

- 1 -

Functionalized Beads and Their Uses Thereof

Technical Field

5 The present invention relates, in general terms, to functionalized beads and their uses thereof.

Background

The increasing occurrences of harmful algal blooms (HABs) of cyanobacteria *Microcystis*
10 *aeruginosa*, caused by eutrophication have been of significant concern worldwide due to
their deleterious effects on water quality and fragile ecosystem. Many methods have been
proposed to solve the HABs problem. Compared with physical approach which requires
heavy manpower or using bioorganism that could lead to upset of eco-diversity, chemicals
such as copper sulfate, permanganate, iron species, ozone and hydrogen peroxide (H₂O₂)
15 can rapidly and effectively mitigate the HABs. Nevertheless, such approaches are only
transient for algae removal because the nutrients for algae growth still remain in the water.

As one of the excess nutrients resulting in eutrophication, ubiquitous phosphorus (P) is a
vital and essential ingredient for algae growth. A strict regulation limit of 0.05 mg·L⁻¹ P has
20 been set in many countries. Inorganic phosphate phosphorus (PO₄-P) which can be easily
utilized by algae is the common form of P in water systems. Therefore, PO₄-P removal is
significant to control HABs from source.

Chemical adsorption of PO₄-P by metals such as lanthanum, zirconium, calcium (Ca), and
25 magnesium, has been proposed as an efficient method and extensively used to remove PO₄-
P. Among the metals, calcium is much cheaper and more abundant in natural waters. Ca-
related materials have been widely applied in phosphorus removal or recovery by forming
Ca-P complexes. To simultaneously remove algae and phosphorus in a very alkaline (pH
around 10) algae-laden environment, CaCl₂ was used in a filtration system to form
30 Ca₅OH(PO₄)₃ precipitant and then algae were agglutinated in flocs cohesion process.
However, CaCl₂ has no oxidation ability to rupture algae cells and the remaining intact algae

- 2 -

cells will rapidly duplicate. To efficiently remove phosphorus and simultaneously remove algae, oxidants of Ca-based chemicals are good candidates.

Calcium peroxide (CaO_2) possessing oxidation ability may be promising. CaO_2 can generate

- 5 Ca^{2+} (or $\text{Ca}(\text{OH})_2$), H_2O_2 and O_2 (see equations, eqs. (1)-(3)) and has been widely applied in environmental remediation or cell rupture.



CaO_2 is relatively inert to ecosystem, and reportedly has a lethal concentration 50 (LC_{50}) value higher than $1500\text{-}2000 \text{ mg}\cdot\text{L}^{-1}$ for some fishes, indicating its nature of eco-friendliness.

- 15 Ca^{2+} is able to coordinate with phosphorus while H_2O_2 can rupture algae cells and O_2 is helpful to improve the water quality due to the anaerobic environment caused by HABs. CaO_2 powders have been directly applied with an extremely high concentration of $150\text{-}200 \text{ mg}\cdot\text{L}^{-1}$ or combined with $\text{Fe}(\text{II})$ to remove *Microcystis aeruginosa* but the phosphorus removal performance was not investigated. However, CaO_2 may not be suitable to be directly added in realistic algae-contaminated scenarios because the Ca-P complex that may
20 settle in the sediment would still be a potential problem for re-release of phosphorous back to the waters. Additionally, a quick efficiency-loss would occur due to its fast decomposition in water, if the CaO_2 powder is directly used.

- 25 It would be desirable to overcome or ameliorate at least one of the above-described problems.

Summary

- 30 Harmful algal blooms (HABs) resulting from eutrophication due to excess phosphorus have been a global threat. Simultaneous removal of phosphorus and inhibition of algae growth is of importance. It is technologically challenging in development of materials and systems for

- 3 -

the algae-contaminated water remediation. In an embodiment of the invention, wet alginate beads functionalized by CaO_2 formed layer by layer were prepared and optimized with an in-situ method for removing phosphate and HABs. A long-lasting and steady H_2O_2 concentration of 0.06 mM could last for at least 26 days. The chromate reduction and permanganate-based titration was used to validate and quantify the -O-O- groups (0.44 mmol·g⁻¹). The phosphorus removal efficiency was 97.2% (pH 3.0-10.0). Different kinetic fitting models and characterization tools (e.g., by XRD, SEM, and XPS) were used for phosphorus removal process analysis; the chemisorption for the phosphorous uptake due to the precipitation process was proposed as the main mechanism. Meanwhile, CaO_2 -encapsulated beads were superior to H_2O_2 liquid and Ca(OH)_2 beads for simulated algae removal with a duration over 170 d. Simultaneously, about 70% of phosphorus removal was obtained. The beads were demonstrated to be efficient in the remediation of realistic algae-laden water (e.g., lakes and reservoirs). Additionally, about 10 to 30% of ammonia in the water can be removed by the bead, even when the initial concentration of ammonia is only 2 to 3 ppm. In summary, this invention provides a promising CaO_2 -functionalized material to be applied for simultaneously removing phosphorus and algae, which will benefit the authentic HABs-contaminated water remediation.

The present invention provides a functionalised bead having a core and a surface, comprising:

- a) peroxide moieties within the core of the bead; and
- b) peroxide particles adhered to the surface of the bead;

wherein the core comprises anionic and hydrophilic polymers crosslinked with multivalent cations;

wherein the functionalized bead has an average diameter of about 0.2 mm to about 10 mm; and

wherein the peroxide particles have an average diameter of about 10 nm to about 900 nm.

Advantageously, the formed cation-functionalized peroxide particles allow for a slow release of an oxidising agent for several applications: inhibition of algae growth, removing algae, disinfection, oxidization of organic compounds, and oxidation/removal of low-valent

- 4 -

inorganic compounds (e.g. As(III) to AS(V)). As the peroxide particles on the surface of the bead are depleted by dissolution, peroxide moieties within the core of the bead are released such that a slow and constant release of oxidising agent is achievable. Further, the presence of multivalent cations within the bead and optionally in the peroxide particles provide a platform for binding phosphates.

In some embodiments, the peroxide moieties within the core of the bead comprises peroxide particles.

10 In some embodiments, the concentration of the peroxide moieties and peroxide particles in the functionalised bead is about $0.3 \text{ mmol} \cdot \text{g}^{-1}$ to about $0.6 \text{ mmol} \cdot \text{g}^{-1}$.

In some embodiments, the multivalent cations are selected from a Group 2 metal or transition metal. In some embodiments, the multivalent cations are selected from Ca^{2+} , Zn^{2+} , Mg^{2+} ,
15 Cu^{2+} , Ce^{3+} , La^{3+} or a combination thereof.

In some embodiments, the functionalised bead is further functionalised with alkaline moieties within the core or the surface of the bead. In some embodiments, an anion of the alkaline is hydroxide.

20

In some embodiments, the anionic and hydrophilic polymers is a polysaccharide. In some embodiments, the anionic and hydrophilic polymers is alginate or a derivative thereof.

In some embodiments, the bead is at least 97% hydrated with an aqueous medium.

25

In some embodiments, H_2O_2 is releasable from the functionalised bead when dispersed in aqueous medium. In some embodiments, H_2O_2 is releasable from the functionalised bead at a rate of about 0.03 mM/g/d to about 0.1 mM/g/d .

30 In some embodiments, a pH of about 6.0 to about 9.0 is maintainable when the functionalised bead is dispersed in aqueous medium.

- 5 -

In some embodiments, the multivalent cation has a dissolution of less than about 1.4 mg/L.

5 In some embodiments, the functionalised bead is for use in inhibiting algae growth, removing algae, disinfecting an aqueous medium, oxidizing organic compounds in an aqueous medium, oxidizing or removing low-valent inorganic compounds in an aqueous medium, or a combination thereof.

10 The present invention provides a method of removing phosphate phosphorus ($\text{PO}_4\text{-P}$) and/or algae from an aqueous medium, comprising:

- a) dispersing the functionalised bead as disclosed herein in the aqueous medium for at least 20 min in order to allow the $\text{PO}_4\text{-P}$ to bind onto the surface of the functionalised bead, and/or
- 15 b) dispersing the functionalised bead in the aqueous medium for at least 4 h in order to release the peroxide from the core of the functionalised bead or from the peroxide particles into the aqueous medium for removing the algae.

20 In some embodiments, the $\text{PO}_4\text{-P}$ binds onto the surface of the functionalised bead as a monolayer and is characterised by a Langmuir constant (k_L) for $\text{PO}_4\text{-P}$ adsorption of about 0.2 L/mg to about 0.4 L/mg.

In some embodiments, the functionalised bead has a pseudo second order rate constant (k_2) for $\text{PO}_4\text{-P}$ adsorption of about 0.15 g/mg/min to about 2 g/mg/min.

25 In some embodiments, a $\text{PO}_4\text{-P}$ removal efficiency of more than about 95% within 40 min.

In some embodiments, the functionalised beads have a selectivity of more than 90% for $\text{PO}_4\text{-P}$ in the presence of at least one of Cl^- , NO_3^- , CO_3^{2-} , and SO_4^{2-} .

30 In some embodiments, the $\text{PO}_4\text{-P}$ bonded on the surface of the functionalised bead is at least 80% recoverable when reacted with an acid.

