

Harmful Algal Bloom Control Using Recycled Concrete Aggregate Coated With Quaternary Ammonium Compounds

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The following quote is from U.S. Geological Survey, “The Science of Harmful Algal Blooms,” October 2016:

“Toxic cyanobacterial harmful algal blooms (cyanoHABs) have caused human and animal illness and death in at least 43 states in the United States (Graham et al., 2009; Hudnell, 2008). In August 2016, there were public health advisories in at least 19 states on cyanoHABs (U.S. Geological Survey, 2016). The toxins produced by some species of cyanobacteria (called cyanotoxins) cause acute and chronic illnesses in humans. The HABs can adversely affect aquatic ecosystem health, both directly through the presence of these toxins and indirectly through the low dissolved oxygen concentrations and changes in aquatic food webs caused by an overabundance of cyanobacteria. Economic damages related to cyanoHABs include the loss of recreational revenue, decreased property values, and increased drinking water treatment costs.”

CyanoHABs result from various human activities (mainly in urban centers), groundwater inflow, and atmospheric deposition, which generate surplus nutrients in water resources (Anderson et al., 2002; Nam et al., 2016). Cyanobacteria are single-celled, colonial, or filamentous widespread notorious bloom formers that have persisted through geochemical and cli-

matic changes by morphological, physiological, and ecological modifications that exist in the widest range of ecological habitats (Gupta et al., 2013; Mur et al., 1999; Paerl et al., 2001). Cyanobacteria flourish in nutrient-enriched freshwater and brackish ecosystems, causing serious ecological problems, and thus, substantial economic losses.

The primary concern of HABs is their production of a wide variety of cyanotoxins that are consumed via ingestion of contaminated drinking water, inhalation during recreational activities, and consumption of contaminated fish and shellfish. CyanoHABs also produce toxic compounds, like geosmin and 2-methylisoborneol, that impart undesirable taste and odor to surface water (Graham et al., 2010), alter the food web by producing bioactive compounds (Table 1), or create anoxic conditions that causes mortality to aquatic life (Glibert and Burkholder, 2011).

Thus, HABs have been an emerging global issue in terms of clean water loss and water quality deterioration, which requires an urgent need for effective algal bloom control. Typically, physical, biological, and chemical methods have been used to control and manage HABs in water bodies (Table 2). Among them, chemical controls are considered the most fast-responsive method (Rosen and Kunjappu, 2012), but may pose a threat to nontarget organisms through

Table 1. Toxic compounds produced by cyanobacteria and U. S. Environmental Protection Agency health advisory levels.

Toxins	Cyanobacteria sp.	USEPA Health Advisory Levels
Microcystin	<i>Anabaena</i> , <i>Oscillatoria</i> , <i>Microcystis</i>	0.3 µg/L for children; 1.6 µg/L for adults
Anatoxins	<i>Anabaena</i> , <i>Aphanizomenon</i> , <i>Oscillatoria</i> , <i>Cylindrospermum</i>	Varies with states
Cylindrospermopsin	<i>Cylindrospermopsis</i>	0.7 µg/L for children; 3 µg/L for adults
Saitoxin	<i>Anabaena</i> , <i>Planktothrix</i>	N/A*
Cytotoxin	Fresh and marine cyanobacteria	N/A*

*N/A: not available

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the uncontrolled release into the environment (Kidwell, 2015). To exploit the effectiveness of chemical controls without the negative impacts to the environment, recent studies have aimed at fixing biocides to substrate surfaces to provide a fixed source of antimicrobial properties (Kidwell, 2015).

Recycled concrete aggregate (RCA) represents an economically viable substrate for coating biocides. It's a major construction waste and has gained considerable attention due to its environmentally friendly nature and economic viability in reuse, and has drawn attention from engineers for nonstructural applications in a variety of sustainable ways (Ding et al., 2016; Nam et al., 2016), such as use for exfiltration and French drain systems. It provides benefits, such as conservation of primary resources, beneficial reduction of landfill disposal (Wagih et al., 2013), and reduction in transportation costs (Sagoe-Crentsil et al., 2001).

