideal method for controlling the HABs, there should be a balance between the effectiveness and costs of these HABs control methods, existing regulations of acceptable toxin levels, and the ecological effects on the organisms in water systems.

2.3 Proposed Sustainable Approach for Controlling Harmful Algal Blooms

2.3.1 Quaternary Ammonium Compounds (Quats)

Quaternary ammonium compounds (Quats) are cationic surfactants (positively charged in aqueous solution) effective, at very low concentrations, against a variety of microorganisms [49, 50]. Quats have low toxicity [51] and corrosivity and are constituents of domestic products for personal care, fabric softeners, emulsifiers, disinfectants, pesticides, and corrosion inhibitors [52, 53]. Quats exhibits antimicrobial and toxicological properties via chemical substitution and thus provide a wide range of its numerous structures [54]. Regardless of high potency of Quats against numerous bacterial strain but are less effective against bacterial spores and viruses.

Quats are chemically structured based on hydrophobic hydrocarbon (aliphatic or aromatic) chain connected to the positively charged central nitrogen atom along with other short chain alkyl groups (Figure 3) [49, 53]. The transport and fate of Quats in environment are generally attributed to their high adsorption affinity and biotransformation in aerobic conditions [49]. Quats easily adsorbs onto surfaces depending on its structure, nature of surface in contact with and environmental parameters [55, 56]. Chemical structure and concentration of Quats determine their biotransformation in aerobic conditions thus their concentrations vary in the environment. It has been reported that increasing alkyl chain length or replacement of a methyl group with a benzyl group decreases biotransformation of Quats [50].

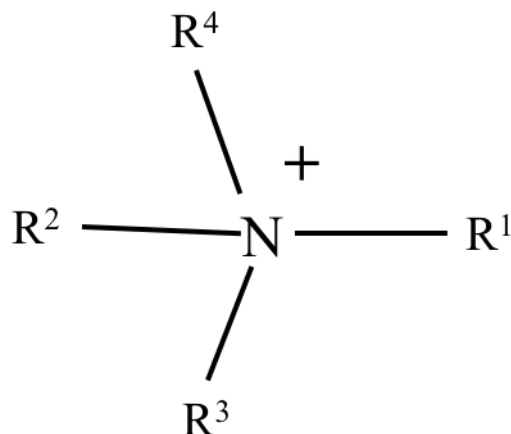


Figure 3. Chemical structure of Quats.

Quats are bacteriostatic in contact with bacteria resulting reversible inactivation of enzymes, disruption of bacterial cell membrane's physical and ionic stability [26, 53, 57] or by hampering cell mechanisms, synthesis and/or cell division. Recent studies have linked its mode of action to cell membrane disruption due to its affinity to negatively charged cell walls of bacteria and algae especially above minimum inhibitory concentration (MIC) [53] where physical and biochemical properties of cell is disrupted by the penetrating long alkyl [58-60] with the charged nitrogen disrupting charge distribution at the surface of the membrane [57]. With Quats recalcitrant property of creating an adsorption layer on the surface of cell, recovery from inhibition by Quats is retarded, compared to other disinfectants, with most cases leading to cell death [51].

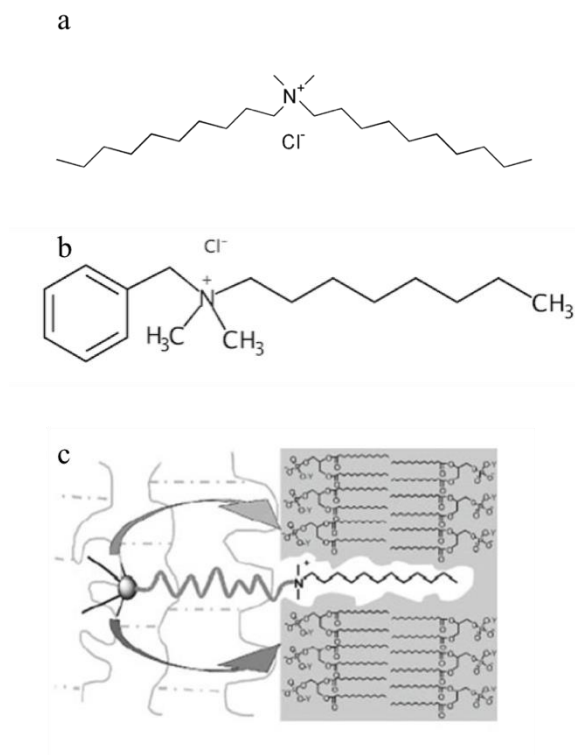


Figure 4. Illustration of two commonly used Quats a. Didecyldimethylammonium chloride (DDAC) b. Benzalkonium chloride (BAC). c. Antimicrobial mechanism of Quats on bacterial cell walls [61].

Table 2. Acronyms and structure of Quats mentioned in this literature

Quats	Acronym	Formula
Benzalkonium chloride	BAC	$C_6H_5CH_2N(CH_3)_2RCl$
Cetrimonium bromide	CTAB	$C_{19}H_{42}BrN$
Cetrimonium chloride	CTAC	$C_{19}H_{42}ClN$
Didecyldimethylammonium chloride	DDAC	$C_{22}H_{48}ClN$
Diethylaminobenzaldehyde	DEAB	$(C_2H_5)_2NC_6H_4CHO$

Andrew et al. [62] and Tezel et al. [63] showed that biocidal activity is the main attribute of Quats which poses a risk for aquatic ecosystems. Quats are toxic to aquatic organisms and a lot of microorganisms including algae are sensitive to the presence of Quats because Quats are readily bound to negatively charged algal cell walls [56]. Nalecz-Jawecki et al. [64] investigated the toxicity of 15 Quats on four bioassays comprising a bacterium, two ciliated protozoa and an anostracean crustacean (*Artemia franciscana*) and found that Quats release into surface water may be harmful as nontarget species, protozoa and crustacean, were severely affected. Jing et al. [65] used quantitative structure–activity relationship (QSAR) for prediction Quats toxicity to algal species and showed that different algal species have different sensitivity to variety of Quats. Liang et al. [66] showed that Quats, Cetrimonium bromide (CTAB), limited the uptake of NH_4^+ and TP by *C. vulgaris* with cell response analyses showing reduced photosynthetic and esterase activity, as well as cell viability. Through analysis of the relative quantum yield of chlorophyll fluorescence by PAM fluorimeter, Sanchez-Fortunet et al. [67] showed that Quats, Diethylaminobenzaldehyde (DEAB), significantly inhibited the photosynthetic performance and diminished the oxygen production of *S. intermedius* and *D. chlorelloides*.

Quats also form mixtures with other contaminants such as heavy metals, aromatic hydrocarbons (AHs) anionic surfactant whose toxic effect differs from Quats individuals [49, 68]. Combination of Quat (CTAC) with low concentrations of AHs showed synergistic toxicity on *Chlorella vulgaris*; however, at high concentrations of AHs, the synergistic effect became antagonistic due to the competitive adsorption between both compounds [68]. Binary mixtures of Quats and glutaraldehyde have also been proven to be synergistic for algae inactivation.

With known drawbacks of with biocidal agents such as silver, copper and other chemical controls (e.g., mammalian cytotoxicity and secondary pollution), antimicrobial layers which are

covalently immobilized on surfaces have recently been the subject of rapidly increasing interest for disinfection [61, 69]. Antimicrobial layers on surfaces prevent cytotoxicity issues with organisms which are not in contact and protects surfaces against biofilm development which is vital in medicine [61]. In addition, the organic antimicrobial compounds such as Quats can be an attractive alternative to metal-based antimicrobials. Due to its high adsorption affinity onto materials, toxicity of Quats can be mitigated especially in real environmental conditions with the surplus existence of sorbents (e.g., clay, sediments and suspended matter) [56].

Using Quats, Saif et al. 2008 [69] prepared a transparent coating via sol-gel technique on glass for testing inactivation of two bacteria, gram negative *E. coli* and gram positive *Staphylococcus aureus* and found that 95% of the viable colonies of both strains were inactivated after 48 hours. This indicated an excellent antibacterial property to the Quat coated surfaces. Contact-killing coating was also prepared by immobilizing Quats on hyperbranched polyuria coatings [70] which produced bactericidal ability. Adherence of Quat to polyuria was tested and it was found that, regardless of extensive washing, the coating still ruptured cell walls of *Staphylococcus epidermidis* without any detectable leaching of antibacterial compounds. Using cold plasma techniques, Soujanya et al. 2008, reported development of Quat surface layers with bactericidal activity on stainless steel and filter paper surfaces [71]. They also ensured the leaching out Quats in aqueous solutions was not occurred with a conclusion that the efficacy of Quats depends on the length of the alkyl chain and a high concentration of N^+ groups leads to high potency of inactivation to bacteria. Dhende et al. 2011 [72] prepared a non-leaching and ultra-thin coating consisting of quaternary polyetheleneimines spray-coating and photocross-linked by UV irradiation on surfaces which include cotton and plastic, and investigated the inactivation of both Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) bacteria. An interesting observation in the

experiment by Dhende et al. 2011 was that almost all the bacteria were killed when polymer layer was above 50 nm, which is a thin film of antimicrobial layer [72].

2.3.2 Recycled Concrete Aggregate (RCA)

Recycled concrete aggregate (RCA) is a material with high quality, well-graded aggregates bonded by a hardened mortar usually from obsolete concrete structures (e.g., buildings and roads). [73]. RCAs are one of the major wastes from demolition sites in developed ,and developing countries made by crushing concrete rubble, and removal of contaminants such as reinforcement, paper, plastics, gypsum [74] and metal which sometimes stays as RCA constituent due to the difficulty in removal from aggregates [73]. A large portion of these aggregates end up in landfills and thus considering the potential of its environmentally friendly nature and economic viability, a lot attention has been drawn for recycling and reuse them in a sustainable way. Construction and demolition wastes cause large waste streams in many countries and, thus, have become a global concern and to increase economic and environmental benefits, reuse of demolished debris can be efficient for nonstructural applications in variety of ways [1, 75, 76] as well as for secondary construction such as drainage, low cost housing road construction [76].

Recycling concrete materials can increase the effectiveness and sustainability of concrete materials by reducing environmental damage [74, 77] as well as energy consumption for concrete production [77]. Other areas which RCA can be used for include the reduction of carbon dioxide emission from resource extraction, soil stabilization, base layer replacing virgin aggregate in pavement foundation in transportation sector, for exfiltration trench and drain fields [73, 78, 79]. Sustainable engineering considers the economic and environmental impacts of designs and is fast becoming a standard for decision making in engineering and with the aforementioned benefits of recycling concrete, opportunities for conservation of primary resources, beneficial reduction of

landfill disposal [74] and transportation costs of waste disposal [80, 81] are achievable limiting the use of natural resources [82].

2.4 Thesis Statement and Tasks

The overall objective of this study was to explore the application of modified RCA as an algistatic media for sustainable water treatment. This is achievable through the following tasks:

- I. **To prepare, by sol-gel, a quaternary ammonium coated RCA process that imparts excellent antibacterial and algistatic properties.** Sol-gel is a process of preparing a protective and functional coatings, for the modification of a substrate surface properties whereas the bulk characteristics remain unchanged [83]. Sol-gel process is a synthetic approach that comprises hydrolysis, condensation and a drying process for the preparation of novel metal oxide nanoparticles and mixed oxide composites [84]. Hydrolysis is achieved by spreading Fixed-Quat on RCA through the process of spin-coating using machine stirrer. This leads to the formation of intermediates silanol groups (Si—OH). Subsequent heating process produce siloxane bonds (Si—O—Si) which leads to formation of a rigid, interconnected three-dimensional network with submicrometer pores and polymeric chains. The thickness of coating is dependent on the coating solution properties such as density, viscosity, and surface tension [85]. Scanning Electron Microscopy / Energy Dispersive X-Ray Spectroscopy (SEM/EDS) will be used for material characterization in this study. SEM aids material inspection by providing a great deal of information to help contrast between materials. SEM will be used for visual observation of both RCA samples (uncoated and Fixed-Quat coated) since Fixed-Quat is expected to alter the surface morphology of RCA. Concurrently, Energy Dispersive X-Ray Spectroscopy

(EDS, EDAX or EDX) will be used to obtain semi-quantitative elemental results about very specific locations within both RCA samples (uncoated and Fixed-Quat coated). Formation of a silica covalent bond between the Fixed-Quat and RCA will be detected using EDS graphs and elemental table.