- 6 -

In some embodiments, a pH of about 6.0 to about 9.0 is maintainable.

Advantageously, this is in line with the regulations from governmental agencies such as

5 National Environment Agency of Singapore (NEA).

In some embodiments, the functionalised bead has an algacide efficiency of more than about 80% after 6 h. In some embodiments, the functionalised bead has an algacide and/or inhibition efficiency of more than about 90% for at least 60 days.

10

In some embodiments, at least about 5 mg/L of PO₄-P is removed from the aqueous medium when the functionalised bead is characterised by a loading of about 1 to 4 g/50-mL of aqueous medium.

15 In some embodiments, at least about 4 x 10⁶ cells/mL of algae is removed from the aqueous medium when the functionalised bead is characterised by a loading of about 1 to 4 g/50-mL of aqueous medium.

In some embodiments, the functionalised bead is dispersed at or near a water surface of the

20 aqueous medium.

The present invention provides a method of inhibiting the growth of algae and/or the accumulation of PO₄-P in an aqueous medium, comprising:

a) dispersing the functionalised bead as disclosed herein in the aqueous medium for at
25 least 20 min.

The present invention provides a method of fabricating a functionalised bead having a core and a surface, comprising:

a) contacting an anionic and hydrophilic polymer solution with a solution comprising a
30 multivalent cation and a peroxide in order to form the core of the bead; and

- 7 -

b) cross-linking the core of the bead of step a) under a predetermined condition in order to grow the core and form the surface of the bead;

wherein the core comprises anionic and hydrophilic polymers crosslinked with the multivalent cation;

- 5 wherein the peroxide is encapsulated within the core of the bead as peroxide moieties;
wherein peroxide is adhered to the surface of the bead as peroxide particles;
wherein the functionalized bead has an average diameter of about 0.2 mm to about 10 mm;
and
wherein the peroxide particles have an average diameter of about 10 nm to about 900 nm.

10

In some embodiments, the polymer solution is added drop-wise into the solution under constant agitation.

In some embodiments, the polymer solution is a sodium alginate solution.

15

In some embodiments, the polymer solution has a concentration of about 2% w/v.

In some embodiments, the polymer solution further comprises an alkaline.

- 20 In some embodiments, the alkaline in the polymer solution is about 0.1 M to about 1.0 M.

In some embodiments, the multivalent cation in the solution is selected from Ca^{2+} , Zn^{2+} , Mg^{2+} , Cu^{2+} , Ce^{3+} , Fe^{3+} , La^{3+} or a combination thereof at about 0.05 M to about 1.0 M.

- 25 In some embodiments, the peroxide in the solution comprises a metal salt and hydrogen peroxide (H_2O_2). In some embodiments, the metal salt is metal chloride.

In some embodiments, the hydrogen peroxide at about 0.05 M to about 0.5 M.

- 30 In some embodiments, the beads are cross-linked for at least 4 h.

- 8 -

The present invention also provides a method of removing nitrate and/or ammonia in an aqueous medium, comprising:

- a) dispersing the functionalised bead according to any one of claims 1 to 10 in the aqueous medium for at least 4 h in order to allow the nitrate and/or ammonia to bind onto the surface of the functionalised bead.

In some embodiments, at least about 20% of ammonia is removed from the aqueous medium when the functionalised bead is characterised by a loading of about 1 to 4 g/50-mL of aqueous medium.

10

In some embodiments, at least about 5% of nitrate is removed from the aqueous medium when the functionalised bead is characterised by a loading of about 1 to 4 g/50-mL of aqueous medium.

15 Brief description of the drawings

Embodiments of the present invention will now be described, by way of non-limiting example, with reference to the drawings in which:

- 20 **Figure 1** shows H_2O_2 detection and confirmation in the presence of functionalised beads versus control. (a) The profile of H_2O_2 released by beads of CaCl_2 , CaO_2 -0, and CaO_2 -2 in solutions of pH 6.0 (solid symbols) and pH 9.0 (hollow symbols), [wet beads] = 1 g/50 mL; (b) Change of H_2O_2 concentration in the systems of H_2O_2 solution, CaCl_2 beads, CaO_2 -1 beads, and CaO_2 -2 beads at initial pH of 6.0, [wet beads] = 2 g/50 mL, $[\text{H}_2\text{O}_2]$ = 0.06 mM.
- 25 (c) Reduction kinetics of Cr(VI) with different initial concentrations by H_2O_2 or CaO_2 -2 beads, [wet bead] = 2 g/50 mL, $[\text{Cr(VI)}]$ = 20, 50, 100 μM . (d) Quantification of peroxo groups in CaO_2 -2 beads through the reduction of known concentration of KMnO_4 , [wet beads] = 1 g, $[\text{H}_2\text{O}_2]$ = 0.44 mM, $[\text{KMnO}_4]$ = 0.10 mM.

- Figure 2** shows XRD patterns of different samples: commercial CaO_2 particles, dried Ca(OH)_2 beads, and dried CaO_2 -2 beads before and after being used for PO_4 -P removal, [wet CaO_2 -2 beads] = 2 g/ 50 mL, $[\text{PO}_4\text{-P}]$ = 10 $\text{mg}\cdot\text{L}^{-1}$.
- 30

- 9 -

Figure 3 shows (a) adsorption kinetics of PO₄-P by wet CaO₂-2 beads and Ca(OH)₂ beads; (b) Pseudo-first-order fitting profile for PO₄-P adsorption kinetics. (c) Pseudo-second-order model fitting profile for PO₄-P adsorption kinetics; (d) Result of PO₄-P adsorption kinetics fitted by intraparticle diffusion model. For the symbols in (b)-(c): t (min) represents the sampling intervals, q_e (mg·g⁻¹) represents the adsorption amount at adsorption equilibrium, q_t (mg·g⁻¹) represents the adsorption amount at t , and k_{d1} , k_{d2} and k_{d3} represent the corresponding kinetic constants (mg·g·min^{-1/2}) obtained using intraparticle diffusion model. Experimental conditions: [wet CaO₂-2 beads] = [wet Ca(OH)₂ beads] = 2 g/ 50 mL, [PO₄-P]₀ = 10 mg·L⁻¹, pH₀ = 7.0.

Figure 4 shows the change of Ca²⁺ dissolution concentration during P adsorption by wet CaO₂-2 beads. Experimental conditions: [wet CaO₂-2 beads] = 2 g/50 mL, [PO₄-P]₀ = 10 mg·L⁻¹, pH₀ = 7.0.

Figure 5 shows (a) the adsorption isotherm of CaO₂-2 beads for PO₄-P adsorption; (b) Langmuir and (c) Freundlich isotherms models fitting for PO₄-P adsorption by CaO₂-2 beads. Experimental conditions: [CaO₂-2 beads] = 40 g·L⁻¹, [PO₄-P]₀ = 10-100 mg·L⁻¹, pH₀ = 7.0.

Figure 6 shows SEM images of different samples: (a-b) Ca(OH)₂ beads; (c-d) CaO₂-2 beads; (e-f) CaO₂-2 beads after being used for phosphorus adsorption.

Figure 7 shows the effect of different initial pH (3.0, 7.0 and 10.0) (a) and coexisting anions (b) on the PO₄-P removal by wet CaO₂-2 beads. (c) Desorption efficiency of PO₄-P from the used CaO₂-2 beads by adopting different solutions of NaOH, NaHCO₃ and HCl as eluents. Experimental conditions of (a)-(c): [CaO₂ beads] = 2 g/ 50 mL, [PO₄-P]₀ = 10 mg·L⁻¹, [HCl] = [NaHCO₃] = [NaOH] = 0.5 M. (b)-(c): pH = 7.0.

Figure 8 shows (a) algae removal by H₂O₂ solution, wet Ca(OH)₂ beads, and different amount of wet CaO₂-2 beads. [H₂O₂] = 0.06 mM, [Ca(OH)₂] = 2 g/ 50 mL, [wet CaO₂-2] = 1, 2, 3 and 4 g/ 50 mL. cell number: $(4.5-5.1) \times 10^6$ cells·mL⁻¹ (OD₆₈₀ = 0.1). (b) Simultaneous PO₄-P removal efficiency in the algae removal process (Figure 8a) by different dosage of wet CaO₂-2 beads. [PO₄-P] = 7.0 mg·L⁻¹, [wet CaO₂-2] = 1, 2, 3 and 4 g/ 50 mL. (c) The change of pH in the algae removal process (Figure 8a) after 12 d. (d) The removal efficiency of algae with different initial concentrations determined by different OD₆₈₀ values.

- 10 -

Figure 9 shows cytometer results of simulated algae-containing water by wet CaO_2 -2 beads after 170 d. [wet beads] = 3 g (upper graph) and 4 g (below graph) / 50 mL. No algae species could be found in the two cytometer results.

Figure 10 shows (a) removal efficiency of algae conducted in flasks with realistic algae-laden water by CaO_2 -2 beads. [wet CaO_2 -2] = 0, 2, and 4 g/ 50 mL. (b) The corresponding pH values in Figure 10a after adding CaO_2 -2 beads for 12 d. (c) Algae removal by wet CaO_2 -2 beads distributed into 3 mesh bags conducted in tanks with a large-scale volume (60 L). [wet CaO_2 -2] = 120 g. (d) Visible spectra (400-800 nm) change of water samples in realistic water treatment process conducted in tank. (e)-(f) Cytometer results on 26th d after realistic algae water being exposed to wet CaO_2 -2 beads. APC means allophycocyanin while PC5.5 is the abbreviation of Phycoerythrin-Cyanin 5.5. -H means the height of intensity.