Quaternary ammonium compounds (Quats) are Food and Drug Administration (FDA)-approved cationic surfactants that are effective at very low concentrations against a variety of microorganisms and leave a residual germicidal effect on surfaces (Garcia et al., 2001; Zhang et al., 2015). Quats have low toxicity compared to metal-based nanomaterials, low corrosivity, and are constituents of disinfectant and/or antiseptic formulations used in homes,

healthcare facilities, agriculture, and industry (Tezel and Pavlostathis, 2015). Quats are chemically structured and based on a hydrophobic hydrocarbon chain connected to the positively charged central nitrogen atom (Tezel and Pavlostathis 2015; Zhang et al., 2015) and can disrupt bacterial cell membrane's physical and ionic stability (Church et al., 2017; Tezel and Pavlostathis, 2015; Wessels and Ingmer, 2013). Toxicity of Quats in the environment can be mitigated due to its high adsorption affinity onto materials (e.g., sediments, clay, and sludge), depending on its structure, the nature of surfaces, and environmental parameters (van Wijk et al., 2009; Ying 2006). Use of a sol-gel process in fixing Quats on surfaces has been effective against microorganisms without undesired chemical release (Church et al., 2017; Saif et al., 2008).

In this study, to establish a point-of-use water treatment system for cyanoHABs control, a composite of silica-quaternary ammonium compound (fixed-Quat) containing didecyl-dimethylammonium chloride (DDAC) was coated to the surface RCA using a sol-gel technique to limit undesired discharge of Quat in suspension, while retaining its antimicrobial viability (Santra et al., 2014; Song et al., 2011). It is expected that fixed-Quat will adhere to the hydrophilic RCA surface (i.e., silica) through solid intermolecular forces, such as hydrogen bonding. In a preliminary test with *E. coli* (1×10^5 CFUs/mL), fixed-Quat RCA exhibited 99 percent reduction of *E. coli* (K-12 strain S 4362, ATCC 29181) within two hours in a 0.01 M phosphate buffer saline (PBS) solution at pH 7 (Church et al., 2017). With the antimicrobial activity of the fixed-Quat RCA, in this study, its algaestatic capability was further investigated for control and mitigation of *Microcystis* sp. in water.

Material Preparation

Recycled Concrete Aggregate

The RCA (1.5–2.5 in. in diameter) was obtained from a local construction and demolition waste recycling facility in Orlando. The aggregate was then washed three times daily with deionized (DI) water for three days to disconnect deleterious materials (i.e., debris and fines) at room temperature (i.e., 23°C). Then, the washed RCA samples were put in an oven (Model 40 GC lab oven, Quincy Lab Inc.) at 50°C for three days to dry the examples until the moisture content under 0.5 percent of the total mass was accomplished (Church et al., 2017).

Preparation of Quaternary Ammonium Gel

The gel-type fixed-Quat was used to coat the RCA, which demonstrated better execution

Table 2. HABs and HABs toxins control and mitigation methods.

Classification	Description	Methods	References
Physical	Use of physical means to remove algal cells or toxins from the water column and limit the spatial extent of a bloom using devices that agitates the water column	Ultrasound; Ultraviolet (UV) irradiation; Mechanical aeration; Destratification; Circulation; Raking	(Ahn et al., 2003; Lee et al., 2001; Park et al., 2017)
Biological	Algicidal microorganisms (e.g., algicidal bacteria, viruses, and plankton grazers capable of inhibiting HAB species)	Algicidal bacterium (<i>Ulva</i> sp., etc.); White-rot fungus	(Kim et al., 2009; Mitra and Flynn, 2006; Tang and Gobler, 2011; Tian et al., 2012; Zeng et al., 2015; Zheng et al., 2013)
Chemical	Artificial and naturally derived compounds that hinder algal cellular growth and/or result in algal cell lysis	Clay; Chitosan modified soil; Sophorolipid; Metals; Oxidants; Nanoparticles; Surfactants	(Li and Pan, 2015; Rodea-Palomares et al., 2010; Sun et al., 2004a; Sun et al., 2004b; Surosz and Palinska, 2004)

and firm adherence to the substrate RCA compared to the particle-type fixed-Quat (Church et al., 2017). The gel-type fixed-Quat is a composite biocide medium in which Quat is integrated with a silica gel matrix. The material was prepared by combining 60 mL of 37 percent sodium silicate (Fisher Scientific) with 910 mL DI water and left to mix at 150 revolutions per minutes (rpm) for six hours. Then, 30 mL DDAC were added to the blend and mixed for an additional 24 hours.