II. To investigate mechanism growth inhibition mechanism on harmful algal bloom organisms, *Microcystis aeruginosa* from reaction with Fixed-Quat coated RCA.

Microcystis aeruginosa a species of freshwater cyanobacteria are the most common toxic cyanobacterial bloom in eutrophic fresh water which can form harmful algal blooms [86]. Characterized by small cells (few micrometers in diameter) without individual sheaths, *Microcystis aeruginosa* possess vesicles that allows buoyancy necessary for to stay at a level within the water column where light and carbon dioxide are accessible for rapid growth [87]. OD₆₆₀ universally known as the absorbance for estimating the concentration of cells in a medium will be used to monitor the growth of *Microcystis aeruginosa*. Cyanobacteria contain chlorophyll-*a* which is the most abundant form of chlorophyll in photosynthetic organisms as it captures sunlight for energy and gives plants their green color [6]. *Microcystis aeruginosa* is a species of photosynthetic cyanobacteria, thus, is capable of producing chlorophyll [6, 10, 23]. Monitoring chlorophyll levels will be conducted using the standard method protocol to help track algal growth and measure the amount of concentration of suspended cyanobacteria.

CHAPTER THREE: MATERIALS AND METHODS

3.1 Preparation of Material

3.1.1 Recycled Concrete Aggregates

RCAs for this study were obtained from a local commercial C&D waste recycling facility in Orlando, FL, USA and the diameters of RCAs were in the ranges of 1.5–2.5 inches. As a cleaning procedure, aggregates were washed three times daily with deionized (DI) water and submerged in DI water for 3 days to detach deleterious materials (i.e., debris and fines) at room temperature (23°C). RCA samples were then placed in an oven (Model 40 GC Lab Oven, Quincy Lab, Inc.) at 50°C for 3 days to dry the samples until a moisture content less than 0.5% of the mass of RCA was achieved.

A series of tests determined the physical properties of RCA which include a specific gravity of 2.16, unit weight of 1,210 kg/m³, and void content of 43%. For mineralogical investigation, X-ray diffractometer (XRD, Rigaku D/MAX XRD II) with CuK α radiation, 2 theta scanning from 5° to 80° with step size of 0.02° were used [73, 74].



Figure 5. Recycled concreted aggregates.

3.1.2 Silica-Quaternary Ammonium Compound (Fixed-Quat)

RCA coating agent used for this study was the gel-type which showed better performance and firm adherence to the substrate RCA in a previous study for inactivation of *E.coli* which was conducted in Dr. Lee's lab [26]. A mixture of coating agent was prepared by adding 60 mL of 37% sodium silicate (Fisher Scientific) to 910 mL of DI water and left to stir at 150 rpm using an overhead machine stirrer (Electric Stirrer 6000, Arrow Engineering) for 6 hours at room temperature. Subsequently, 30 mL didecyldimethylammonium (DDAC, EMD Millipore) was then added to the mixture and stirring continued for 24 hours.

3.1.3 Antimicrobial Coating of Fixed-Quat on RCA

Total 23 RCA samples were kept in a 4L container which 2L of coating mixture (Fixed-Quat gel) was subsequently added. Mixture was stirred at 150 rpm for 72 hours using an overhead

machine stirrer (Electric Stirrer 6000, Arrow Engineering). Samples were subsequently taken out gradually and rinsed gently to get rid of the samples from superficially bound froth of the coating agents. To complete the process, condensation of samples was achieved by heating in an oven (isotemp oven Model 615f, Fisher Scientific) for 48 hours at 50°C.

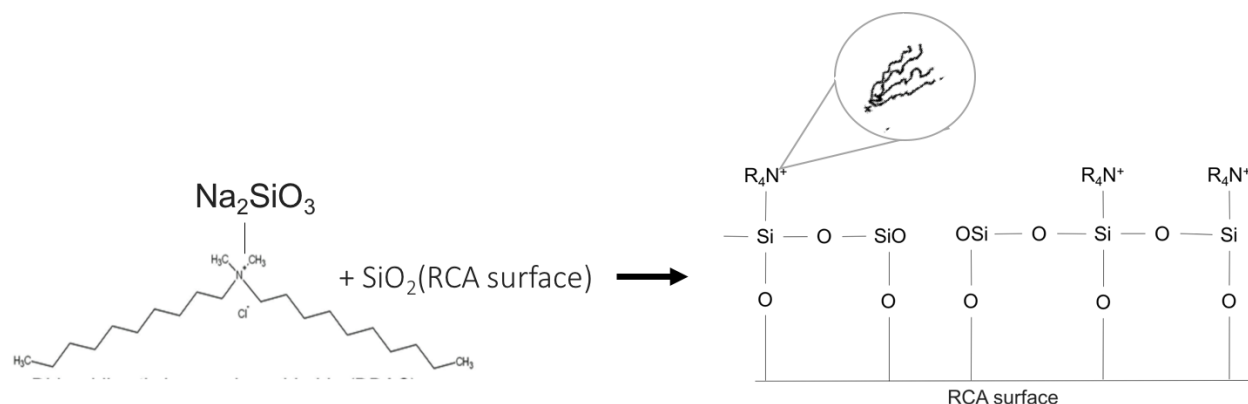


Figure 6. Schematic for the covalent bonding of Fixed-Quat on RCA surface.

For characterization of RCA samples, scanning electron microscope (SEM; JEOL JSM – 6480) with X-Ray Energy-Dispersive Spectrometer (EDS) was used for surface analysis after samples (uncoated and Fixed-Quat RCA) were gold coated in a sputter coater (Emitech K550) for 3 min at 20 mA.

3.1.4 Algae preparation

Bold's basal medium (BBM) for maintaining optimal growth conditions was prepared using the protocol from Canadian Phycological Culture Centre (CPCC), University of Waterloo [88]. The enriched BBM (1L) used as nutrients for the algae cultivation contained 175 mg/L KH_2PO_4 , 25 mg/L $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 75 g/L $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 250 mg/L NaNO_3 , 75 mg/L K_2HPO_4 , 25 mg/L NaCl , 5 mg/L $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 1 mg/L H_2SO_4 (concentrated), 11.5 mg/L H_3BO_3 , 10 mg/L $\text{Na}_2\text{EDTA} \cdot 2\text{H}_2\text{O}$, 6.2 mg/L KOH , trace metals solution containing 2.86 mg/L H_3BO_3 , 1.8 mg/L $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 0.0222 mg/L $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.039 mg/L $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$, 0.0079 mg/L

CuSO₄·5H₂O, 0.00494 Co(NO₃)₂·6H₂O. Cyanobacteria culture of *Microcystis aeruginosa* (UTEX 2385) was grown at 28°C in photobioreactors with continuous white fluorescent light illumination of 2,000 lux. For experiments, 1L aliquots of the BBM medium containing exponentially growing *M. aeruginosa* with the initial density of **3.6 × 10⁶ cells/mL** (concentrations of algal blooms range between 10⁵-10⁷ cells/mL) were conveyed into the two reactors which were subsequently occupied by uncoated and Fixed Quat coated RCA samples.

3.2. Experimental Procedure

3.2.1 Biomass Determination

Biomass was determined with cell density by determining cell counts using hemocytometers (Incyto c-chip, Fischer Scientific) and optical density (OD₆₆₀) using a portable spectrophotometer (DR1900, Hach). 10 µL of cell suspension applied to the hemocytometer (Incyto c-chip, Fischer Scientific) and inspected under a microscope (Revelation III Binocular, LW Scientific) using a 10× objective lens. Cell counts were conducted using a hand tally counter. Average cell count from each of the sets of 16 corner squares multiplied by 10,000 (10⁴) and the dilution factor. The coverslip sits 0.1mm over the chamber. Therefore, the volume of each square, contained under the coverslip is: 1mm × 1mm × 0.1mm = 0.1mm³ or 10⁻⁴ml.

3.2.2 Reactor Configuration

Two rectangular reactors were constructed using acrylic plastic materials with dimensions 14.5 cm (height), 21.4 cm (length) and 15 cm (width) (Figure 6). The volume of each reactor was 1.5 L. Reactor configuration includes recycle stream lines and peristaltic pump (Masterflex, L/S).



Figure 7. Reactor construction.

Contact-killing requires cells of target species to approach the surface which contains sufficient accessible Quats to kill bacteria [89]. Thus, effluent was recycled at 0.6 L/min of flowrate to increase contact time between RCA samples and cyanobacteria cells. The reactors were exposed to continuous light conditions 1,550 lux using fluorescent lamp to ensure algal photosynthesis and enable phototaxis, a property that allows for HAB species to reach the surface during the daytime [12, 31].

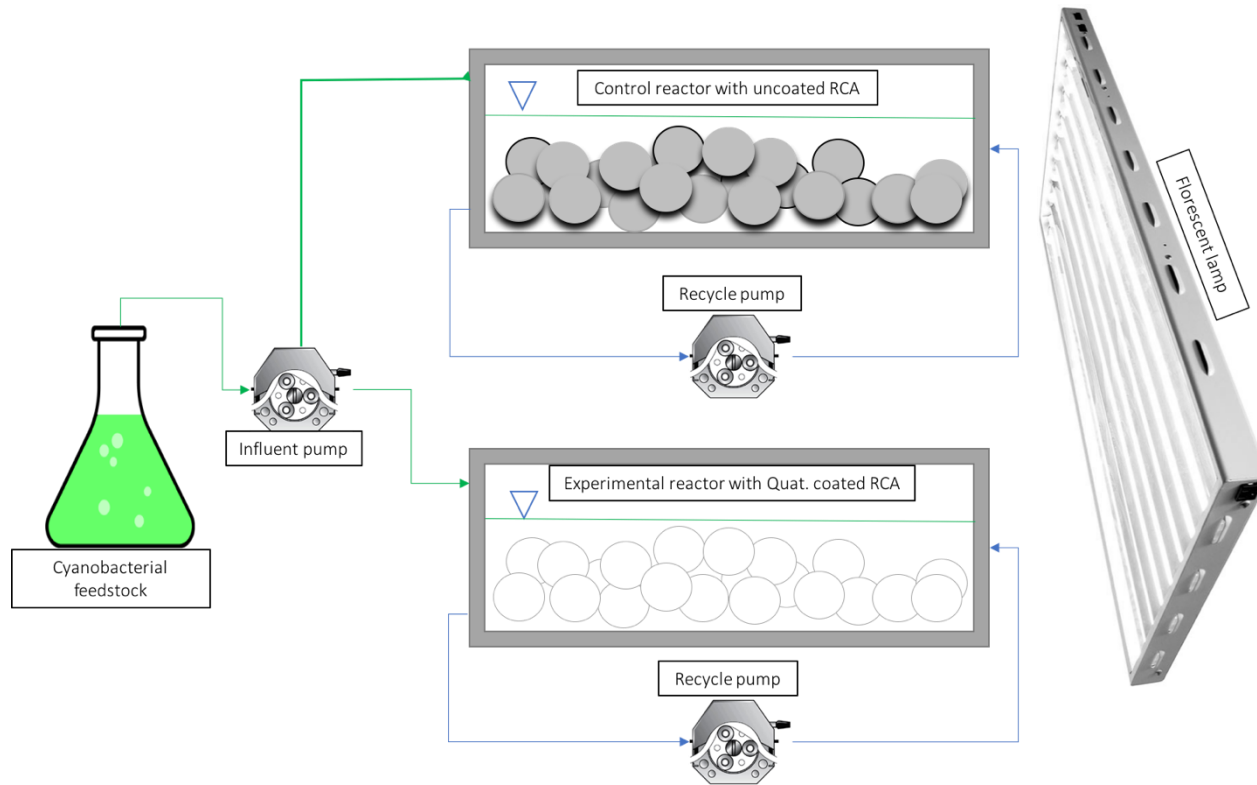


Figure 8. Schematic of reactor setup for algal interaction with uncoated and Fixed-Quat coated RCA samples.

3.2.3 pH of Medium

pH of test solution was measured prior to each experiment to monitor the possible pH changes due to RCA rehydration during experiment. RCA produces a substantial amount of calcium carbonate when exposed to moisture and carbon dioxide (CO₂) [41]. The crushing process to generate RCA exposes fines that are not hydrated which leads to cementation when these fines are exposed to humid conditions changing the physical properties of the RCA [73]. The pH in both RCA samples was measured using a pH meter (DR1900, Hach) continuously to monitor any pH increase from the cement hydration which may cause experimental bias. The batch tests were initiated when RCA samples aged (when no significant increase in pH was observed).