Figure 11 shows photos of raw algae-contaminated water (blank) and water after being treated by CaO_2 -2 for 12 d and 26 d.

Figure 12 shows a mechanism diagram for PO_4 -P and algae removal by CaO_2 -functionalized alginate beads. The bead comprises alginate, which is cross-linked by the calcium ions.

Figure 13 shows a flow diagram of the CaO_2 -encapsulated alginate beads preparation process.

Figure 14 shows a plot of removal of phosphate by wet CaO_2 -2 beads. [wet beads] = 40 g/L, $[\text{PO}_4\text{-P}]$ = 10, 25 and 50 mg/L.

Figure 15 shows (left) the mesh bag that was used to hold the beads; and (right) a diagram to show how the beads can be used in realistic water treatment.

Detailed description

Few in-situ prepared CaO_2 -functionalized materials have been carried out and rare studies about using heterogeneous materials in the simultaneous removal of algae and phosphorus have been reported. For example, materials that have a sustained-release ability with encapsulated CaO_2 particles in beads have been developed to remediate environment or kill tumor cells. However, such encapsulated CaO_2 particles in beads result in uneven CaO_2 dispersion and uncontrollable particle sizes. Additionally, direct use of purchased CaO_2 powders to prepare CaO_2 -functionalized materials resulted in uneven CaO_2 dispersion and uncontrollable particle sizes.

- 11 -

To fill this gap and develop a multi-purpose material, CaO₂-functionalized alginate beads was prepared by in-situ preparation method using alginate, CaCl₂ and H₂O₂. CaO₂ nanoparticles can be formed from the reaction of CaCl₂ and H₂O₂. The in-situ formed CaO₂ particles can be encapsulated layer by layer during the alginate beads formation process due to the cross-link role between alginate and Ca²⁺. The performance and mechanism of algae and PO₄-P removal by prepared beads was investigated. The use of the synthesized beads in realistic algae-lead water was also evaluated in a large tank. The CaO₂-functionalized alginate beads can be used in simultaneously remediating the realistic phosphorus and algae polluted water environment.

Accordingly, the present invention provides a functionalised bead having a core and a surface, comprising:

- a) peroxide moieties within the core of the bead; and
 - b) peroxide particles adhered to the surface of the bead;
- wherein the core comprises anionic and hydrophilic polymers crosslinked with multivalent cations.

As used herein, "peroxide" refers to a compound having a formula in which two oxygen atoms are linked together by a single covalent bond.

A moiety refers to a part of a molecule that has a particular characteristic or functionality. A moiety can also be part of or extending from a backbone of a hydrocarbon molecule like a side chain or substituent. In this regard, and as used herein, "peroxide moieties within the core of the bead" refer to single moieties which can be free-standing or linked to the polymeric core.

A peroxide particle refers to an agglomeration of molecules having peroxide moieties. As peroxide moieties are negatively charged, the agglomerates can also comprise the positively charged counterions. These agglomerates can be amorphous, or can be crystalline.

- 12 -

Advantageously, the peroxide particles allow for a slow release of an oxidising agent to inhibit the growth of algae. The H₂O₂ that is released is decomposed by metals in algal suspensions to form strong oxidants such as hydroxyl radical, singlet oxygen. As the peroxide particles on the surface of the bead are depleted by dissolution, peroxide moieties
5 within the core of the bead are released such that a slow and constant release of oxidising agent is achievable. Further, the presence of multivalent cations within the bead and optionally in the peroxide particles provide a platform for binding phosphates.

In some embodiments, the functionalized bead has an average diameter of about 0.2 mm to
10 about 10 mm. In other embodiments, the average diameter is about 0.2 mm to about 9 mm, about 0.2 mm to about 8 mm, about 0.2 mm to about 7 mm, about 0.2 mm to about 6 mm, about 0.2 mm to about 5 mm, about 0.2 mm to about 4 mm, about 0.2 mm to about 3 mm, or about 0.2 mm to about 2 mm. In other embodiments, the average diameter is about 1 mm
15 to about 9 mm, about 1 mm to about 8 mm, about 1 mm to about 7 mm, about 1 mm to about 6 mm, about 1 mm to about 5 mm, about 1 mm to about 4 mm, about 1 mm to about 3 mm, or about 1 mm to about 2 mm. In other embodiments, the average diameter of about 3 mm.

In some embodiments, the peroxide particles have an average diameter of about 10 nm to
20 about 900 nm. In other embodiments, the average diameter is about 10 nm to about 800 nm, about 10 nm to about 700 nm, about 10 nm to about 600 nm, about 10 nm to about 500 nm, about 10 nm to about 400 nm, about 10 nm to about 300 nm, about 10 nm to about 200 nm, about 10 nm to about 100 nm, about 20 nm to about 100 nm, about 30 nm to about 100 nm, about 40 nm to about 100 nm, about 50 nm to about 100 nm, about 60 nm to about 100 nm, or about 70 nm to about 100 nm.

25

Accordingly, in some embodiments, the functionalised bead has a core and a surface, comprising:

- a) peroxide moieties within the core of the bead; and
 - b) peroxide particles adhered to the surface of the bead;
- 30 wherein the core comprises anionic and hydrophilic polymers crosslinked with multivalent cations;

- 13 -

wherein the functionalized bead has an average diameter of about 0.2 mm to about 10 mm;
and

wherein the peroxide particles have an average diameter of about 10 nm to about 900 nm.

5 In some embodiments, the functionalised bead has a core and a surface, comprising:

a) peroxide moieties within the core of the bead; and

b) peroxide particles adhered to the surface of the bead;

wherein the core comprises anionic and hydrophilic polymers crosslinked with multivalent cations;

10 wherein the functionalized bead has an average diameter of about 1 mm to about 10 mm;
and

wherein the peroxide particles have an average diameter of about 10 nm to about 900 nm.

In some embodiments, the peroxide particles are homogenously distributed on the surface
15 of the bead. In some embodiments, the surface of the bead further comprises hydroxide particles. In other embodiments, a ratio of peroxide particles to hydroxide particles is about 80:20 to about 99:1. In other embodiments, the ratio is about 85:15 to about 99:1, about 90:10 to about 99:1, or about 95:5 to about 99:1.

20 In some embodiments, the peroxide moieties within the core of the bead comprises peroxide particles.

In some embodiments, the concentration of the peroxide moieties and peroxide particles in the functionalised bead is about $0.3 \text{ mmol} \cdot \text{g}^{-1}$ to about $0.6 \text{ mmol} \cdot \text{g}^{-1}$. In other embodiments,
25 the concentration is about $0.3 \text{ mmol} \cdot \text{g}^{-1}$ to about $0.5 \text{ mmol} \cdot \text{g}^{-1}$, or $0.3 \text{ mmol} \cdot \text{g}^{-1}$ to about $0.4 \text{ mmol} \cdot \text{g}^{-1}$.

In some embodiments, the peroxide is selected from calcium peroxide, zinc peroxide, magnesium peroxide, lanthanum peroxide, copper peroxide, cerium peroxide, iron (III)
30 peroxide, or a combination thereof. In other embodiments, the peroxide is calcium peroxide.

- 14 -

In some embodiments, the multivalent cations are selected from a Group 2 metal or transition metal. In some embodiments, the multivalent cations are selected from Ca^{2+} , Zn^{2+} , Mg^{2+} , Cu^{2+} , Ce^{3+} , La^{3+} or a combination thereof. In other embodiments, the multivalent cation is Ca^{2+} .

5

In some embodiments, the functionalised bead is further functionalised with alkaline moieties within the core of the bead. In some embodiments, the functionalised bead is further functionalised with alkaline moieties on the surface of the bead. In some embodiments, an anion of the alkaline is hydroxide. Alkaline condition in beads preparation is found to be
10 favourable for the formation of peroxide.

As used herein, "anionic polymer" refers to a polymer which is generally capable of being ionised under basic conditions through loss of a proton. For example, a carboxylic acid functional group may form a carboxylate anion under basic conditions. Other anionic
15 charged groups include but not limited to chlorate, sulphate, phosphate, acetate, carboxyl, hydroxide.

Hydrophilic polymers contain polar or charged functional groups, rendering them soluble in water. The charged functional groups can be anionic or cationic. The hydrophilic polymer
20 can interact with or be dissolved by water or other polar substances. Examples of hydrophilic polymers include poly(carboxymethyl cellulose) and polyvinyl alcohol. Natural hydrophilic polymers include polysaccharides (e.g., dextran, alginate, chitosan, agarose, and pullulan) and proteins (e.g., albumin, gelatin, collagen, lectin, legumine, and viciline).

25 Polymers such as straight chain polymers and branched polymers can be used. The degree of cross linking can alter the concentration of peroxide moieties within the core of the bead. Accordingly, in order to increase the concentration of peroxide within the bead, in some embodiments, straight chain polymers are used.