Biocide Coating on Recycled Concrete Aggregate Substrate

Twenty-three RCAs were submerged in 2 liters of the prepared quaternary ammonium gel and mixed at 150 rpm for 72 hours using a machine stirrer (Electric Stirrer 6000, Arrow Engineering). Afterwards, RCA samples were washed carefully to remove superficially bound quaternary ammonium gel. To firmly develop the siloxane bond between fixed-Quat and RCA surface, the samples were oven-heated (Fisher Scientific isotemp oven, Model 615f) for 48 hours at 50°C (Church et al., 2017). For surface characterization of Quat-coated RCA, a scanning electron microscope (SEM; JEOL JSM – 6480) with an X-ray energy-dispersive spectrometer (EDS) was utilized for surface investigation after samples (uncoated and fixed-Quat RCA) were sputter-coated with gold (Emitech K550) for three minutes at 20 mA.

Algal Cultivation

To maintain optimal algal growth conditions, Bold's basal medium (BBM) was prepared using the protocol from Canadian Phycological Culture Centre (CPCC), University of Waterloo (Stein, 1973). Cyanobacteria culture of *Microcystis aeruginosa* (UTEX 2385) was grown at 28°C in two photobioreactors with continuous white-fluorescent-light illumination of 2,000 lux (Figure 1). Two reactors (Fixed-Quat gel RCA and uncoated RCA) were colonized with 1 liter of nutrient-enriched exponentially growing *M. aeruginosa* with the initial density of 5.8×10^6 cells/mL (concentrations of algal blooms targeting between 10^5 – 10^6 cells/mL).

Algal Growth Inhibition Tests and Water Analysis

For the test of algaestatic activity, fixed-Quat RCA samples were first washed to avoid inactivation by fixed-Quat in suspension. The RCA samples were rinsed 10 times with 1 liter of deionized (DI) water in a 4-liter flask, gently swaying for 10 minutes, and followed by 20 minutes of undisturbed submersion (Church et al., 2017). Then, the RCA samples were immersed into a photobioreactor with a one-to-one ratio (w/w) of RCA to cyanoHABs culture solution (total of 23 rocks per reactor). A peristaltic pump was installed to recycle the water in reactors and provide continuous contact between RCA samples and cyanobacteria cells.

Cell density was tallied with a disposable

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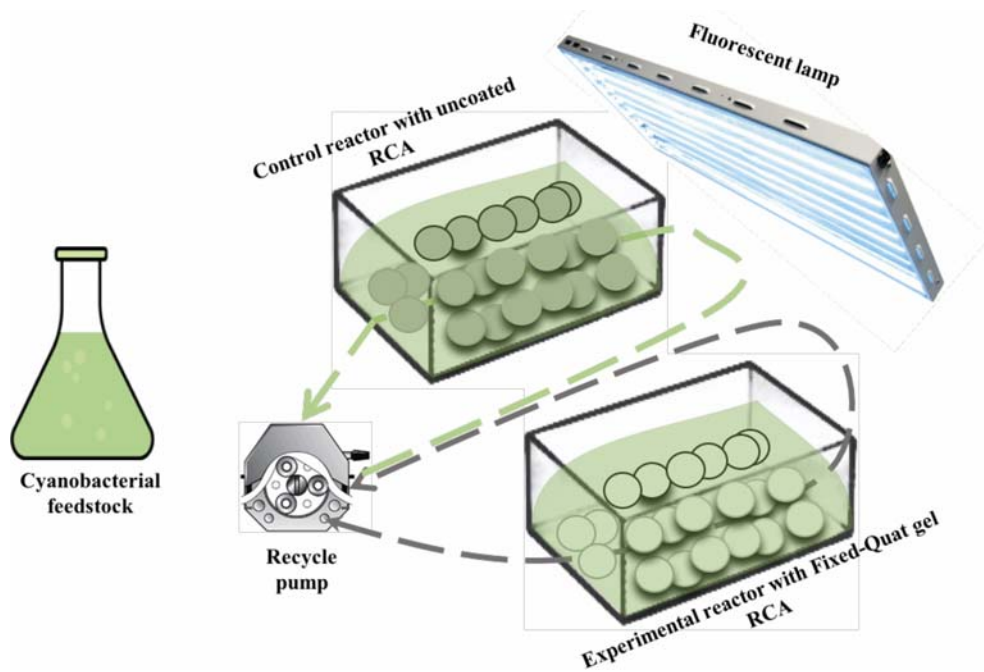


Figure 1. A schematic diagram of photobioreactors with fixed-Quat-coated RCA versus uncoated RCA samples.