3.2.4 Surface Adhesion of Coating Material

It is impossible to ensure coatings to have no leachable biocides as even small amounts of leachable can be enough to inactivate both target and non-target species. Intensive washing is thus required to ensure the absence of any leachable component. To avoid inactivation of algae by reacting with coating materials in suspension, RCA samples were rinsed with DI water (1L) in 4L Pyrex volumetric flask by gently swaying for 10 min followed by 20 min of undisturbed submersion. Rinse water was inoculated with *E. coli* until growth was observed which was around the 5th rinse followed by the previous study in the lab [26].

3.2.5 Algistatic activity of Fixed-Quat coated RCA

Chlorophyll-*a* was used to estimate changes in the efficiency of photosynthesis in each sampling time. Saturated magnesium carbonate solution was prepared by mixing 1.0 g finely powdered MgCO₃ in 100 mL distilled water. 90 parts aqueous acetone solution (reagent-grade BP 56°C) was mixed with 10 parts saturated magnesium carbonate solution.

Chlorophyll-*a* from cyanobacteria was extracted with 90% acetone by agitating the solution containing cyanobacteria using an ultrasonic probe (Model 505 sonic dismembrator, Fisher Scientific) in fume hood (Hemco Independence, Missouri CAT: 4940-CF-031019). Samples were first filtered using glass microfiber filter media (934-AH, Hach), macerated using 90% acetone and ultrasonification followed. Agitated samples were transferred to a screw-cap centrifuge tube (Fischer Scientific) and adjusted total volume to 10 mL, with 90% aqueous acetone, steeped overnight at 4°C in the dark.

Extracts were clarified by centrifuging in closed tubes for 20 min at 5000×g (225a, Fisher Scientific). The acetone extracts of chlorophyll-*a* were transferred to cuvettes and analyzed using a portable spectrophotometer (DR 1900, Hach). Chlorophyll-*a* was calculated using standard methods (19th Edition, 1995) as follows:

$$\text{Chlorophyll-}a \text{ (mg/m}^3\text{)} = \frac{26.7(664b-665a) \times V1}{V2 \times L} \quad (1)$$

Where, $V1$ = volume of extract,

$V2$ = volume of sample, m^3 ,

L = light path length or width of cuvette, cm, and

$664b, 665a$ = optical densities of 90% acetone extract before and after acidification respectively. The value 26.7 is the absorbance correction and equals $A \times K$, where A is the absorbance coefficient of chlorophyll- a at 664 nm = 11 and K is the ratio expressing correction for acidification = 2.43 (19th Edition, 1995).

Efficiency of chlorophyll- a removal was calculated per the following:

$$\text{Inhibition ratio (\%)} = \frac{(Co-Ct)}{Co} \times 100 \quad (2)$$

where Co and Ct represent chlorophyll- a initial content and treatment groups, respectively [41] .

Measurements were conducted in triplicate, at ambient room temperature with glass wares which were autoclaved at 121°C for 20 minutes.

CHAPTER FOUR: RESULTS AND DISSCUSSION

4.1 Results

4.1.1 Characterization of RCA samples

RCA constitutes of hydrated cement, sand, and limestone which were analyzed using XRD [41, 42]. SEM micrographs of both uncoated and Fixed-Quat coated RCA (shown in Figure 9a and 9b respectively) shows surface RCA was altered in the Fixed-Quat coated RCA. This alteration is due to the amorphous nature of the Quats which appeared dense and homogeneous compared to the uncoated RCA sample. Since Si is a common element for both RCA and the Quats, it plays an important role in bonding the Quat and the surface of RCA.

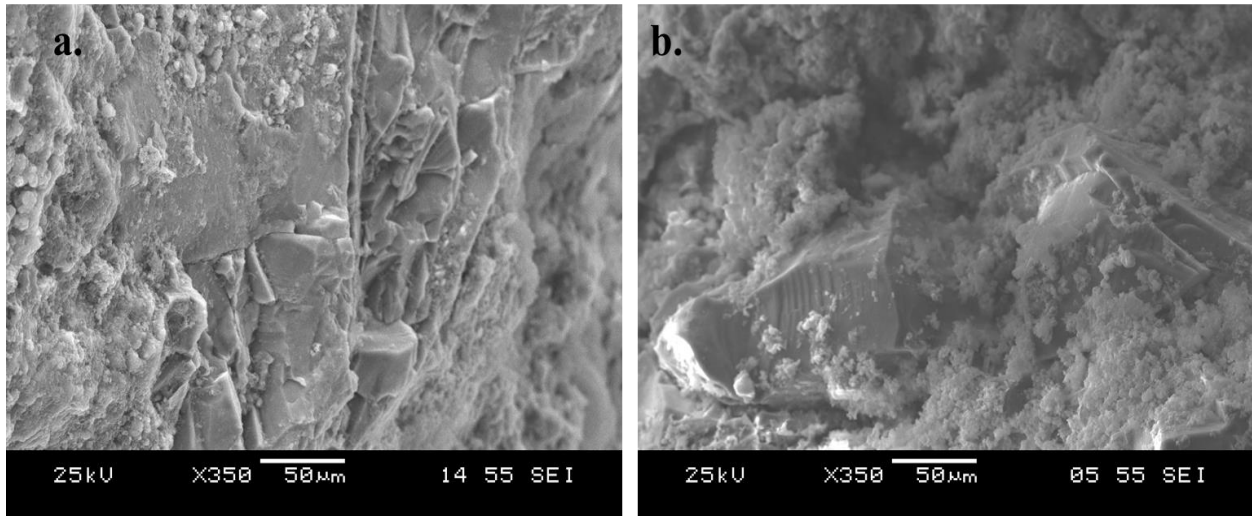


Figure 9. SEM micrographs of RCA. a. Uncoated RCA. b. Fixed-Quat coated RCA. $\times 350$ magnification at 25 kV.

The EDS analysis (Figure 10; Table 3) reveals atomic and weight percentage values (at % and wt. % respectively) in both samples. Weight percentages (wt. %) are related to concentrations of the element, thus, an increase in wt. % after coating Fixed-Quat to RCA (13.2% to 32.05%),

indicating the formation of covalent silica bond between the coating material and RCA. SEM/EDS analysis of samples surfaces confirms successful coating.

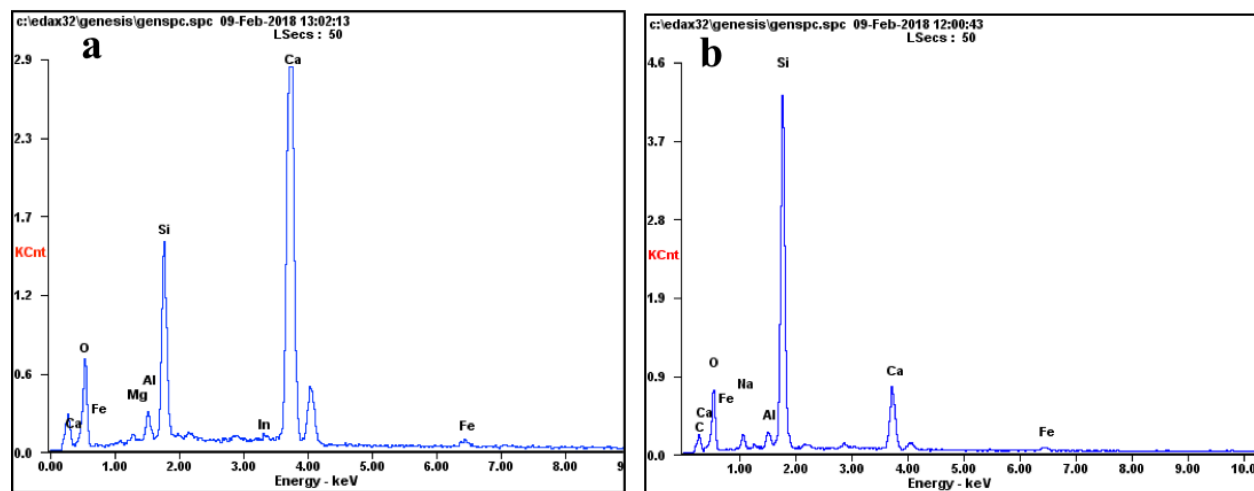


Figure 10. Graphs of the EDS analysis of RCA samples. a. Uncoated RCA. b. Fixed-Quat coated RCA.

Table 3. EDS table showing the element compositions of both RCA samples.

Element	Uncoated RCA		Fixed-Quat. RCA	
	Wt. %	At %	Wt. %	At %
O	40.32	60.58	29.95	34.27
C	-	-	24.79	37.78
Mg	00.98	00.97	-	-
Al	02.57	02.29	01.55	01.05
Si	13.42	11.48	32.05	20.88
InL	01.72	00.36	-	-
Ca	39.47	23.67	08.16	03.73
Fe	01.51	00.65	01.06	00.35
Na	00.00	00.00	02.45	01.95

4.1.2 Effects of pH on Uncoated and Fixed-Quat Coated RCA

4.1.2.1 Initial pH Changes from Hydration Prior to Experiment

Prior to the experiment, the uncoated and Fixed-Quat gel coated RCA samples were immersed in DI water and samples were monitored continuously for 3 days. For accuracy in pH

reading, a 1:1 weight to volume ratio was used for pH measurement to maintain a solid-liquid ratio consistency. Figure 11 showed that uncoated RCA samples reached pH 7.4 and dropped remaining almost within same pH range, whereas in Fixed-Quat coated sample, pH was maintained at 10. High pH in Fixed-Quat coated RCA mixture showed similarity to the pH of Quat in solution measured at 11.3, thus the relatively higher increase in pH when compared to the mixture containing uncoated RCA samples is attributed to Quats in suspension and in solution (dissolved Quats). Stable pH was achieved in both mixtures indicating the stabilization of cement hydration.

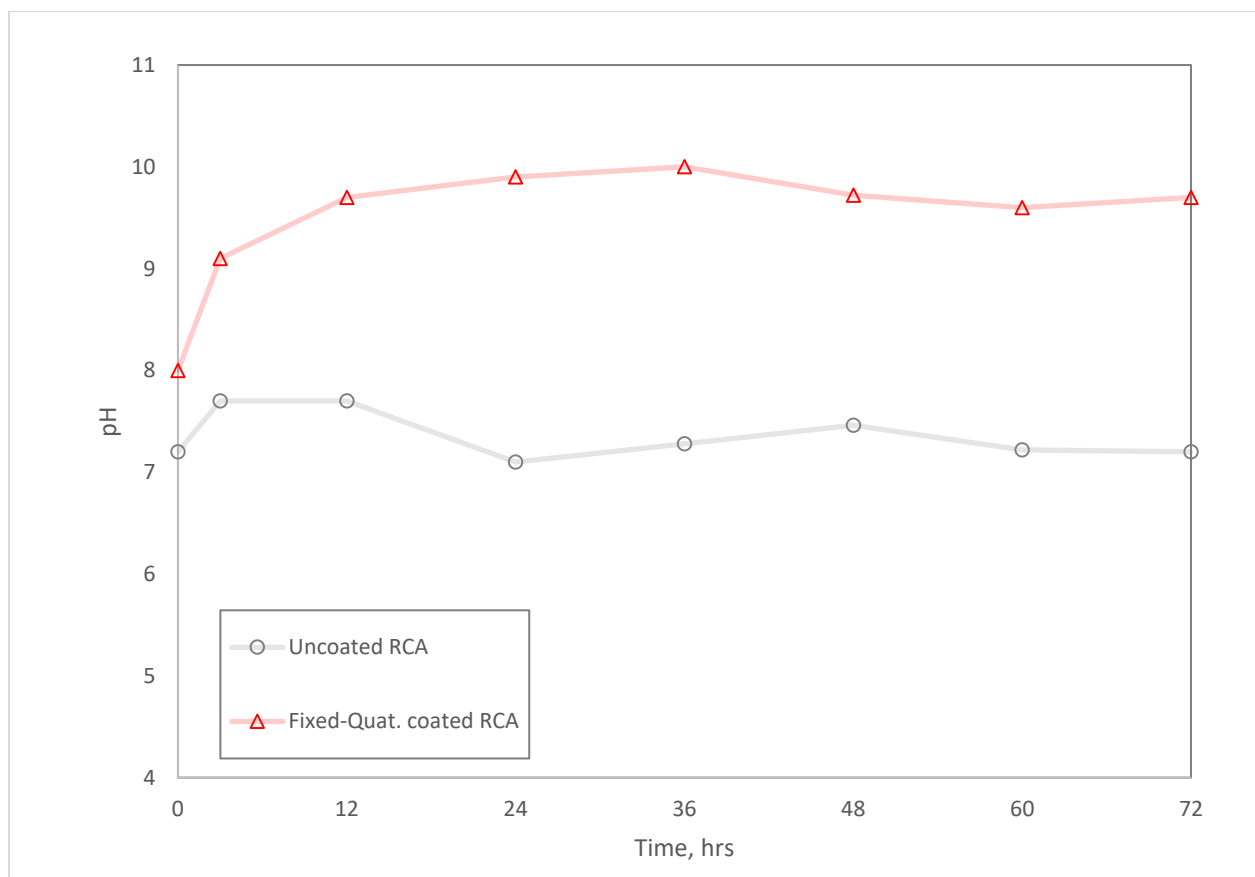


Figure 11. pH changes in uncoated and Fixed-Quat coated RCA samples.