30 In some embodiments, the anionic and hydrophilic polymers is a polysaccharide. In some embodiments, the anionic and hydrophilic polymers is alginate or a derivative thereof. The

- 15 -

alginate can be alginate acid sodium salt produced from brown algae. The concentration of the alginate can about 1% w/v to about 5% w/v. The alginate can have a viscosity of about 250 cp. Alginate salts with higher cp values can also be used.

- 5 A “derivative” is a compound that is derived from a similar compound by a chemical reaction. Derivative also meant a compound that can be imagined to arise from another compound, if one atom or group of atoms is replaced with another atom or group of atoms. Accordingly, alginate or a derivative thereof can refer to alginate which has various pendant groups attached to it at various locations of the polysaccharide (such as OH and COOH
- 10 moieties), and can also refer to modifications within the backbone of the polysaccharide, such as the number and type of atoms in the glycosidic bonds connecting the β -D-mannonic acids and α -L-guluronic acids, and the ratios of the β -D-mannonic acids and α -L-guluronic acids.
- 15 Hydrophobic polymers can also be used. In some embodiments, the hydrophobic polymer is polyvinylidene fluoride (PVDF) or a derivative thereof. Experiments using PVDF modified by CaO_2 to remove algae were shown to work.

In some embodiments, the bead is at least 97% hydrated with an aqueous medium. This is

20 in part due to the hydrophilicity of the polymers, and can act to provide a sustained release of peroxide from the functionalised beads. In other embodiments, the bead is at least 99%, 98%, 96%, 95%, 94%, 93%, 92%, 91% or 90% hydrated.

In some embodiments, H_2O_2 is releasable from the functionalised bead when dispersed in

25 aqueous medium. This is shown in Equation (1) – (3).

In some embodiments, H_2O_2 is releasable from the functionalised bead at a rate of about 0.03 mM/g/d to about 0.1 mM/g/d. In other embodiments, the rate is about 0.03 mM/g/d to about 0.09 mM/g/d, about 0.03 mM/g/d to about 0.08 mM/g/d, about 0.03 mM/g/d to about

30 0.07 mM/g/d, or about 0.03 mM/g/d to about 0.06 mM/g/d. In some embodiments, H_2O_2 is releasable from the functionalised bead at a rate of about 0.06 mM/g/d.

- 16 -

In some embodiments, when a concentration of functionalised bead in an aqueous medium is about 0.04 g/mL, a H₂O₂ concentration of more than about 0.04 mM is maintainable for at least 9 days. In other embodiments, the H₂O₂ concentration is maintainable for at least 14
5 days, 21 days, 28 days, 1 month or 2 months.

In some embodiments, a pH of about 6.0 to about 9.0 is maintainable when the functionalised bead is dispersed in aqueous medium. In other embodiments, the pH is about 6.5 to about 9.0, about 7.0 to about 9.0, about 7.5 to about 9.0, or about 8 to about 9.0.

10

In some embodiments, the multivalent cation has a dissolution of less than about 1.4 mg·L⁻¹ (mg/L). In other embodiments, the dissolution is less than about 1.3 mg·L⁻¹, about 1.2 mg·L⁻¹, about 1.1 mg·L⁻¹, about 1.0 mg·L⁻¹, or about 1.4 mg·L⁻¹. In this regard, the multivalent cation can also be released or leached from the bead.

15

In some embodiments, the functionalised beads is characterised by an initial burst release of peroxide, followed by a sustained release of peroxide. In some embodiments, the burst release of peroxide occurs over about 10 min. In other embodiments, the sustained release of peroxide occurs over at least 8 days.

20

The present invention provides a method of fabricating a functionalised bead having a core and a surface, comprising:

- a) contacting an anionic and hydrophilic polymer solution with a solution comprising a multivalent cation and a peroxide in order to form the core of the bead; and
- 25 b) incubating the core of the bead of step a) under a predetermined condition in order to grow the core and form the surface of the bead;
wherein the core comprises anionic and hydrophilic polymers crosslinked with the multivalent cation;
wherein the peroxide is encapsulated within the core of the bead as peroxide moieties; and
30 wherein peroxide is adhered to the surface of the bead as peroxide particles.

- 17 -

During the step of preparing the bead in the presence of anionic and hydrophilic polymer solution, a multivalent cation and a peroxide (step b), the core of the bead continues to grow via crosslinking of the polymers. As the anionic and hydrophilic polymer is depleted, peroxide particles are formed and are adhered to the surface of the beads or embedded inside.

- 5 In this regard, the beads that are formed in step a) reacts with the anionic and hydrophilic polymer solution, the multivalent cations and peroxides to further grow the bead, and subsequently reacts with the multivalent cations and peroxides to form the peroxide particles on the surface of the beads. As used herein, "incubating" the core of the bead in the aqueous medium refers to placing the core of the bead in the aqueous medium for cross-linking to
10 occur.

Accordingly, in some embodiments, the step of incubating the core of the bead is a step of cross-linking the core of the bead.

- 15 In some embodiments, the method of fabricating a functionalised bead having a core and a surface, comprising:
- a) contacting an anionic and hydrophilic polymer solution with a solution comprising a multivalent cation and a peroxide in order to form the core of the bead; and
 - b) cross-linking the core of the bead of step a) under a predetermined condition in order
20 to grow the core and form the surface of the bead;
- wherein the core comprises anionic and hydrophilic polymers crosslinked with the multivalent cation;
- wherein the peroxide is encapsulated within the core of the bead as peroxide moieties; and
- wherein peroxide is adhered to the surface of the bead as peroxide particles.

25

In some embodiments, the method of fabricating a functionalised bead having a core and a surface, comprises:

- a) contacting an anionic and hydrophilic polymer solution with a solution comprising a multivalent cation and a peroxide in order to form the core of the bead; and
- 30 b) incubating or crosslinking the bead under a predetermined condition in order to grow the core and form the surface of the bead;

- 18 -

wherein the core comprises anionic and hydrophilic polymers crosslinked with the multivalent cation;

wherein the peroxide is encapsulated within the core of the bead as peroxide moieties;

wherein peroxide is adhered to the surface of the bead as peroxide particles;

- 5 wherein the functionalized bead has an average diameter of about 0.2 mm to about 10 mm;
and

wherein the peroxide particles have an average diameter of about 10 nm to about 900 nm.

- In some embodiments, the method of fabricating a functionalised bead having a core and a
10 surface, comprises:

a) contacting an anionic and hydrophilic polymer solution with a solution comprising a multivalent cation and a peroxide in order to form the core of the bead; and

b) incubating or crosslinking the core of the bead of step a) under a predetermined condition in order to grow the core and form the surface of the bead;

- 15 wherein the core comprises anionic and hydrophilic polymers crosslinked with the multivalent cation;

wherein the peroxide is encapsulated within the core of the bead as peroxide moieties;

wherein peroxide is adhered to the surface of the bead as peroxide particles;

wherein the functionalized bead has an average diameter of about 1 mm to about 10 mm;

- 20 and

wherein the peroxide particles have an average diameter of about 10 nm to about 900 nm.

- In some embodiments, step (a) comprises drop-wise adding the polymer solution into the solution of multivalent cation and a peroxide under constant agitation. The size of the
25 functionalised beads can be controlled by for example, controlling the gauge size of the output, the droplet addition speed, the concentration of polymer, or the concentration of the crosslinker (multivalent cation). In other embodiments, the constant agitation is stirring the solution of multivalent cation and a peroxide at a rate of about 400 rpm. In other embodiments, step (a) is performed at about 15°C to about 30°C.

30

- 19 -

In some embodiments, the polymer solution is an alginate solution. The counterion associated with alginate can be any cation, as long as the alginate is dispersible in an aqueous medium to form a solution. In some embodiments, the cation is a monovalent cation. In some embodiments, the polymer solution is a sodium alginate solution.

5

In some embodiments, the polymer solution has a concentration of about 2% w/v. In other embodiments, the concentration is about 1% w/v to about 5% w/v, about 1% w/v to about 4% w/v, about 1% w/v to about 3% w/v, or about 1% w/v to about 2% w/v.

10 In some embodiments, the polymer solution further comprises an alkaline. In some embodiments, the alkaline in the polymer solution is about 0.1 M to about 1 M. In other embodiments, the concentration is about 0.1 M to about 0.9 M, about 0.1 M to about 0.8 M, about 0.1 M to about 0.7 M, about 0.1 M to about 0.6 M, about 0.1 M to about 0.5 M, about 0.1 M to about 0.4 M, about 0.1 M to about 0.3 M, or about 0.2 M to about 0.3 M.

15

In some embodiments, the multivalent cation in the solution is selected from Ca^{2+} , Zn^{2+} , Mg^{2+} , Cu^{2+} , Ce^{3+} , Fe^{3+} , La^{3+} or a combination thereof. In other embodiments, the multivalent cation is Ca^{2+} . In other embodiments, the concentration of the multivalent cation is about 0.05 M to about 1 M, about 0.05 M to about 0.9 M, about 0.05 M to about 0.8 M, about 0.05 M to about 0.7 M, about 0.05 M to about 0.6 M, about 0.05 M to about 0.5 M, about 0.05 M to about 0.4 M, about 0.05 M to about 0.3 M, about 0.05 M to about 0.2 M, or about 0.05 M to about 0.1 M.