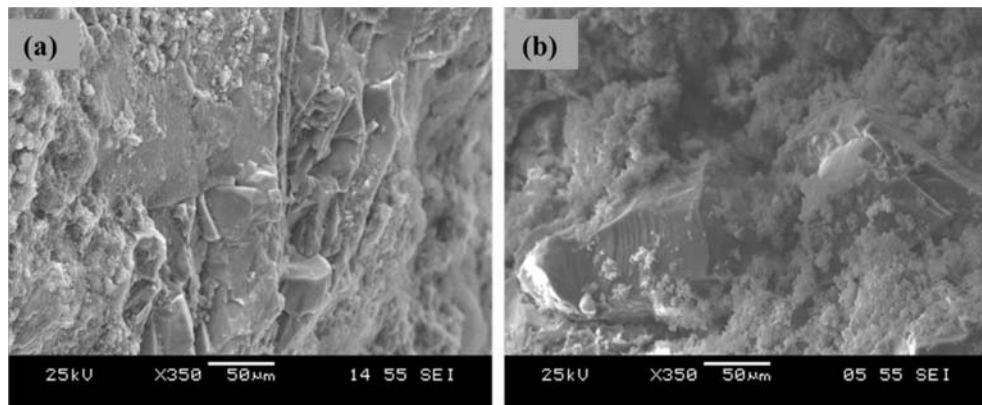


Figure 2. SEM images of the surface of RCA samples: (a) uncoated RCA (x350 magnification) and (b) fixed-Quat-coated RCA (x350 magnification) at 25 kV.

Table 3. EDS analysis results with element compositions of RCA samples.

Element	Uncoated RCA	Fixed-Quat gel coated RCA
	wt%	wt%
O	40.32	29.95
C	N/A	24.79
Mg	00.98	N/A
Al	02.57	01.55
Si	13.42	32.05
In	01.72	N/A
Ca	39.47	08.16
Fe	01.51	01.06
Na	00.00	02.45

Continued from page 31

hemocytometer (Fischer Scientific) under a microscope (LW Scientific Revelation III Binocular). Chlorophyll-*a* (Chl-*a*), an important molecule in the conversion of light to chemical energy, was used to estimate changes in the efficiency of photosynthesis in each sampling time. Cyanobacterial Chl-*a* content was extracted with 90 percent acetone after disruption using an ultrasonic probe (Fisher Scientific, Model 505 sonic dismembrator) in a fume hood (Hemco, Independence, Mo.; CAT: 4940-CF-031019). The acetone extracts of Chl-*a* were transferred to cuvettes and analyzed using a portable spectrophotometer (HQD, Hach) and Chl-*a* was calculated using Standard Methods (19th edition, 1995). All measurements were conducted in triplicate with glass wares autoclaved at 121°C for 20 minutes. Efficiency of Chl-*a* was calculated per the following:

$$\text{Inhibition Ratio (IR)} = (\text{Co} - \text{Ct}) / \text{Co} \times 100$$

where, Co and Ct represent Chl-*a* (mg/m³) at initial time and at time=t, respectively (Zeng et al., 2015).

The RCA produces a substantial amount of calcium carbonate when exposed to moisture and carbon dioxide (Nam et al., 2014). Thus, both RCA samples (uncoated and fixed-Quat-coated) were immersed in DI water and pH was measured continuously using a pH meter (DR1900, Hach) to monitor pH changes probably due to the cement rehydration that may cause experimental bias.

OD₆₆₀ was used to estimate algal cell concentrations (DR1900, Hach).

Results and Discussion

Surface Characterization of Recycled Concrete Aggregate

The XRD analysis confirmed the constituents of RCA to be hydrated cement, sand, and limestone (Kim et al., 2014; Nam et al., 2014). The SEM images of uncoated and fixed-Quat-coated RCA (Figure 2) displayed the undefined nature of the Quats on the surface RCA, which signifies the modification of the RCA surface. The EDS examination (Table 3) shows an increase in Si concentration (wt/percent), which validates the covalent silica bond between the coating blend and RCA. The SEM and EDS analysis of the surface of RCA samples confirmed successful coating of fixed-Quat gel on the RCA surface.