4.1.2.2 pH Measurements during Experiment

The initial pH of the media (BBM) was 5.8 and 3 hours into the experiment both reactors containing the aggregates increased by approximately 2 units when compared to the reactor

containing no RCA sample. This increase of pH 3 hours into the experiment seems to be possible due to the effect of concrete hydration in a new solution (Figure 12) and with semblance of a parabola it affirms that recycle concrete aggregate follows parabolic rate law during hydration. pH did not undergo any significant changes afterwards and was maintained between 7 and 8 throughout the entire duration of the experiment (32 hours). With similar trends in pH changes in both reactors, algal growth was favored within these pH ranges, indicated by OD₆₆₀ curve of the control reactor containing uncoated RCA, thus it is safe to conclude that cement hydration had no effect on the experiment.

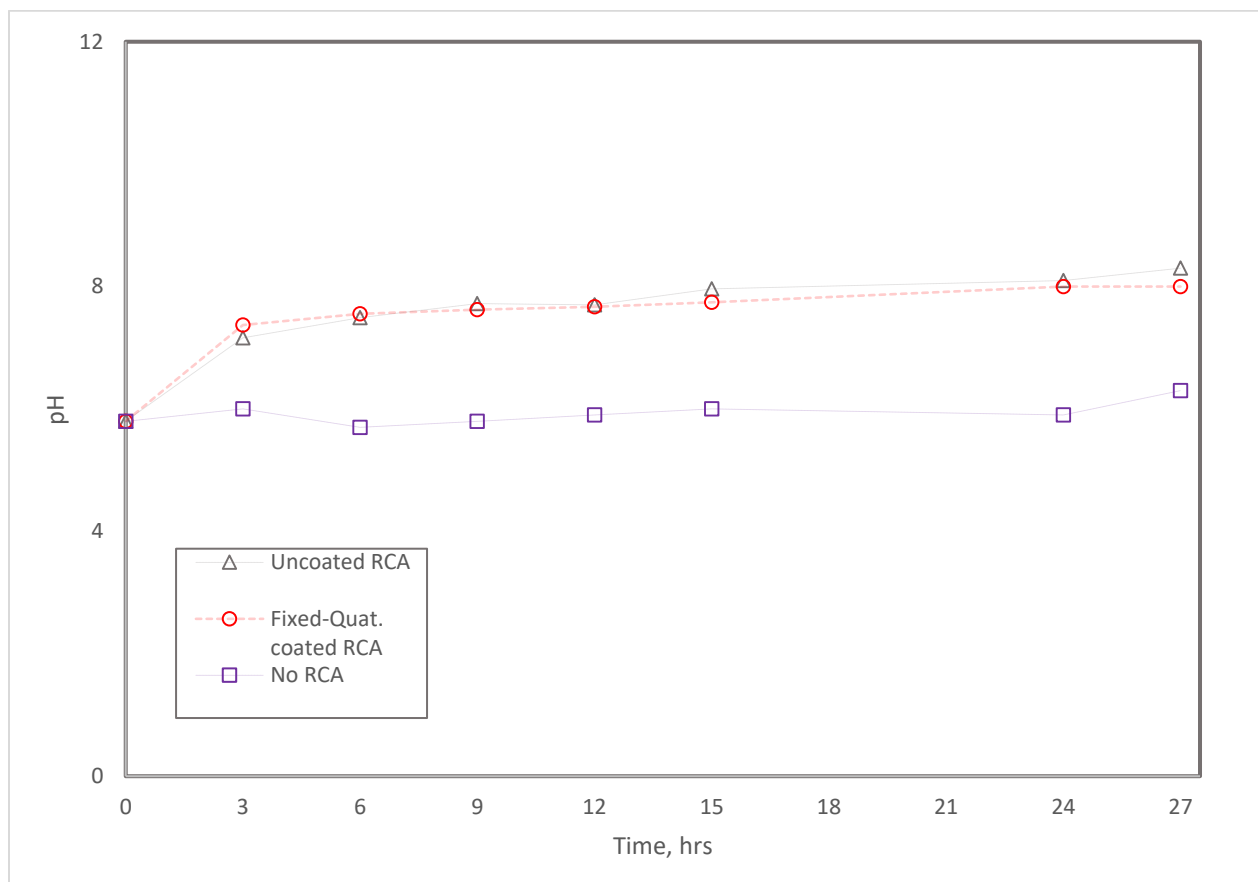


Figure 12. pH changes in uncoated, Fixed-Quat coated and no RCA (reactor without RCA samples) during experiment.

4.1.3 Pretreatment of Fixed-Quat coated RCA

The rinse water of both RCA samples (uncoated and Fixed-Quat coated) were inoculated with *E. coli* and monitored to ensure that the algistatic effect resulted from surface bound and not from unbound Quats (suspended and dissolved Quats). As shown in figure 13, the rinse water from uncoated samples maintained *E. coli* at the inoculated concentration for all washings whereas detectable growth of *E. coli* at the same level of inoculation concentration was observed after the 5th rinse in the Fixed-Quat coated RCA rinse water. RCA samples were further washed 10 times which was sufficient to completely remove unbound Quats in previous study. This result confirmed that the washing steps was effective in the removal of unbound Quats.

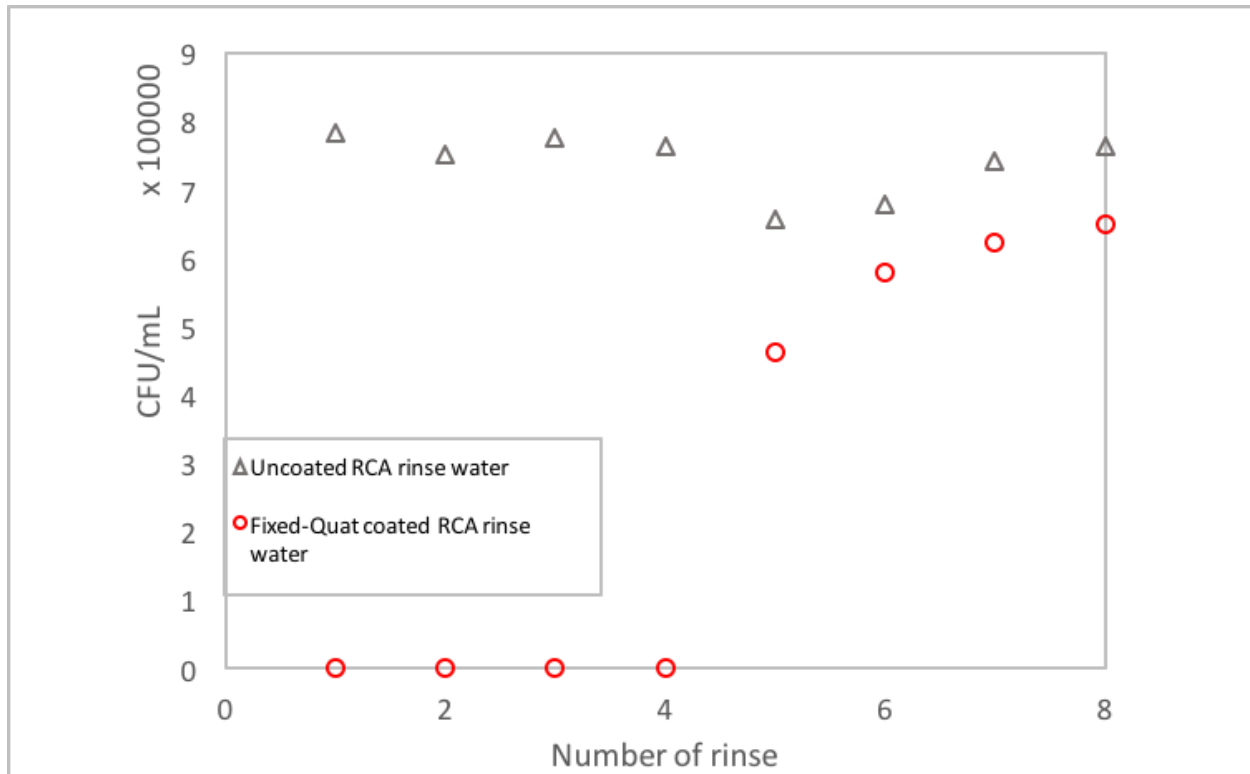


Figure 13: Growth of *E. Coli* in rinse water of uncoated and Fixed-Quat coated RCA samples.

4.1.4 Algistatic Activity

4.1.4.1 Biomass Accumulation and Chlorophyll-*a* Determination

During the experiment, the cyanobacterial cells showed tendency to accumulate at the media surface. The differences observed in the biomass of in the reactors was statistically significant with the rapid decrease in OD₆₆₀ measurements in the reactor containing Fixed-Quat coated RCA samples as shown in figure 13. *Microcystis* sp. regulates buoyancy by adjusting position within water column to overcome vertical separation between light and nutrients [47]. Figure 14 shows the data for biomass growth during exposure to both uncoated, Fixed-Quat coated and reactor without RCA samples. The increasing growth of culture in the reactor containing uncoated RCA and the reactor without RCA (0.16 to 0.31 and 0.61 to 0.28 respectively) which is in stark contrast to the decreasing growth of culture exposed to Fixed-Quat RCA samples (0.16 - 0.01) indicates growth inhibition and a possible loss of vertical migration by *Microcystis aeruginosa*.