20

In some embodiments, solution comprising a multivalent cation and a peroxide comprises a metal salt and hydrogen peroxide (H_2O_2). In some embodiments, the metal salt is metal chloride. In some embodiments, the hydrogen peroxide at about 0.01 M to about 0.5 M. In other embodiments, the concentration is about 0.05 M to about 0.5 M, about 0.05 M to about 0.4 M, about 0.05 M to about 0.3 M, about 0.05 M to about 0.2 M, or about 0.05 M to about 0.1 M.

25

30

- 20 -

In some embodiments, the core of the beads is incubated or crosslinked for at least 4 h. In other embodiments, the core of the functionalised beads is incubated or crosslinked at about 1°C to about 10°C. A low temperature was found to be advantageous for reducing the decomposition of the peroxides, as well as to obtain good control over the size distributions of the beads and the peroxide particles.

In some embodiments, the method further comprises a step of purifying the functionalised beads. The functionalised beads can be purified by washing them in an aqueous medium, such as ultrapure water.

10

The present invention provides a method of removing phosphate phosphorus ($\text{PO}_4\text{-P}$) from an aqueous medium, comprising:

- a) dispersing the functionalised bead as disclosed herein in the aqueous medium; and
- b) incubating the functionalised bead in the aqueous medium for at least 20 min in order to allow the $\text{PO}_4\text{-P}$ to bind onto the surface of the functionalised bead.

15

The functionalised bead is placed in the aqueous medium to allow reaction between the beads and the $\text{PO}_4\text{-P}$ such that $\text{PO}_4\text{-P}$ binds onto the surface of the functionalised bead. The reaction condition is maintained at least at a favourable temperature in order to promote this reaction. As used herein, "incubating" the functionalised bead in the aqueous medium refers to placing the functionalised bead in the aqueous medium for allowing the peroxide to react.

20

Accordingly, in some embodiments, the step of incubating the functionalised bead is a step of placing the functionalised bead in the aqueous medium.

25

In some embodiments, the method of removing phosphate phosphorus ($\text{PO}_4\text{-P}$) from an aqueous medium, comprising:

- a) dispersing the functionalised bead as disclosed herein in the aqueous medium for at least 20 min in order to allow the $\text{PO}_4\text{-P}$ to bind onto the surface of the functionalised bead.

30

- 21 -

The presence of multivalent cations in the functionalised beads provide a platform for phosphorus to bind. For example, calcium, iron and aluminium cations can cause phosphorus to precipitate on the surface of the functionalised beads, thus removing it from the water. Additionally, as the cations are not released into the water, the water hardness is not altered.

- 5 As the phosphorus can be subsequently released, the beads can be used as fertilizer for plant growth. The beads can also remove nitrogen species (such as nitrate and ammonia) from wastewater.

- 10 In some embodiments, the $\text{PO}_4\text{-P}$ binds onto the surface of the functionalised bead as a monolayer. In some embodiments, the $\text{PO}_4\text{-P}$ is chemisorbed on the surface of the functionalised beads. In some embodiments, the $\text{PO}_4\text{-P}$ diffuses into a pore of the functionalised bead.

- In some embodiments, the functionalised bead has a pseudo second order rate constant (k_2) for $\text{PO}_4\text{-P}$ adsorption of about $0.15 \text{ g}\cdot\text{mg}^{-1}\cdot\text{min}^{-1}$ ($\text{g}/\text{mg}/\text{min}$) to about $2 \text{ g}\cdot\text{mg}^{-1}\cdot\text{min}^{-1}$. In other embodiments, the pseudo second order rate constant (k_2) for $\text{PO}_4\text{-P}$ adsorption is about $0.15 \text{ g}\cdot\text{mg}^{-1}\cdot\text{min}^{-1}$ to about $1.8 \text{ g}\cdot\text{mg}^{-1}\cdot\text{min}^{-1}$, about $0.15 \text{ g}\cdot\text{mg}^{-1}\cdot\text{min}^{-1}$ to about $1.6 \text{ g}\cdot\text{mg}^{-1}\cdot\text{min}^{-1}$, about $0.15 \text{ g}\cdot\text{mg}^{-1}\cdot\text{min}^{-1}$ to about $1.4 \text{ g}\cdot\text{mg}^{-1}\cdot\text{min}^{-1}$, about $0.15 \text{ g}\cdot\text{mg}^{-1}\cdot\text{min}^{-1}$ to about $1.2 \text{ g}\cdot\text{mg}^{-1}\cdot\text{min}^{-1}$, about $0.15 \text{ g}\cdot\text{mg}^{-1}\cdot\text{min}^{-1}$ to about $1 \text{ g}\cdot\text{mg}^{-1}\cdot\text{min}^{-1}$, about $0.15 \text{ g}\cdot\text{mg}^{-1}\cdot\text{min}^{-1}$ to about $0.8 \text{ g}\cdot\text{mg}^{-1}\cdot\text{min}^{-1}$, about $0.15 \text{ g}\cdot\text{mg}^{-1}\cdot\text{min}^{-1}$ to about $0.6 \text{ g}\cdot\text{mg}^{-1}\cdot\text{min}^{-1}$, about $0.15 \text{ g}\cdot\text{mg}^{-1}\cdot\text{min}^{-1}$ to about $0.4 \text{ g}\cdot\text{mg}^{-1}\cdot\text{min}^{-1}$, or about $0.15 \text{ g}\cdot\text{mg}^{-1}\cdot\text{min}^{-1}$ to about $0.2 \text{ g}\cdot\text{mg}^{-1}\cdot\text{min}^{-1}$.

- In some embodiments, the functionalised bead has a first adsorption constant (k_{d1}) for $\text{PO}_4\text{-P}$ adsorption of about $1 \text{ mg}\cdot\text{g}^{-1}\cdot\text{min}^{-1/2}$ to about $1.2 \text{ mg}\cdot\text{g}^{-1}\cdot\text{min}^{-1/2}$. In some embodiments, the functionalised bead has a second adsorption constant (k_{d2}) for $\text{PO}_4\text{-P}$ adsorption of about $0.3 \text{ mg}\cdot\text{g}^{-1}\cdot\text{min}^{-1/2}$ to about $0.5 \text{ mg}\cdot\text{g}^{-1}\cdot\text{min}^{-1/2}$. In some embodiments, the functionalised bead has a third adsorption constant (k_{d3}) for $\text{PO}_4\text{-P}$ adsorption of about $0.005 \text{ mg}\cdot\text{g}^{-1}\cdot\text{min}^{-1/2}$ to about $0.02 \text{ mg}\cdot\text{g}^{-1}\cdot\text{min}^{-1/2}$.

30

- 22 -

In some embodiments, the functionalised bead has a Langmuir constant (k_L) for $\text{PO}_4\text{-P}$ adsorption of about $0.2 \text{ L}\cdot\text{mg}^{-1}$ to about $0.4 \text{ L}\cdot\text{mg}^{-1}$.

5 In some embodiments, when a concentration of functionalised bead in the aqueous medium is about 0.04 g/mL (or 2 g/50 mL or 40 g/L), about 5 mg/L to about 20 mg/L of $\text{PO}_4\text{-P}$ is removable from the aqueous medium. In some embodiments, at least about 5 mg/L of $\text{PO}_4\text{-P}$ is removed from the aqueous medium when the functionalised bead is characterised by a loading of about 1 g/50-mL to about 4 g/50-mL of aqueous medium.

In some embodiments, a $\text{PO}_4\text{-P}$ removal efficiency of more than about 95% within 40 min.
10 In other embodiments, the $\text{PO}_4\text{-P}$ removal efficiency of more than about 95% within 35 min, 30 min, 25 min, or 20 min.

In some embodiments, the removal of $\text{PO}_4\text{-P}$ is performed at a pH of about 3 to about 10. In other embodiments, the pH is about 4 to about 10, about 5 to about 10, about 6 to about 10,
15 about 7 to about 10, about 8 to about 10, or about 9 to about 10. While the final removal efficiency at different pH values is similar after about 20 min, it was found that under alkaline conditions the kinetics is higher.

In some embodiments, the functionalised beads have a selectivity of more than 90% for $\text{PO}_4\text{-P}$ in the presence of at least one of Cl^- , NO_3^- , CO_3^{2-} , and SO_4^{2-} . In other embodiments, the
20 selectivity is more than 92%, 94%, 96%, 98% or 99%.

In some embodiments, the $\text{PO}_4\text{-P}$ bonded on the surface of the functionalised bead is at least 80% recoverable when reacted with an acid. In this regard, the $\text{PO}_4\text{-P}$ bonded on the surface
25 of the functionalised bead is releasable. In other embodiments, the bonded $\text{PO}_4\text{-P}$ is at least 82%, 84%, 86%, 88% or 90% recoverable. In other embodiments, the bonded $\text{PO}_4\text{-P}$ is recoverable at a pH of about 1 to about 10, about 1 to about 9, about 1 to about 8, about 1 to about 7, about 1 to about 6, about 1 to about 5, about 1 to about 4, about 1 to about 3, or about 1 to about 2.

30

The present invention provides a method of killing algae in an aqueous medium, comprising:

- 23 -

- a) dispersing the functionalised bead as disclosed herein in the aqueous medium; and
- b) incubating the functionalised bead in the aqueous medium for at least 4 h in order to release the peroxide from the core of the functionalised bead or from the peroxide particles into the aqueous medium for killing the algae.