Continued on page 34

Algaestatic Activity

Time course measurements of Chl-*a* in the reactor containing uncoated RCA samples revealed no inhibition on the photosynthetic activity *M. aeruginosa* in the uncoated RCA mixture (208.3 ± 16 to 213.6 ± 7.6 mg/m³), as shown in Figure 3; however, the fixed-Quat-coated RCA successfully decreased the Chl-*a* contents in the reactor. It seems that during a period of nine hours, photosynthetic activity would be damaged by the contact between *M. aeruginosa* and fixed-Quat-coated RCA and no Chl-*a* content was detected after nine hours. Chl-*a* concentrations were reduced 36, 67, and 100 percent proportionally after three, six, and nine hours of treatment, respectively (Figure 4).

The pH measurements during the experiments showed an initial dramatic pH increase in the presence of concrete compared to the reactor without RCA samples (Figure 5). The initial pH of the media (BBM) was only 5.8 and was increased by approximately two units after three hours of duration; the pH was then maintained within the 7–8 range for 27 hours of the experiments. The pH increase at the initial time was possibly from the result of cement rehydration; however, OD₆₆₀ measurements showed that cement rehydration had no significant impact on the algal growth or inhibition within this pH range (Figure 6).

Figure 6 shows algal growth of *M. aeruginosa* in three reactors over 32 hours: uncoated RCA (positive control), fixed-Quat-coated RCA (negative control), and no RCA (negative control) reactors.

fixed-Quat-coated RCA, and no RCA (negative control) reactors. It was observed that the algal biomass in the reactor containing fixed-Quat-coated RCA samples decreased over time and remained at a certain level (within 0.01–0.02 of OD₆₆₀) after a 24-hour contact period, whereas the reactor with uncoated RCA showed a remarkable increase of algal biomass in the same time frame. Microscopic observation of the fixed-Quat-coated RCA samples showed lower algal cell population at the surface compared to the bottom of the reactor, indicating that cell membranes would be damaged from contact with the fixed-Quat-coated RCA.

At initial time (t=0), two reactors (fixed-Quat gel RCA and uncoated RCA) were filled with *M. aeruginosa* with the initial density of

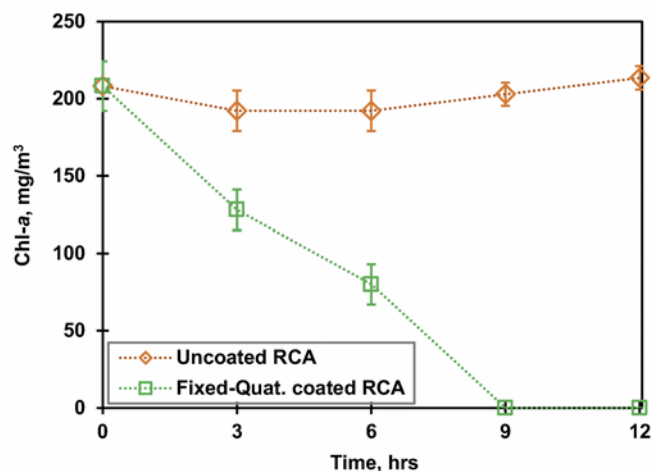


Figure 3. Changes in Chl-*a* contents between uncoated and fixed-Quat-coated RCA samples.

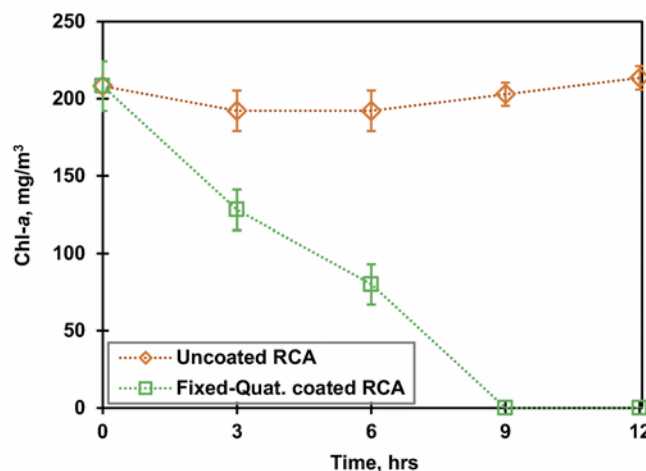


Figure 4. Algaestatic efficiency of fixed-Quat-coated RCA samples in contact with *M. aeruginosa*.