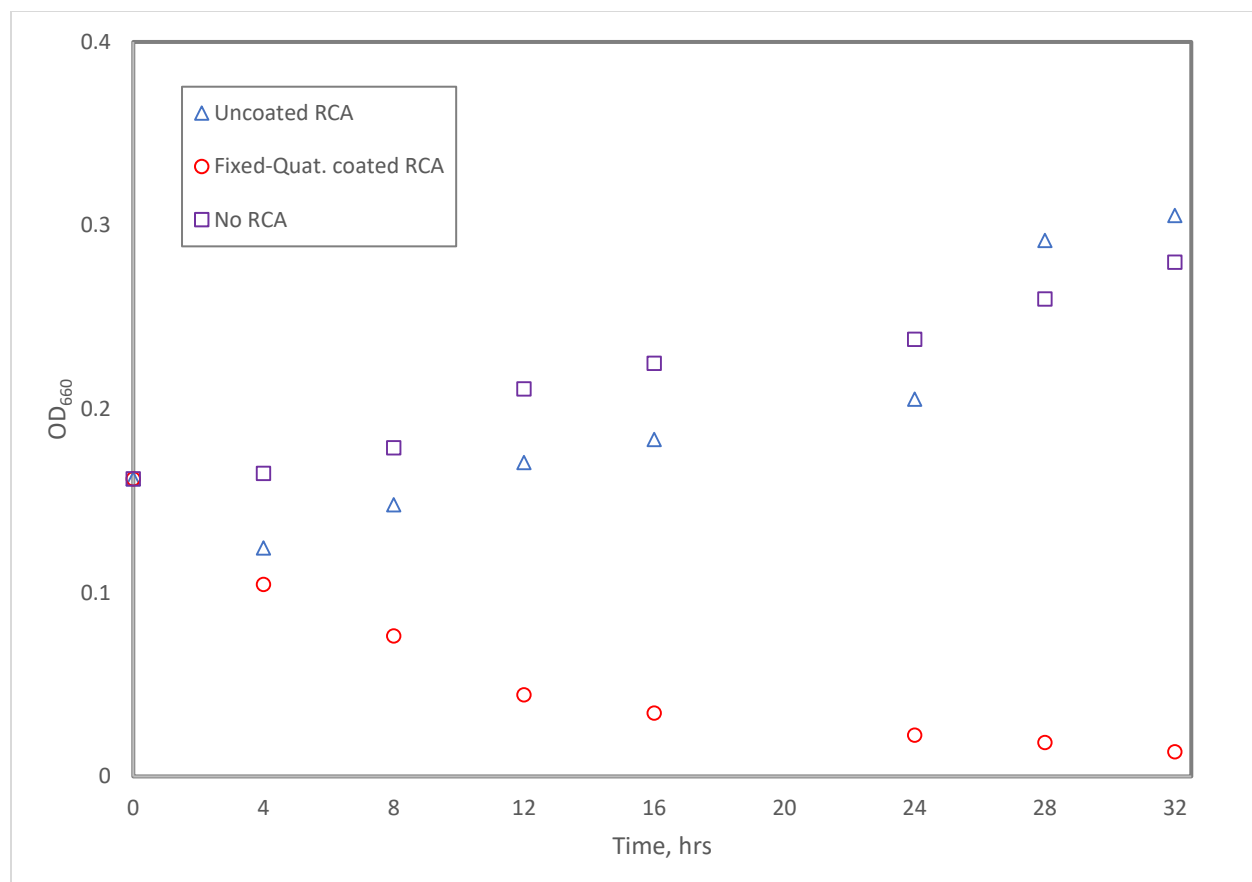


Figure 14. Comparisons of algal growth curves of *M. aeruginosa* between uncoated RCA, Fixed-Quat coated RCA, and no RCA (reactor without RCA samples).

Chlorophyll-*a* (chl-*a*) plays an important role in conversion of light to chemical energy and therefore is a content of all photosynthetic plant, algae and cyanobacteria. Chl-*a* was evaluated after exposure to uncoated and Fixed-Quat coated RCA samples. Time courses measurements of chlorophyll-*a* concentrations are shown in Figure 15. Chl-*a* of *M. aeruginosa* culture exposed to uncoated RCA (i.e. control) remained nearly constant from 208.3 ± 16 to 213.6 ± 7.6 mg/m³ which indicates the culture retained its photosynthetic activity. The *M. aeruginosa* culture exposed to uncoated RCA did not show any visible changes or discoloration retaining their intense blue-green color and exhibited proper vitality i.e. OD₆₆₀ (Figure 14). This indicates no inhibition ability of the

RCA rocks, however decrease in chl-*a* concentration was observed in the reactor containing the Fixed-Quat coated with complete removal of chl-*a* seen within of 7-9 hours of contact with Fixed-Quat coated RCA samples. Chl-*a* content reduced 36%, 61% and 100% after 2, 6 and 9 hours of contact respectively with Fixed-Quat coated RCA (Figure 16) which confirms the algistaitic ability of the Fixed-Quat coated RCA.

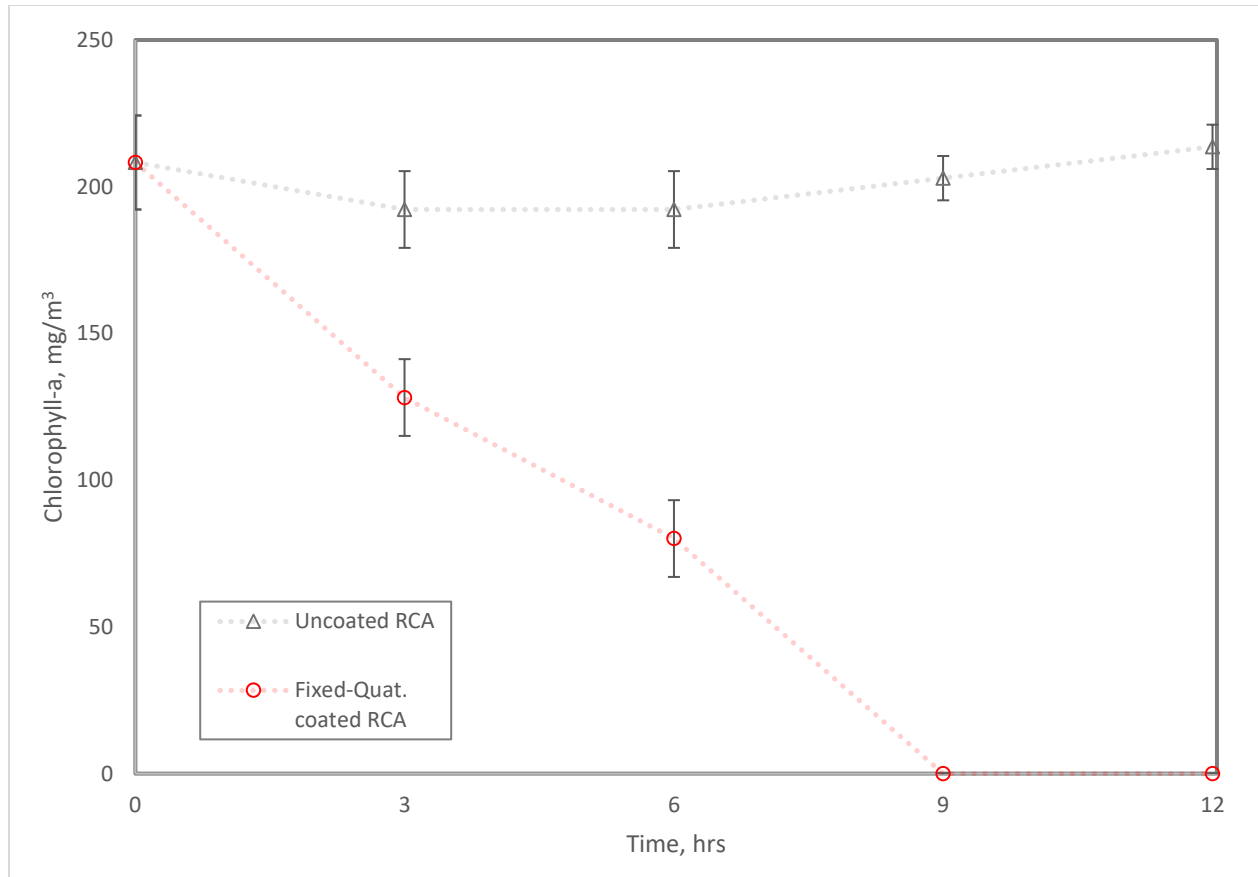


Figure 15. Changes in chlorophyll-*a* content from uncoated and Fixed-Quat coated RCA samples. The vertical bars indicate the standard deviation.

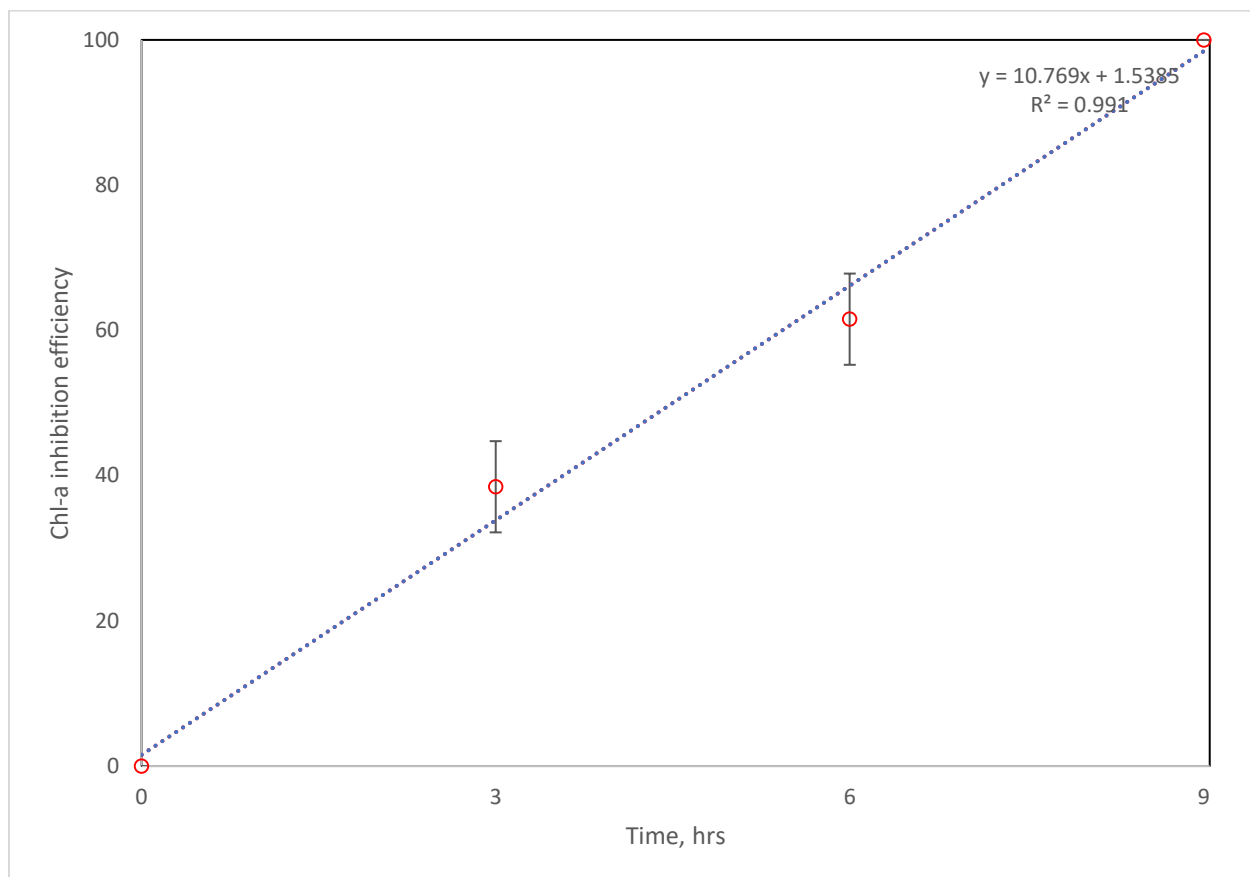


Figure 16. Algistatic efficiency of Fixed-Quat coated RCA against *Microcystis aeruginosa*. The vertical bars indicate the standard deviation.

Images of the cultures are shown in figure 17. After 32 hours, culture with uncoated RCA samples became opaque with dense green coloration indicating the presence of chlorophyll (Figure 17a). On the other hand, the culture containing the Fixed-Quat coated RCA was transparent (Figure 17b). Microscopic observations showed cyanobacterial cells in Fixed-Quat coated RCA reactor had possibly been ruptured causing sedimentation to the bottom of the reactor. This result showed the possibility of effective usage of the Fixed-Quat coated RCA for algal growth inhibition.