5

The functionalised bead is placed in the aqueous medium to allow reaction between the beads and the algae such that the released peroxide kills the algae. The reaction condition is maintained at least at a favourable temperature in order to promote this reaction. The temperature can be about 0 °C to about 40 °C, or preferably about 4 °C to about 30 °C.

10

Accordingly, in some embodiments, the step of incubating the functionalised bead is a step of placing the functionalised bead in the aqueous medium.

In some embodiments, the method of killing algae in an aqueous medium, comprising:

- 15 a) dispersing the functionalised bead as disclosed herein in the aqueous medium for at least 4 h in order to release the peroxide from the core of the functionalised bead or from the peroxide particles into the aqueous medium for killing the algae.

20 The present invention provides a method of removing algae in an aqueous medium, comprising:

- a) dispersing the functionalised bead as disclosed herein in the aqueous medium; and
- b) incubating the functionalised bead in the aqueous medium for at least 4 h in order to release the peroxide from the core of the functionalised bead or from the peroxide particles into the aqueous medium for removing the algae.

25

In some embodiments, the step of incubating the functionalised bead is a step of placing the functionalised bead in the aqueous medium.

In some embodiments, the method of removing algae in an aqueous medium, comprising:

- 24 -

a) dispersing the functionalised bead as disclosed herein in the aqueous medium for at least 4 h in order to release the peroxide from the core of the functionalised bead or from the peroxide particles into the aqueous medium for removing the algae.

- 5 The beads can be dispersed in the aqueous medium in the form of a permeable and/or porous bag. Materials that can be used to fabricate the bag can be for example be cotton muslin, or silken plastic or cheese cloth. The bag can be positioned at about 100 cm below the water level, or preferably at about 0 cm to about 50 cm below the water level/surface. It was found that by positioning or placing the bag of beads at or near the water surface of the aqueous
- 10 medium, algae growth which occurs at an area with higher CO₂ levels can be at least suppressed. In this sense, the functionalised beads are within the aqueous medium and in close proximity a top exposed surface of the aqueous medium. This would reduce the cost as less functionalised beads can be used and improve utility as algae require sunlight to grow.
- 15 In some embodiments, the functionalised bead is placed in the aqueous medium for at least 4 h. In other embodiments, the functionalised bead is placed in the aqueous medium for at least 5 h, 6 h, 7 h, or 8 h.

In some embodiments, a pH of about 6.0 to about 9.0 is maintainable. In other embodiments,

20 the pH is about 6.0 to about 8.0, or about 7.0 to about 8.0.

Advantageously, this is in line with the regulations from governmental agencies such as National Environment Agency of Singapore (NEA).

- 25 In some embodiments, when a concentration of functionalised bead in the aqueous medium is 0.02 g/mL (or 1 g/50 mL or 20 g/L), about 4×10^6 cells/mL to about 5.5×10^6 cells/mL of algae can be killed. In some embodiments, at least about 4×10^6 cells/mL of algae is removed from the aqueous medium when the functionalised bead is characterised by a loading of about 1 to 4 g/50-mL of aqueous medium.

30

- 25 -

In some embodiments, the functionalised bead has an algaecide efficiency of more than about 80% after 6 h. In other embodiments, the algaecide or inhibition efficiency is more than about 85%, 90%, 95%, 98%, or 99%.

- 5 In some embodiments, the functionalised bead has an algaecide efficiency of more than about 90% for at least 60 days. In other embodiments, the algaecide efficiency is more than about 92%, 94%, 95%, 98%, or 99%.

The present invention also provides a method of inhibiting the accumulation of $\text{PO}_4\text{-P}$ and/or
10 growth of algae in an aqueous medium, comprising:

- a) dispersing the functionalised bead as disclosed herein in the aqueous medium; and
- b) incubating the functionalised bead in the aqueous medium for at least 20 min.

In some embodiments, the step of incubating the functionalised bead is a step of placing the
15 functionalised beads in the aqueous medium.

In other embodiments, the method of inhibiting the accumulation of $\text{PO}_4\text{-P}$ and/or growth of algae in an aqueous medium, comprising:

- a) dispersing the functionalised bead as disclosed herein in the aqueous medium for at
20 least 20 min.

In some embodiments, the incubation is for at least 40 min, 1 h, 2 h, 4 h, 6 h, 8 h, 10 h, 12 h, or 1 day.

- 25 In some embodiments, the functionalised bead is capable of inhibiting the accumulation of $\text{PO}_4\text{-P}$ and/or algae growth for at least 5 days, 10 days, 20 days, 30 days, 40 days, 50 days, 60 days, 70 days, 80 days, 90 days, or 120 days.

The present invention provides a method of removing $\text{PO}_4\text{-P}$ and killing algae in an aqueous
30 medium, comprising:

- a) dispersing the functionalised bead as disclosed herein in the aqueous medium; and

- 26 -

b) incubating the functionalised bead in the aqueous medium for at least 4 h in order to allow the $\text{PO}_4\text{-P}$ to bind onto the surface of the functionalised bead and to release the peroxide from the core of the functionalised bead or from the peroxide particles into the aqueous medium for killing the algae.

5

In some embodiments, the step of incubating the functionalised bead is a step of placing the functionalised beads in the aqueous medium.

In some embodiments, the method of removing $\text{PO}_4\text{-P}$ and killing algae in an aqueous medium, comprising:

10

a) dispersing the functionalised bead as disclosed herein in the aqueous medium for at least 4 h in order to allow the $\text{PO}_4\text{-P}$ to bind onto the surface of the functionalised bead and to release the peroxide from the core of the functionalised bead or from the peroxide particles into the aqueous medium for killing the algae.

15

The present invention provides a method of removing $\text{PO}_4\text{-P}$ and removing algae in an aqueous medium, comprising:

a) dispersing the functionalised bead as disclosed herein in the aqueous medium; and
b) incubating the functionalised bead in the aqueous medium for at least 4 h in order to allow the $\text{PO}_4\text{-P}$ to bind onto the surface of the functionalised bead and to release the peroxide from the core of the functionalised bead or from the peroxide particles into the aqueous medium for removing the algae.

20

In some embodiments, the step of incubating the functionalised bead is a step of placing the functionalised beads in the aqueous medium.

25

In some embodiments, the method of removing $\text{PO}_4\text{-P}$ and removing algae in an aqueous medium, comprising:

a) dispersing the functionalised bead as disclosed herein in the aqueous medium for at least 4 h in order to allow the $\text{PO}_4\text{-P}$ to bind onto the surface of the functionalised bead and

30

- 27 -

to release the peroxide from the core of the functionalised bead or from the peroxide particles into the aqueous medium for removing the algae.

The present invention provides a method of removing $\text{PO}_4\text{-P}$ and inhibiting algae growth in an aqueous medium, comprising:

- a) dispersing the functionalised bead as disclosed herein in the aqueous medium; and
- b) incubating the functionalised bead in the aqueous medium for at least 4 h in order to allow the $\text{PO}_4\text{-P}$ to bind onto the surface of the functionalised bead and to release the peroxide from the core of the functionalised bead or from the peroxide particles into the aqueous medium for inhibiting the algae growth.

In some embodiments, the step of incubating the functionalised bead is a step of placing the functionalised beads in the aqueous medium.

In some embodiments, the method of removing $\text{PO}_4\text{-P}$ and inhibiting algae growth in an aqueous medium, comprising:

- a) dispersing the functionalised bead as disclosed herein in the aqueous medium for at least 4 h in order to allow the $\text{PO}_4\text{-P}$ to bind onto the surface of the functionalised bead and to release the peroxide from the core of the functionalised bead or from the peroxide particles into the aqueous medium for inhibiting the algae growth.

In some embodiments, the step of dispersing the functionalised bead (step a) comprises containing the functionalised bead in a mesh bag.

The present invention also provides a method of removing nitrate and/or ammonia in an aqueous medium, comprising:

- a) dispersing the functionalised bead as disclosed herein in the aqueous medium; and
- b) placing the functionalised bead in the aqueous medium for at least 4 h in order to allow the nitrate and/or ammonia to bind onto the surface of the functionalised bead.

30

- 28 -

In other embodiments, the method of removing nitrate and/or ammonia in an aqueous medium, comprising:

- a) dispersing the functionalised bead as disclosed herein in the aqueous medium for at least 4 h in order to allow the nitrate and/or ammonia to bind onto the surface of the functionalised bead.

In some embodiments, about 20 to about 30 % of ammonia is removed from the aqueous medium. In some embodiments, at least about 20% of ammonia is removed, or at least about 30% of ammonia is removed. The contaminated waters tested has an ammonia concentration of about 1 to 5 mg-NH₄/L. This removal can be achieved using beads with a loading of about 1 to 4 g/50-mL solution, for contacting time of about 4 to about 36 hours. Preferably, the bead loading is about 1.6 to 3.5 g/50-mL.

Ammonia is a colourless, pungent gaseous compound of hydrogen and nitrogen that is highly soluble in water. It is a biologically active compound found in most waters as a normal biological degradation product of nitrogenous organic matter (protein). It can exist in water as NH₃ and/or NH₄⁺. As used herein, "ammonia" refers to both ionised and un-ionised forms. Environmental limits for ammonia in surface water in the US range from 0.25 to 32.5 mg/L (ppm). The National Academy of Science recommends, and many European nations have adopted, a drinking water standard of 0.5 mg/L (ppm).