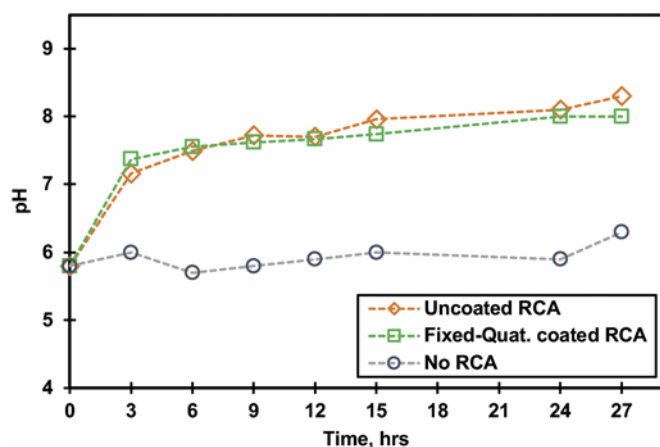


Figure 5. The pH changes in three reactors for 27 hours of algal growth: uncoated RCA (positive control), fixed-Quat-coated RCA, and no RCA (negative control) reactors.

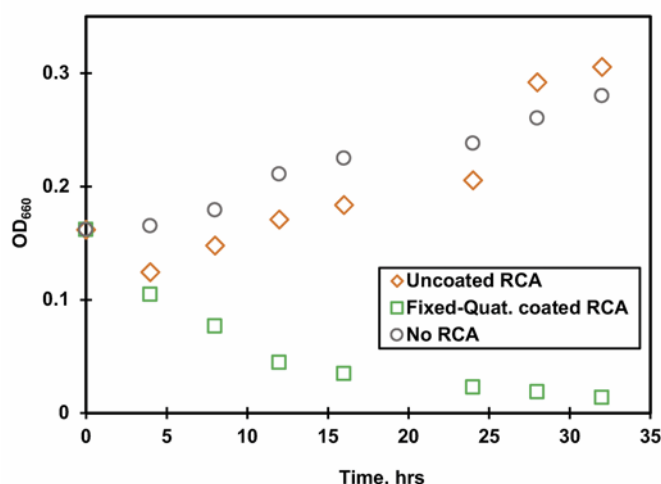


Figure 6. Growth of *M. aeruginosa* in three reactors: uncoated RCA (positive control), fixed-Quat-coated RCA, and no RCA (negative control) reactors.

5.8×10^6 cells/mL (Figure 7[a] and [b]). After 32 hours of experiments, the reactor in the presence of the fixed-Quat-coated RCA samples lost green color, indicating algicidal activity against *M. aeruginosa*, while the bulk solution in the reactor containing uncoated RCA samples showed dark green coloration, indicating algal growth over time without any inhibition (Figure 7[c] and [d]). It was also observed that the algal biomass without the fixed-Quat RCA samples is in good suspension (Figure 8[c]), while inactivated algal cells from the reactor with the fixed-Quat RCA samples are precipitated at the bottom of the reactor (Figure 8[b]).

The results showed that physical contact between antimicrobial-coated RCA and cyanobacteria species is required to inhibit the algal growth. This was also confirmed by the observation of attached growth of algal biomass on the uncoated RCA samples (Figure 8[c]) compared to the fixed-Quat-coated RCA samples without algal growth on the surface (Figure 8[d]). Overall, this study showed the excellent performance of the fixed-Quat-coated RCA for algal growth control and mitigation.

Conclusions

In eutrophic rivers or lakes, HABs can lead to significant water quality degradation and algal toxin production, affecting drinking water quality. There is an urgent need to develop a novel and sustainable method for effective HABs control and mitigation without second-hand pollution or hazardous byproducts generation. In this study, antimicrobial-fixed Quat was successfully coated onto the surface of RCA by sol-gel technique to control *M. aeruginosa*. Approximately 61 percent reduction of Chl-*a* was achieved within six hours of exposure and the complete removal of Chl-*a* was possible within nine hours, showing that the fixed-Quat-coated RCA could be an efficient solution for algal growth inhibition of harmful algal species (e.g., *M. aeruginosa*). Overall, by potentially eliminating regulated disinfection byproducts (DBPs) formation and minimizing release of nanomaterials (NMs) into the environment, the ammonia-Quat-coated materials could be a promising and sustainable alternative to conventional disinfection methods in engineered aquatic systems and HABs mitigation in natural water systems.