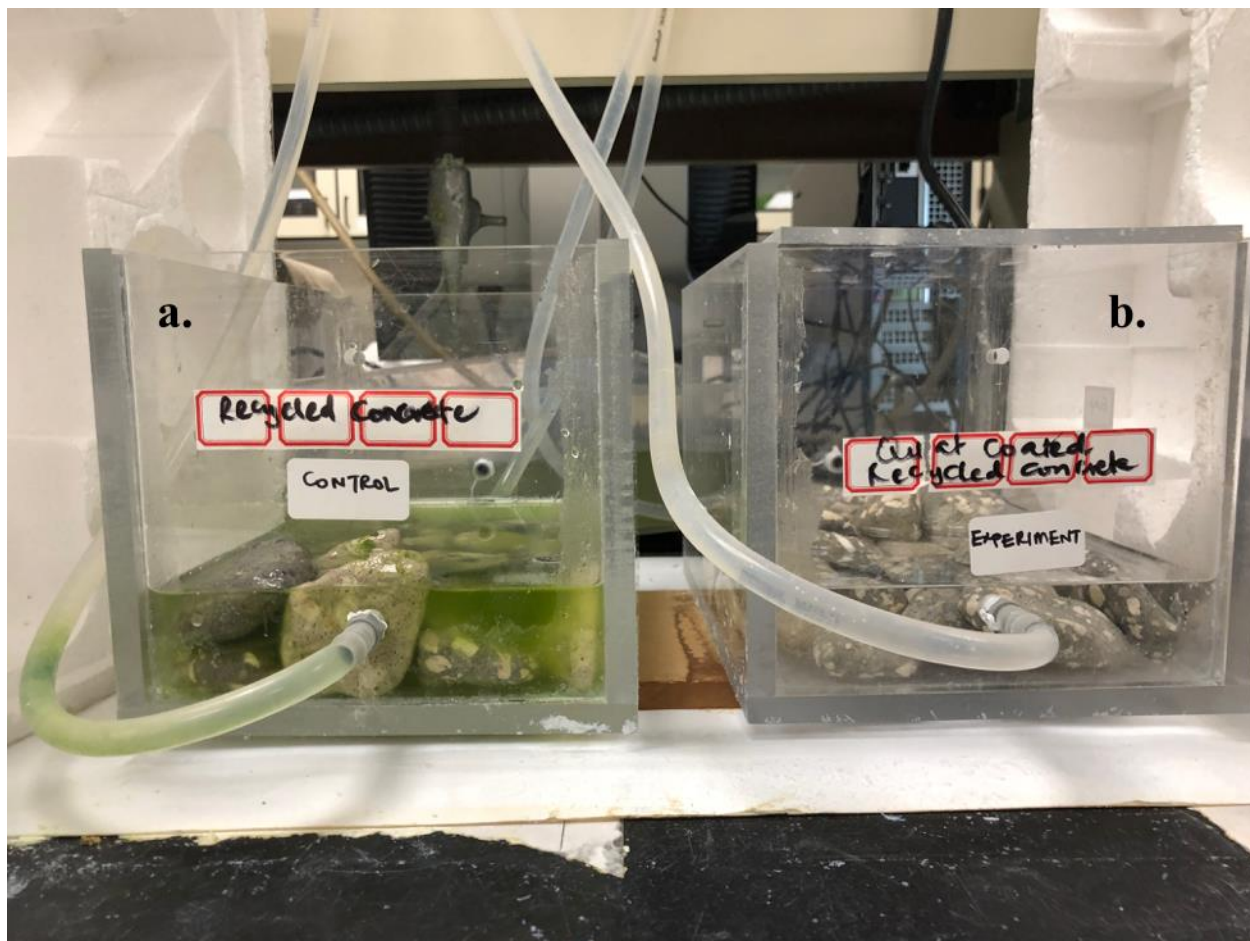


Figure 17. Images of algal growth with a. Uncoated RCA samples. b. Fixed-Quat coated RCA sample, with initial algal concentration of 3.6×10^6 cells/ml after 32 hours contact in continuous light intensity of 1,550 lux.

4.2 Discussion

This study demonstrated the reduced biomass yield of harmful cyanobacterium *Microcystis aeruginosa* when in contact with Fixed-Quat coated RCA. As reviewed previously in chapter 2 of this study, cyanobacterial growth is related to factors which include light, carbon dioxide, temperature and availability of certain key nutrients (phosphorus particularly). With provisions made for light through high fluorescent and a modified enriched BBM used for culture growth, Fixed-Quat RCA still inhibited the growth of *Microcystis aeruginosa*.

The major adverse effect of Fixed-Quat coated RCA on cyanobacteria is attributed to the attachment of long alkyl chain to negatively charged cell walls which leads to rupturing of cell wall and subsequently sedimentation and the prevention of photosynthesis. Contact-killing was confirmed as attached growth of algal biomass was physically observed on the surface of the uncoated RCA samples when compared to the Fixed-Quat coated which retained its physical appearance (Figure 18).

a.



b.



Figure 18. Images of RCA samples a. Uncoated RCA samples. b. Fixed-Quat coated RCA sample, after 32-hours exposure to algal culture of *Microcystis aeruginosa*.

It was also observed that photosynthesis is affected by degradation of photosynthetic pigments. The results indicate that the Fixed-Quat coated RCA has a great influence on the chlorophyll-*a* content. The chl-*a* content and cell growth decreased simultaneously when exposed to Fixed-Quat coated RCA indicating growth inhibition by cell lysis and sedimentation of the

cyanobacterial cells after losing its ability of vertical migration. This study shown Fixed-Quat coated RCA is a convenient and efficient tool for the inhibition of cyanobacterial growth.

CHAPTER FIVE: CONCLUSION AND PRACTICAL APPLICATION

HABs are global nuisance, mainly fueled by man's needs to produce food to meet increasing population demands via agriculture and other sewage disposal techniques. Various methods developed for the mitigation of these blooms and their shortcomings were explored. HAB prevention strategies should be thoroughly considered to curtail rapid increase of nutrients from sewage disposal, use of chemical fertilizers in agriculture, and fossil fuel combustion, into waters. Use of small scale biological nutrient removal plants, implementing and encouraging sewage reduction strategies, as well as controlled agricultural practices can help prevent excessive accumulation of nutrients.

Chemical techniques cause secondary pollution and finding a means to curtail this problem by minimizing the risk on unintended species in aquatic environment, is key to developing a sustainable approach for HABs control. Factors considered during selection of HAB controlling methods include effectiveness, cost and practicability [90]. Herein, algistatic capabilities of imparting Quats to the surface of RCA via sol-gel technique was presented. Fixed-Quat coated RCA promises a good measure for controlling HABs by efficiently inhibiting growth and photosynthetic pigment of harmful algae species *Microcystis aeruginosa*. Preparation of material and process of application are very simple and easy and Quat could successfully be immobilized on the surface of the RCA.

Practicability can be in several ways. Construction and demolition (C&D) wastes cause large waste streams in many countries have become a global concern. Since Fixed-Quat enhanced algicidal properties of concrete surfaces without leaching out into the algal culture in this study, Fixed-Quat can be used in a mortar mix for curbing algal growth on concrete, walls of reservoirs, biological growth on buildings and conveyance structures. RCA also promises a good material for

the construction of French drain and the application of Fixed-Quat coated RCA to French drain platform can prevent clogging from biofilm buildup in French drain. With majority of these blooms occurring in coastal areas, filling up a constructed contour trench rather than landfills with Fixed-Quat RCA can be used to recover algal infested water for other beneficial uses like irrigation, water supply for firefighting etc.

In eutrophic rivers or lakes, algal blooms lead to anoxia and fish kills during especially in summer periods. The odors and aesthetically unappealing nature of algal blooms is of great concern to human as it depreciates recreational value of reservoirs, lakes, and streams causing a decline in lakeside or riverside property values [86]. Introduction of the Fixed-Quat coated RCA in a river or lakes through free-floating permeable enclosures can thus reduce the concentrations of chlorophyll-*a* significantly under sunlight. The enclosures could be made of permeable material, such as ventilated filters or screens, equipped with flotation collars (to float in water to enhance the close contact with algae), tethered and deployed in boats or tethered to a support (poles or posts) in the case of lakeside blooms as shown in figures 19 and 20.

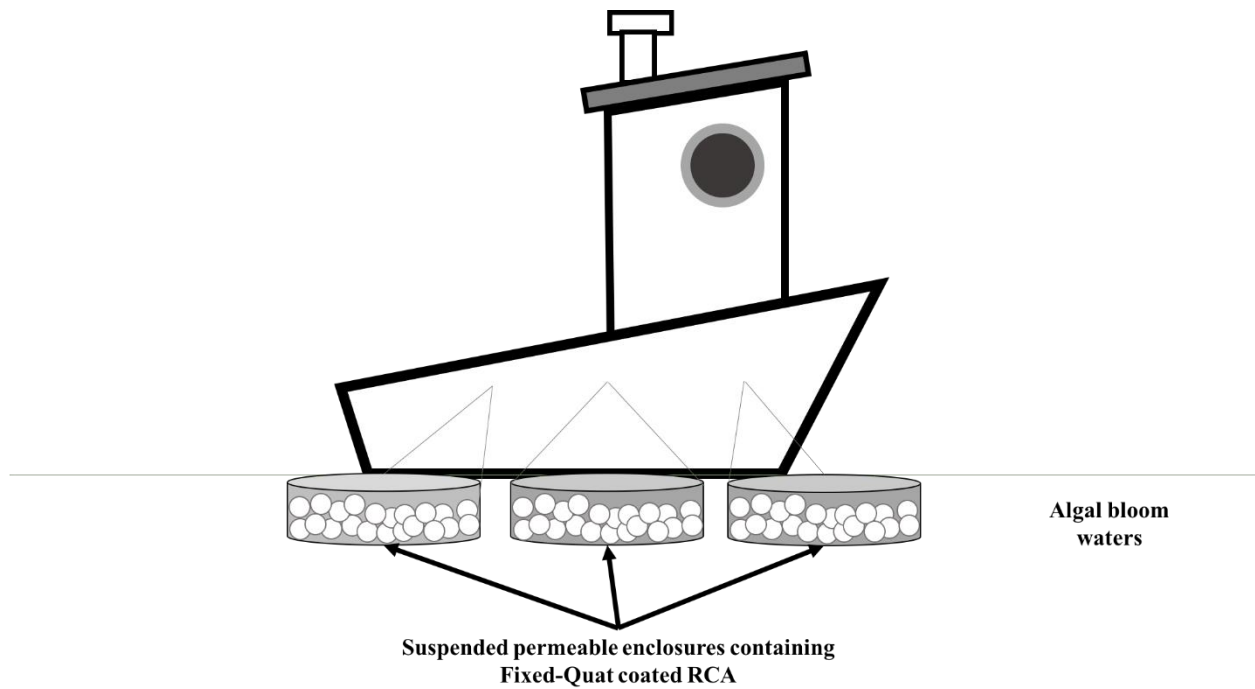


Figure 19. Schematic of the free-floating enclosures deployed in boats on algal bloom rivers.

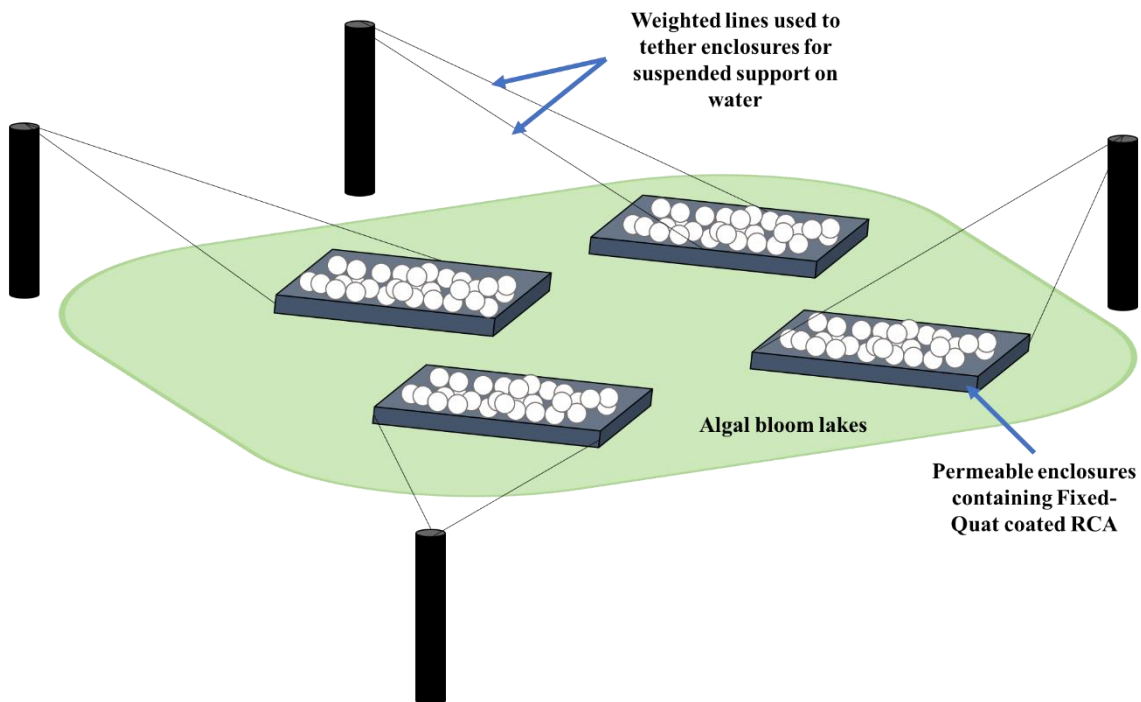


Figure 20. Schematic of the free-floating enclosures tethered to onshore support system on algal bloom lakes.