In some embodiments, about 5% to about 50% of nitrate is removed from the aqueous medium. In other embodiments, about 5% to about 45% of nitrate is removed, or about 5% to about 10% of nitrate is removed. In other embodiments, at least about 5% of nitrate is removed, or at least about 10% of nitrate is removed. The contaminated waters tested has a nitrate concentration of about 44 to 950 mg/L of nitrate. This removal can be achieved using beads with a loading of about 1 to 4 g/50-mL solution, for contacting time of about 4 to about 36 hours. Preferably, the bead loading is about 1.6 to 3.5 g/50-mL.

The principal sources of nitrate contamination in water are fertilizers, animal waste and septic tanks. The water supplies most vulnerable to nitrate contamination are in agricultural

- 29 -

areas and in well waters having a close or hydraulic relationship to septic tanks. Nitrate in drinking water can be responsible for a temporary blood disorder in infants called methemoglobinemia (blue baby syndrome). It can exist in water as nitrate (NO_3^-) and/or nitrite (NO_2^-). As used herein, "nitrate" refers to both forms. The U.S. Environmental Protection Agency (EPA) standard for nitrate in drinking water is 10 milligrams of nitrate (measured as nitrogen) per liter of drinking water (mg/L).

Examples

In-situ Synthesis CaO_2 Beads and Optimization. To obtain beads with sustainable H_2O_2 release ability, wet beads (Table 1) of CaO_2 -0, CaO_2 -1, and CaO_2 -2 were prepared and compared with CaCl_2 and $\text{Ca}(\text{OH})_2$.

Table 1. Chemical compositions of prepared alginate beads.

Name of samples	Solution A (50 mL)		Solution B (100 mL)	
	Alginate (2% w/v)	NaOH	CaCl_2 (M)	H_2O_2
CaCl_2	50	0	0.1	0.00
$\text{Ca}(\text{OH})_2$	50	0.2	0.1	0.00
CaO_2 -0	50	0	0.1	0.10
CaO_2 -1	50	0.2	0.1	0.01
CaO_2 -2	50	0.2	0.1	0.10

15

As shown in Figure 1a, there is no H_2O_2 release by CaCl_2 beads. For CaO_2 -0 beads (without alkali in synthesis process), the H_2O_2 concentration was steady in the first five days and gradually decreased to zero on the eighth day. However, CaO_2 -2 beads showed a sustainable H_2O_2 concentration for a longer time since alkaline condition in beads preparation process is favorable for the formation of CaO_2 (eqs. 4-6). The H_2O_2 release at pH 6.0 behaved similarly to that at pH 9.0, suggesting that the common water environment pH (6.0-9.0) would pose negligible effect on the steady H_2O_2 release by synthesized beads.

20

- 30 -



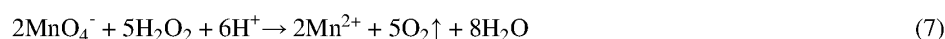
5 To further investigate the influence of different synthesis compositions and the release sustainability, beads with lower concentrations of H_2O_2 and NaOH (CaO_2 -1 in Table 1) in synthesis process were synthesized and longer-term H_2O_2 release running were conducted (Figure 1b). As illustrated in Figure 1b, CaO_2 -2 exhibited longer-lasting and steadier H_2O_2 release compared to CaO_2 -1. The wet beads of CaO_2 -2 could maintain a H_2O_2 concentration
10 of 0.06 mM over 26 days while the H_2O_2 concentration generated by CaO_2 -1 was only around 0.01 mM and decreased rapidly within 5 days. H_2O_2 of 0.06 mM was totally decomposed in 9 days, which validated the sustainable release of H_2O_2 by CaO_2 -2 beads. Overall, CaO_2 -2 beads prepared under alkaline condition exhibited a slow and persistent H_2O_2 release ability, which showed a promising application potential.

15

H_2O_2 possessing -O-O- group has the ability to reduce Cr(VI) , thus Cr(VI) was used to test peroxy group released by CaO_2 -2 beads. As illustrated in Figure 1c, 99.8% of Cr(VI) with an initial concentration of 20 μM was reduced within 90 min by wet CaO_2 -2 beads. On the other hand, H_2O_2 solution of 0.06 mM could only reduce about 30% of 20 μM Cr(VI) ,
20 indicating a higher total amount of H_2O_2 generated by CaO_2 -2 beads. The reduction rate decreased with increasing Cr(VI) concentration from 20-100 μM . The reaction rate decreased quickly and turned steady after the steep gradient in the first 10 min (Figure 1c), meaning H_2O_2 could be slowly released and then reduce Cr(VI) after the rapid consumption of H_2O_2 . Therefore, the Cr(VI) reduction by CaO_2 -2 beads validated the existence of -O-O-
25 group and further demonstrated the slow release ability of beads.

The amount of -O-O- on CaO_2 beads was quantified by a potassium permanganate-based titration method. As exhibited by the inserted photo in Figure 1d, the color of permanganate (MnO_4^-) in CaO_2 beads or H_2O_2 acidic solution disappeared due to the eq. 7. The determined
30 amount of -O-O- in CaO_2 beads is 0.44 $\text{mmol}\cdot\text{g}^{-1}$ according to the titration result, meaning

the total concentration of -O-O- could be high to 17.6 mM with 2 g wet beads put into 50 mL water.



5

To provide a direct evidence on the formation of CaO_2 on beads, XRD patterns of dried materials were investigated. As shown in Figure 2, the pattern of CaO_2 particles exhibits characteristic peaks at $2\theta = 30.6^\circ$, 35.9° and 47.7° , corresponding to (002), (110), and (112) of CaO_2 (JCPDS-03-0865), respectively. The small peaks in dried CaO_2 -2 beads are well indexed to CaO_2 , indicating that CaO_2 particles were successfully formed on CaO_2 -2 beads. There was no characteristic CaO_2 peaks observed by Ca(OH)_2 beads since no H_2O_2 was used during the preparation process.

Table 2. Kinetics values, constants and correlation coefficients of the intraparticle diffusion model used for fitting PO_4 -P adsorption by Ca(OH)_2 beads and CaO_2 -2 beads obtained from Figure 3d.*

Sample	k_{d1}	k_{d2}	k_{d3}	C_1	C_2	C_3	R_1^2	R_2^2	R_3^2
	(mg·g·min ^{-1/2})								
Ca(OH)_2	0.586	0.327	0.125	0.237	0.578	1.490	0.999	0.998	0.980
CaO_2 -2	1.039	0.412	0.010	1.264	3.012	4.838	0.963	0.987	0.983

*Values of k_d are kinetics constants of PO_4 -P adsorption while C is the corresponding constants obtained by fitting the experimental data in Figure 2d. R^2 is the R square values of the fitting results.

20

Phosphorus Removal Performance and Kinetics Fitting. The adsorption kinetics experiments were conducted to understand the phosphorus adsorption process and mechanism by wet CaO_2 -2 beads. As illustrated in Figure 3a, the phosphorus removal efficiency by wet Ca(OH)_2 and CaO_2 -2 beads are 48.8% and 97.2% within 40 min, respectively, demonstrating the excellent PO_4 -P removal performance by CaO_2 -2 beads. The corresponding concentration of Ca^{2+} dissolution by CaO_2 -2 beads is lower than $1.4 \text{ mg}\cdot\text{L}^{-1}$

25

- 32 -

(Figure 4); this indicates that the use of CaO₂-2 beads would not cause the increase in water hardness.

Pseudo-first-order (Figure 3b) and pseudo-second-order models (Figure 3c) were both used to fit the experimental data and the resultant parameters are listed in Table 3.

Table 3. Kinetic fitting parameter results with different models in PO₄-P adsorption process by Ca(OH)₂ beads and CaO₂-2 beads.

Beads	pseudo-first-order model			pseudo-second-order model		
	R^2	k_1 (min ⁻¹)	q_e (mg·g ⁻¹)	R^2	k_2 (g·mg ⁻¹ ·min ⁻¹)	q_e (mg·g ⁻¹)
Ca(OH) ₂	0.965	0.059	1.779	0.999	0.051	2.733
CaO ₂ -2	0.776	0.082	1.551	0.999	0.172	5.037

According to the R^2 values, the adsorption kinetics of the two adsorbents followed the pseudo-second-order model, which meant that the removal of PO₄-P by wet beads should be a chemisorption process. The pseudo-second-order rate constant (k_2 , g·mg⁻¹·min⁻¹) of PO₄-P removal by CaO₂-2 beads (0.172) was 3.37 times that of Ca(OH)₂ beads (0.051), showing a much higher increase of phosphorus removal efficiency.