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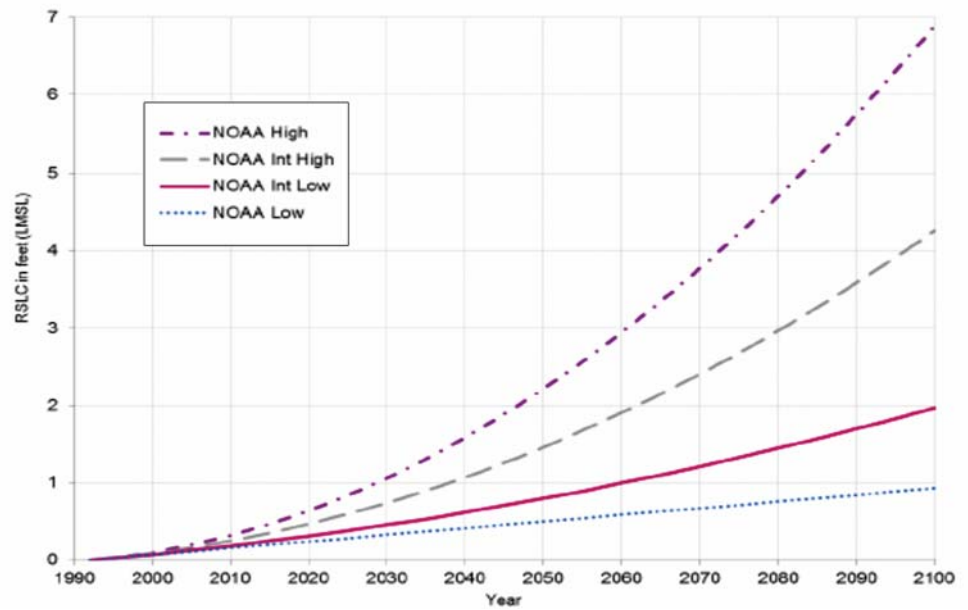


Figure 7. Pictures of the reactors with (a) uncoated RCA and (b) fixed-Quat-coated RCA at initial time ($t=0$) and the reactors with (c) uncoated RCA and (d) fixed-Quat-coated RCA at 32 hours of exposure.

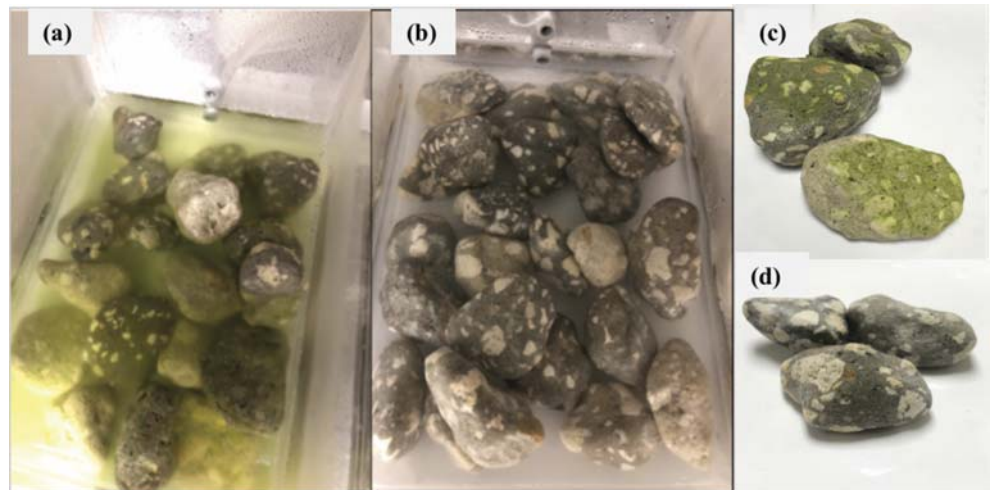


Figure 8. Pictures taken after 32 hours exposure of *M. aeruginosa* to RCA samples between (a) uncoated RCA and (b) fixed-Quat-coated RCA. Comparison after drying the RCA samples in air between (c) uncoated RCA and (d) fixed-Quat-coated RCA.

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Continued on page 36

Continued from page 35

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