After displaying a successful antimicrobial characteristic against *E. coli*, Fixed-Quat RCAs has the capability to prevent the potential transmission of pathogens in wastewater. Without formation of disinfection by-products [91], notable in the well-known chlorination processes, Fixed Quat RCA can be used for storm water and greywater disinfection and other small scale unattended system without the need for continuous addition of chemicals or testing. Two-stage systems of coarse filtration along with disinfection is the most commonly used technology in greywater treatment. To minimize cost of physical and chemical non-potable reuse and/or greywater treatment, coarse filtration presented by the shape and size of RCA along with the synergy with Quats can thus improve removal of microorganisms and suspended solids in the low strength grey water especially when preceded by coagulation. Domestic rainwater harvesting system provides a renewable clean water source ideal for non-potable domestic uses. Fixed-Quat RCA can also be applied to harvested rainwater in a slow sand filtration configuration to improve its microbiological quality.

Overall, the antimicrobial coated RCA would be a promising and sustainable alternative to conventional HABs mitigation methods in various aquatic systems.

REFERENCES

- [1] G. S. Nam, E. H. Lee, and K. H. Chang, "Application of an Algal Bloom Control Technique using Large Zooplankton Predators in An Eutrophic Agricultural Reservoir: A Preliminary Study," *Irrigation and Drainage*, vol. 65, no. S2, pp. 230-238, 2016.
- [2] D. M. Anderson, P. M. Glibert, and J. M. Burkholder, "Harmful algal blooms and eutrophication: nutrient sources, composition, and consequences," *Estuaries*, vol. 25, no. 4, pp. 704-726, 2002.
- [3] A. Alum, A. Rashid, B. Mobasher, and M. Abbaszadegan, "Cement-based biocide coatings for controlling algal growth in water distribution canals," *Cement and Concrete Composites*, vol. 30, no. 9, pp. 839-847, 2008.
- [4] T. M. Mata, A. A. Martins, and N. S. Caetano, "Microalgae for biodiesel production and other applications: a review," *Renewable and sustainable energy reviews*, vol. 14, no. 1, pp. 217-232, 2010.
- [5] N. Takamura and M. M. Watanabe, "Seasonal changes in the biomass of four species of *Microcystis* in Lake Kasumigaura," *Japanese Journal of Limnology (Rikusuigaku Zasshi)*, vol. 48, no. Special, pp. 139-144, 1987.
- [6] H. W. Paerl, R. S. Fulton, P. H. Moisander, and J. Dyble, "Harmful freshwater algal blooms, with an emphasis on cyanobacteria," *The Scientific World Journal*, vol. 1, pp. 76-113, 2001.
- [7] L. Campanella, F. Cubadda, M. Sammartino, and A. Saoncella, "An algal biosensor for the monitoring of water toxicity in estuarine environments," *Water Research*, vol. 35, no. 1, pp. 69-76, 2001.
- [8] D. M. ANDERSON, "The expanding global problem of harmful algal blooms," in *Society And Structures: (Full Title: Society and Structures: Historical Perspectives—Culture and Ideology; National and Regional Geopolitical Issues; Globalisation—Economy and Culture; Human Rights—Freedom and Democracy Debate; Confrontations and Countermeasures: Present and Future Confrontations; Psychology of Terrorism; Defensive Countermeasures; Preventive Countermeasures; General Debate; Science and Technology: Emergencies; Pollution; Climate—Greenhouse Effect; Desertification, Water Pollution, Algal Bloom; Brain and Behaviour Diseases; The Cultural Emergency: General Debate and Conclusions; Permanent Monitoring Panel Reports; Information Security Workshop; Kangaroo Mother's Care Workshop; Brain and Behaviour Diseases Workshop)*, 2003, pp. 372-393: World Scientific.
- [9] D. M. Anderson, "Approaches to monitoring, control and management of harmful algal blooms (HABs)," *Ocean & coastal management*, vol. 52, no. 7, pp. 342-347, 2009.
- [10] R. Mur, O. M. Skulberg, and H. Utkilen, ". CYANOBACTERIA IN THE ENVIRONMENT," 1999.
- [11] V. Gupta, S. K. Ratha, A. Sood, V. Chaudhary, and R. Prasanna, "New insights into the biodiversity and applications of cyanobacteria (blue-green algae)—prospects and challenges," *Algal research*, vol. 2, no. 2, pp. 79-97, 2013.
- [12] Q. Hu *et al.*, "Microalgal triacylglycerols as feedstocks for biofuel production: perspectives and advances," *The plant journal*, vol. 54, no. 4, pp. 621-639, 2008.
- [13] I. M. Machado and S. Atsumi, "Cyanobacterial biofuel production," *Journal of biotechnology*, vol. 162, no. 1, pp. 50-56, 2012.

- [14] A. Parmar, N. K. Singh, A. Pandey, E. Gnansounou, and D. Madamwar, "Cyanobacteria and microalgae: a positive prospect for biofuels," *Bioresource technology*, vol. 102, no. 22, pp. 10163-10172, 2011.
- [15] J. L. Graham, K. A. Loftin, M. T. Meyer, and A. C. Ziegler, "Cyanotoxin mixtures and taste-and-odor compounds in cyanobacterial blooms from the Midwestern United States," *Environmental science & technology*, vol. 44, no. 19, pp. 7361-7368, 2010.
- [16] P. M. Glibert and J. M. Burkholder, "Harmful algal blooms and eutrophication: "strategies" for nutrient uptake and growth outside the Redfield comfort zone," *Chinese Journal of Oceanology and Limnology*, vol. 29, no. 4, pp. 724-738, 2011.
- [17] National Geographic Sites. (2013, 6 Dec). *Pictures: Extreme Algae Blooms Expanding Worldwide*. Available: <https://news.nationalgeographic.com/news/2013/04/pictures/130423-extreme-algae-bloom-fertilizer-lake-erie-science/>
- [18] K. Christoffersen and H. Kaas, "Toxic cyanobacteria in water. A guide to their public health consequences, monitoring, and management," ed: Wiley Online Library, 2000.
- [19] J. O'neil, T. Davis, M. Burford, and C. Gobler, "The rise of harmful cyanobacteria blooms: the potential roles of eutrophication and climate change," *Harmful algae*, vol. 14, pp. 313-334, 2012.
- [20] C. Reynolds and A. Walsby, "Water-blooms," *Biological reviews*, vol. 50, no. 4, pp. 437-481, 1975.
- [21] J. Ringelberg and R. Baard, "Growth and decline of a population of *Microcystis aeruginosa* in mesotrophic Lake Maarsseveen I (The Netherlands)," *Archiv für Hydrobiologie*, vol. 111, no. 4, pp. 533-545, 1988.
- [22] O. M. Skulberg, G. A. Codd, and W. W. Carmichael, "Toxic blue-green algal blooms in Europe: a growing problem," *Ambio: journal of human environment*, vol. 13, no. 4, pp. 245-7, 1984.
- [23] T. W. Davis, D. L. Berry, G. L. Boyer, and C. J. Gobler, "The effects of temperature and nutrients on the growth and dynamics of toxic and non-toxic strains of *Microcystis* during cyanobacteria blooms," *Harmful algae*, vol. 8, no. 5, pp. 715-725, 2009.
- [24] R. Dastjerdi and M. Montazer, "A review on the application of inorganic nano-structured materials in the modification of textiles: focus on anti-microbial properties," *Colloids and Surfaces B: Biointerfaces*, vol. 79, no. 1, pp. 5-18, 2010.
- [25] B. H. Nam, Z. Behring, J. Kim, and M. Chopra, "Evaluate the use of reclaimed concrete aggregate in French drain application," *Rep. No. FDOT-BDK78-977-12, Florida Dept. of Transportation, Tallahassee, FL*, 2014.
- [26] J. Church, H. Kannan, J. An, W. Lee, S. Santra, and B. Nam, "Recycled concrete aggregate coated with quaternary ammonium compounds for water disinfection," *International Journal of Environmental Science and Technology*, vol. 14, no. 3, pp. 543-552, 2017.
- [27] M. J. Rosen and J. T. Kunjappu, *Surfactants and interfacial phenomena*. John Wiley & Sons, 2012.
- [28] Y. Hu and L.-K. Ju, "Purification of lactonic sophorolipids by crystallization," *Journal of biotechnology*, vol. 87, no. 3, pp. 263-272, 2001.
- [29] X.-X. Sun, K.-N. Han, J.-K. Choi, and E.-K. Kim, "Screening of surfactants for harmful algal blooms mitigation," *Marine pollution bulletin*, vol. 48, no. 9, pp. 937-945, 2004.

- [30] K. Kim, Y. Dalsoo, K. Youngbum, L. BAEKSEOK, S. DOONHOON, and K. EUN-KI, "Characteristics of sophorolipid as an antimicrobial agent," *Journal of microbiology and biotechnology*, vol. 12, no. 2, pp. 235-241, 2002.
- [31] S.-H. Baek *et al.*, "Mitigation of harmful algal blooms by sophorolipid," *Journal of microbiology and biotechnology*, vol. 13, no. 5, pp. 651-659, 2003.
- [32] X.-X. Sun, J.-K. Choi, and E.-K. Kim, "A preliminary study on the mechanism of harmful algal bloom mitigation by use of sophorolipid treatment," *Journal of Experimental Marine Biology and Ecology*, vol. 304, no. 1, pp. 35-49, 2004.
- [33] I. Rodea-Palomares *et al.*, "Physicochemical characterization and ecotoxicological assessment of CeO₂ nanoparticles using two aquatic microorganisms," *Toxicological Sciences*, vol. 119, no. 1, pp. 135-145, 2010.
- [34] T. Ochiai *et al.*, "Photocatalytic inactivation and removal of algae with TiO₂-coated materials," *Journal of applied electrochemistry*, vol. 40, no. 10, pp. 1737-1742, 2010.
- [35] R. H. Pierce, M. S. Henry, C. J. Higham, P. Blum, M. R. Sengco, and D. M. Anderson, "Removal of harmful algal cells (*Karenia brevis*) and toxins from seawater culture by clay flocculation," *Harmful Algae*, vol. 3, no. 2, pp. 141-148, 2004.
- [36] H. Li and G. Pan, "Simultaneous removal of harmful algal blooms and microcystins using microorganism-and chitosan-modified local soil," *Environmental science & technology*, vol. 49, no. 10, pp. 6249-6256, 2015.
- [37] H. Kim, "Mitigation and controls of HABs," in *Ecology of harmful algae*: Springer, 2006, pp. 327-338.
- [38] D. Kidwell, "Mitigation of harmful algal blooms: The way forward," *PICES Press*, vol. 23, no. 2, p. 22, 2015.
- [39] C. Tian, X. Liu, J. Tan, S. Lin, D. Li, and H. Yang, "Isolation, identification and characterization of an algicidal bacterium from Lake Taihu and preliminary studies on its algicidal compounds," *Journal of Environmental Sciences*, vol. 24, no. 10, pp. 1823-1831, 2012.
- [40] X. Zheng *et al.*, "A marine algicidal actinomycete and its active substance against the harmful algal bloom species *Phaeocystis globosa*," *Applied microbiology and biotechnology*, vol. 97, no. 20, pp. 9207-9215, 2013.
- [41] G. Zeng, P. Wang, and Y. Wang, "Algicidal efficiency and mechanism of *Phanerochaete chrysosporium* against harmful algal bloom species," *Algal Research*, vol. 12, pp. 182-190, 2015.
- [42] Z. A. Mohamed, M. Hashem, and S. A. Alamri, "Growth inhibition of the cyanobacterium *Microcystis aeruginosa* and degradation of its microcystin toxins by the fungus *Trichoderma citrinoviride*," *Toxicon*, vol. 86, pp. 51-58, 2014.
- [43] E. J. Espinosa-Ortiz, E. R. Rene, K. Pakshirajan, E. D. van Hullebusch, and P. N. Lens, "Fungal pelleted reactors in wastewater treatment: applications and perspectives," *Chemical Engineering Journal*, vol. 283, pp. 553-571, 2016.
- [44] Y. Z. Tang and C. J. Gobler, "The green macroalga, *Ulva lactuca*, inhibits the growth of seven common harmful algal bloom species via allelopathy," *Harmful Algae*, vol. 10, no. 5, pp. 480-488, 2011.

- [45] C. Nan, H. Zhang, S. Lin, G. Zhao, and X. Liu, "Allelopathic effects of *Ulva lactuca* on selected species of harmful bloom-forming microalgae in laboratory cultures," *Aquatic Botany*, vol. 89, no. 1, pp. 9-15, 2008.
- [46] S. S. Mangiafico and M. Haberland, "Pond and Lake Management Part VI: Using Barley Straw to Control Algae," *Rutgers NJAES Cooperative Extension*, 2011.
- [47] T. Lee, K. Nakano, and M. Matsumara, "Ultrasonic irradiation for blue-green algae bloom control," *Environmental technology*, vol. 22, no. 4, pp. 383-390, 2001.
- [48] C.-Y. Ahn, M.-H. Park, S.-H. Joung, H.-S. Kim, K.-Y. Jang, and H.-M. Oh, "Growth inhibition of cyanobacteria by ultrasonic radiation: laboratory and enclosure studies," *Environmental science & technology*, vol. 37, no. 13, pp. 3031-3037, 2003.
- [49] C. Zhang *et al.*, "Quaternary ammonium compounds (QACs): a review on occurrence, fate and toxicity in the environment," *Science of the Total Environment*, vol. 518, pp. 352-362, 2015.
- [50] M. Garcia, I. Ribosa, T. Guindulain, J. Sanchez-Leal, and J. Vives-Rego, "Fate and effect of monoalkyl quaternary ammonium surfactants in the aquatic environment," *Environmental Pollution*, vol. 111, no. 1, pp. 169-175, 2001.
- [51] O. Rahn and W. P. Van Eseltine, "Quaternary ammonium compounds," *Annual Reviews in Microbiology*, vol. 1, no. 1, pp. 173-192, 1947.
- [52] M. Garcia, E. Campos, J. Sanchez-Leal, and I. Ribosa, "Effect of the alkyl chain length on the anaerobic biodegradability and toxicity of quaternary ammonium based surfactants," *Chemosphere*, vol. 38, no. 15, pp. 3473-3483, 1999.
- [53] U. Tezel and S. G. Pavlostathis, "Quaternary ammonium disinfectants: microbial adaptation, degradation and ecology," *Current opinion in biotechnology*, vol. 33, pp. 296-304, 2015.
- [54] E. Fu, K. McCue, and D. Boesenberg, "Chemical Disinfection of Hard Surfaces—Household, Industrial and Institutional Settings," in *Handbook for cleaning/decontamination of surfaces*: Elsevier, 2007, pp. 573-592.
- [55] G.-G. Ying, "Fate, behavior and effects of surfactants and their degradation products in the environment," *Environment international*, vol. 32, no. 3, pp. 417-431, 2006.
- [56] D. van Wijk, M. Gyimesi-van den Bos, I. Gattener-Arends, M. Geurts, J. Kamstra, and P. Thomas, "Bioavailability and detoxification of cationics: I. Algal toxicity of alkyltrimethyl ammonium salts in the presence of suspended sediment and humic acid," *Chemosphere*, vol. 75, no. 3, pp. 303-309, 2009.
- [57] S. Wessels and H. Ingmer, "Modes of action of three disinfectant active substances: a review," *Regulatory toxicology and pharmacology*, vol. 67, no. 3, pp. 456-467, 2013.
- [58] J. Lin, S. Qiu, K. Lewis, and A. M. Klibanov, "Mechanism of bactericidal and fungicidal activities of textiles covalently modified with alkylated polyethylenimine," *Biotechnology and Bioengineering*, vol. 83, no. 2, pp. 168-172, 2003.
- [59] C. J. Waschinski, V. Herdes, F. Schueler, and J. C. Tiller, "Influence of satellite groups on telechelic antimicrobial functions of polyoxazolines," *Macromolecular bioscience*, vol. 5, no. 2, pp. 149-156, 2005.
- [60] C. J. Ioannou, G. W. Hanlon, and S. P. Denyer, "Action of disinfectant quaternary ammonium compounds against *Staphylococcus aureus*," *Antimicrobial agents and chemotherapy*, vol. 51, no. 1, pp. 296-306, 2007.

- [61] J. Loontjens, "Quaternary ammonium compounds," in *Biomaterials associated infection*: Springer, 2013, pp. 379-404.
- [62] A. J. McBain, R. G. Ledder, L. E. Moore, C. E. Catrenich, and P. Gilbert, "Effects of quaternary-ammonium-based formulations on bacterial community dynamics and antimicrobial susceptibility," *Applied and environmental microbiology*, vol. 70, no. 6, pp. 3449-3456, 2004.
- [63] U. Tezel, J. A. Pierson, and S. G. Pavlostathis, "Fate and effect of quaternary ammonium compounds on a mixed methanogenic culture," *Water research*, vol. 40, no. 19, pp. 3660-3668, 2006.
- [64] G. Nałęcz-Jawecki, E. Grabińska-Sota, and P. Narkiewicz, "The toxicity of cationic surfactants in four bioassays," *Ecotoxicology and environmental safety*, vol. 54, no. 1, pp. 87-91, 2003.
- [65] G. Jing, Z. Zhou, and J. Zhuo, "Quantitative structure–activity relationship (QSAR) study of toxicity of quaternary ammonium compounds on *Chlorella pyrenoidosa* and *Scenedesmus quadricauda*," *Chemosphere*, vol. 86, no. 1, pp. 76-82, 2012.
- [66] Z. Liang, F. Ge, H. Zeng, Y. Xu, F. Peng, and M. Wong, "Influence of cetyltrimethyl ammonium bromide on nutrient uptake and cell responses of *Chlorella vulgaris*," *Aquatic toxicology*, vol. 138, pp. 81-87, 2013.
- [67] S. Sánchez-Fortún, F. Marv, A. D'ors, and E. Costas, "Inhibition of growth and photosynthesis of selected green microalgae as tools to evaluate toxicity of dodecylethyldimethyl-ammonium bromide," *Ecotoxicology*, vol. 17, no. 4, pp. 229-234, 2008.
- [68] F. Ge, Y. Xu, R. Zhu, F. Yu, M. Zhu, and M. Wong, "Joint action of binary mixtures of cetyltrimethyl ammonium chloride and aromatic hydrocarbons on *Chlorella vulgaris*," *Ecotoxicology and environmental safety*, vol. 73, no. 7, pp. 1689-1695, 2010.
- [69] M. J. Saif, J. Anwar, and M. A. Munawar, "A novel application of quaternary ammonium compounds as antibacterial hybrid coating on glass surfaces," *Langmuir*, vol. 25, no. 1, pp. 377-379, 2008.
- [70] L. A. Asri *et al.*, "A Shape-Adaptive, Antibacterial-Coating of Immobilized Quaternary-Ammonium Compounds Tethered on Hyperbranched Polyurea and its Mechanism of Action," *Advanced Functional Materials*, vol. 24, no. 3, pp. 346-355, 2014.
- [71] S. N. Jampala, M. Sarmadi, E. Somers, A. Wong, and F. Denes, "Plasma-enhanced synthesis of bactericidal quaternary ammonium thin layers on stainless steel and cellulose surfaces," *Langmuir*, vol. 24, no. 16, pp. 8583-8591, 2008.
- [72] V. P. Dhende, S. Samanta, D. M. Jones, I. R. Hardin, and J. Locklin, "One-step photochemical synthesis of permanent, nonleaching, ultrathin antimicrobial coatings for textiles and plastics," *ACS applied materials & interfaces*, vol. 3, no. 8, pp. 2830-2837, 2011.
- [73] B. H. Nam, Z. Behring, J. Kim, and M. Chopra, "Evaluate the use of reclaimed concrete aggregate in french drain applications," *Rep. No. FDOT-BDK78-977-12, Florida Dept. of Transportation, Tallahassee, FL*, 2014.
- [74] A. M. Wagih, H. Z. El-Karmoty, M. Ebid, and S. H. Okba, "Recycled construction and demolition concrete waste as aggregate for structural concrete," *HBRC Journal*, vol. 9, no. 3, pp. 193-200, 2013.
- [75] T. Ding, J. Xiao, and V. W. Tam, "A closed-loop life cycle assessment of recycled aggregate concrete utilization in China," *Waste Management*, vol. 56, pp. 367-375, 2016.

- [76] M. Limbachiya, A. Koulouris, J. Roberts, and A. Fried, "Performance of recycled aggregate concrete," in *Proceeding of RILEM International Symposium on Environment-Conscious Materials and Systems for Sustainable Development*, 2004, pp. 127-136.
- [77] M. Limbachiya, M. S. Meddah, and Y. Ouchagour, "Use of recycled concrete aggregate in fly-ash concrete," *Construction and Building Materials*, vol. 27, no. 1, pp. 439-449, 2012.
- [78] J. E. Hiller, Y. S. Deshpande, Y. Qin, C. J. Shorkey, and K. Peterson, "Efficient use of recycled concrete in transportation infrastructure," Michigan Technological University 2011.
- [79] A. Rana, P. Kalla, H. Verma, and J. Mohnot, "Recycling of dimensional stone waste in concrete: A review," *Journal of cleaner production*, vol. 135, pp. 312-331, 2016.
- [80] Z. Behring, J. Kim, B. Nam, M. Chopra, and J. Shoucair, "Drainage performance evaluation of reclaimed concrete aggregate," in *Innovative and sustainable use of geomaterials and geosystems*, 2014, pp. 33-40.
- [81] J. Donalson, R. Curtis, and F. T. Najafi, "Sustainable assessment of recycled concrete aggregate (RCA) used in highway construction," in *Proceedings of the 90th annual meeting of the transportation research board*, 2011.
- [82] K. K. Sagoe-Crentsil, T. Brown, and A. H. Taylor, "Performance of concrete made with commercially produced coarse recycled concrete aggregate," *Cement and concrete research*, vol. 31, no. 5, pp. 707-712, 2001.
- [83] A. Duran, Y. a. Castro, M. Aparicio, A. Conde, and J. De Damborenea, "Protection and surface modification of metals with sol-gel coatings," *International materials reviews*, vol. 52, no. 3, pp. 175-192, 2007.
- [84] B. G. Rao, D. Mukherjee, and B. M. Reddy, "Chapter 1 - Novel approaches for preparation of nanoparticles A2 - Fical, Denisa," in *Nanostructures for Novel Therapy*, A. M. Grumezescu, Ed.: Elsevier, 2017, pp. 1-36.
- [85] L. M. Muresan, "Chapter 17 - Corrosion Protective Coatings for Ti and Ti Alloys Used for Biomedical Implants," in *Intelligent Coatings for Corrosion Control* Boston: Butterworth-Heinemann, 2015, pp. 585-602.
- [86] P. Oberholster, A. Botha, and J. Grobbelaar, "Microcystis aeruginosa: source of toxic microcystins in drinking water," *African Journal of Biotechnology*, vol. 3, no. 3, 2004.
- [87] L. Frangeul *et al.*, "Highly plastic genome of Microcystis aeruginosa PCC 7806, a ubiquitous toxic freshwater cyanobacterium," *BMC genomics*, vol. 9, no. 1, p. 274, 2008.
- [88] J. R. Stein, *Handbook of phycological methods: physiological and biochemical methods*. Cambridge University Press, 1973.
- [89] T. F. Moriarty, S. A. Zaat, and H. J. Busscher, *Biomaterials associated infection: immunological aspects and antimicrobial strategies*. Springer Science & Business Media, 2012.
- [90] M. R. Sengco, A. Li, K. Tugend, D. Kulis, and D. M. Anderson, "Removal of red-and brown-tide cells using clay flocculation. I. Laboratory culture experiments with Gymnodinium breve and Aureococcus anophagefferens," *Marine ecology progress series*, vol. 210, pp. 41-53, 2001.

[91] J. Church, M. E. Verbyla, W. H. Lee, A. A. Randall, T. J. Amundsen, and D. J. Zastrow, "Dishwashing water recycling system and related water quality standards for military use," *Science of the Total Environment*, vol. 529, pp. 275-284, 2015.