15

The intraparticle diffusion kinetic model was then used to further analyze the specific adsorption process. As shown in Figures 3d and Table 2, three linear segments in PO₄-P adsorption processes by Ca(OH)₂ and CaO₂-2 beads were fitted, indicating that three different steps took place. The first stage was the external surface adsorption period when PO₄-P with a high initial concentration acted as the driving diffusion force. The PO₄-P in solution was chemically adsorbed on the beads surface in this process. The adsorption of PO₄-P by CaO₂-2 beads was rapidly completed within 10 min at a k_{d1} (mg·g⁻¹·min^{-1/2}) of 1.039 which was 1.77 times that of Ca(OH)₂ beads (0.586). The second adsorption phase was an intrapore diffusion process which was the limiting step for PO₄-P adsorption. Kinetics constants in the second stage became much slower than those in the first one (Table 2). The k_{d2} (mg·g⁻¹·min^{-1/2}) value (0.412) in CaO₂-2 beads system experienced a 60% decrease

25

- 33 -

compared with k_{d1} (1.039) while the decrease trend in $\text{Ca}(\text{OH})_2$ beads system was about 44%. The decreased k_{d2} values should be caused by the internal structural resistance of beads, in consistent with increased C values which indicated the mass transfer resistance. Since the fitting lines did not pass the zero point, the chemical surface adsorption also took place in the second stage. This implied that there were chemicals of CaO_2 encapsulated inside beads structure. The final phase is the equilibrium stage, of which the kinetic rates (k_{d3}) were quite slow due to the limitation of low phosphorus residual.

Adsorption isotherm was used to evaluate the adsorption capacity of CaO_2 -2 beads (q_e). As exhibited in Figure 5a, the q_e of CaO_2 -2 beads rapidly increased with higher concentrations of $\text{PO}_4\text{-P}$ and then reached a steady state. The Langmuir and Freundlich isotherm models (Figures 5b and 7c) were both used for fitting the experimental data and the corresponding isotherm parameters were obtained and showed in Table 4.

Table 4. Isotherm parameters of P adsorption by CaO_2 beads.*

Sample	Langmuir models			Freundlich models		
	R^2	k_L ($\text{L} \cdot \text{mg}^{-1}$)	q_m ($\text{mg} \cdot \text{g}^{-1}$)	R^2	k_F ($\text{mg} \cdot \text{g}^{-1}$)	n
CaO_2 beads	0.995	0.282	16.835	0.976	5.795	3.774

* Values of k_L and k_F represent the kinetic values fitted by the corresponding models.

The adsorption process of phosphorus on CaO_2 -2 beads is better fitted and interpreted by the Langmuir model than Freundlich model. It demonstrated that the phosphorus was bonded on the surface of CaO_2 -2 beads and formed a monolayer. Needed to be noticed is that the beads we used are in wet state and composed of over 99% water. The q_m value of $\text{PO}_4\text{-P}$ uptake by wet CaO_2 -2 beads was $16.84 \text{ mg} \cdot \text{g}^{-1}$, which was slightly higher than that obtained by talc encapsulated La-alginate ($16.4 \text{ mg} \cdot \text{g}^{-1}$).

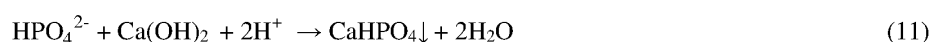
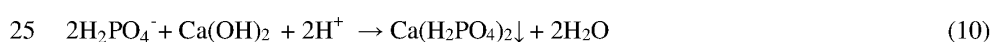
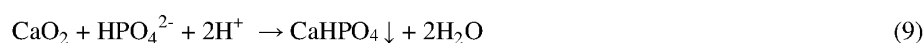
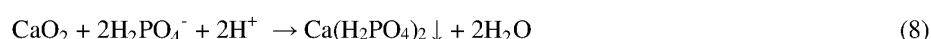
Phosphorus Removal Mechanism Analysis. According to the kinetic fitting analysis results, chemisorption took place between phosphorus and wet $\text{Ca}(\text{OH})_2$ and CaO_2 -2 beads. As shown in Figures 6a and 6b, the SEM images of $\text{Ca}(\text{OH})_2$ beads exhibited tight and thick

- 34 -

surface that was interspersed by some particles, which should be $\text{Ca}(\text{OH})_2$ particles (eq. 5) due to the existence of Ca and O elements. Unlike $\text{Ca}(\text{OH})_2$ beads, the surface of raw CaO_2 -2 beads is layered and three-dimensional (Figures 6c and 6d). Many uniform particles were evenly distributed on its surface. According to the results in Figure 2, the particles should be
5 mainly CaO_2 including some $\text{Ca}(\text{OH})_2$.

After being used for phosphorus removal, the small particles in raw beads disappeared and turned into a much tighter structure (Figures 6e and 6f), demonstrating that the chemical reaction between $\text{CaO}_2/\text{Ca}(\text{OH})_2$ and phosphorus occurred on the surface of CaO_2 -2 beads.
10 This is consistent with the disappearance of CaO_2 characteristic peaks on the XRD pattern of CaO_2 -2 beads after reacting with phosphorus (Figure 2). Furthermore, the EDS further confirms the presence of phosphorus on CaO_2 -2 beads after reaction.

In XPS spectra, the P 2p_{3/2} and P 2p_{1/2} peaks are observed at 129.7 and 131.2 eV on CaO_2
15 beads after the phosphorus adsorption, respectively. The presence of P 2p spectrum indicates that phosphorus was immobilized on functionalised beads. Two characteristic peaks appeared at 347.4 and 346.8 eV corresponding to CaHPO_4 (or $\text{Ca}(\text{H}_2\text{PO}_4)_2$) and $\text{Ca}(\text{OH})_2$ species, respectively. It suggests that PO_4 -P reacted with $\text{CaO}_2/\text{Ca}(\text{OH})_2$ species and formed CaHPO_4 or $\text{Ca}(\text{H}_2\text{PO}_4)_2$ (Eqs. 8-11). According to the result of MINEQL 5.0, HPO_4^{2-} and
20 H_2PO_4^- are the main two forms at pH 7.0, which could directly react with the calcium-based chemicals ($\text{Ca}(\text{OH})_2$ and CaO_2) to produce Ca-P compounds (eqs. 8-11).



Effect of Different Factors on Phosphorus Removal. The solution pH is an important factor since phosphorus may exist in different water circumstances, especially in some algae-laden scenarios or eutrophic water where the pH could rise above 9.0 due to the consumption
30 of CO_2 by algae growth. As shown in Figure 7a, the removal efficiency of phosphorus all

- 35 -

reached up to 97.2% within 20 min in a pH range from 3.0 to 10.0. The workable pH range of CaO₂-2 beads is much larger than that of the extensively studied La(OH)₃ or La₂(CO₃)₃-based material, of which the phosphorus removal efficiency decreased almost 50% when initial solution pH increased from 3.0 to 10.0. For the talc-encapsulated La-alginate beads, the phosphorus decreased almost 75% with pH increasing from 3.0 to 10.0. Nevertheless, the PO₄-P removal rate by CaO₂-2 beads increased with higher initial solution pH of 10.0 (Figure 7a). The superior performance of Ca-based materials to La-based ones at higher pH should be caused by the lower solubility product of calcium phosphate salts ($k_{sp, Ca_2(PO_4)_3} = 2.07 \times 10^{-33}$) than lanthanum phosphate salts ($k_{sp, La_2(PO_4)_3} = 1.0 \times 10^{-26.16}$). At initial pH around 3.0, phosphorus exists part (about 25%) of neutral molecular form of H₃PO₄ (Figure S7), which makes it harder to precipitate with Ca²⁺ compared to negative phosphorus at higher pHs. Even though the phosphorus removal rate decreased, the final phosphorus removal ratios were the same regardless of initial pH, indicating the excellent phosphorus removal performance by CaO₂-2 materials.

Figure 7b reveals the effect of common coexisting anions (Cl⁻, NO₃⁻, CO₃²⁻ and SO₄²⁻) on the removal of phosphorus by CaO₂-2 beads. The presence of common anions with a concentration as high as to 10 mM showed negligible effects on the immobilization of PO₄-P. It suggested that CaO₂-2 beads showed a good selectivity to phosphorus adsorption in simulated water.

Since phosphorus is a not renewable resource, its recovery and reuse has been an increasing and significant concern. The recovery ability of the used CaO₂-2 beads were tested and illustrated in Figure 7c. About 89.7% of phosphorus could be recovered from the used CaO₂-2 beads by using 0.5 M HCl while the desorption efficiencies by 0.5 M NaHCO₃ and NaOH were only 8.4% and 21.3%, respectively. This was consistent with the conclusion that phosphorus removal by CaO₂-2 beads was more favorable at higher pH value (Figure 7a). In addition, we can conclude that the prepared CaO₂-2 beads can be efficiently used for phosphorus removal and recovery by using HCl solution as the eluent and may be beneficial for being utilized in alkaline algae-containing environment due to its high PO₄-P removal at pH of 10.0.

HABs Removal by CaO₂-2 beads. To figure out the performance of wet CaO₂-2 beads on cyanobacteria removal, Ca(OH)₂ beads and H₂O₂ solution were used as comparison (Figure 8a). Algae started to proliferate within 1 day after being incubated into the fresh medium.

5 The addition of Ca(OH)₂ beads showed no inhibition but slight enhancement on algae growth. Even though the single lime addition has been reported to remove algae by forming flocculation, the required pH was quite high approaching 11.0. The initial pH in the experiment by beads was around 7.0 and thus no removal of algae was recorded by Ca(OH)₂ beads.

10

H₂O₂ is a widely used algaecide that is very effective to kill cyanobacteria by posing oxidative pressure to algae cells. The direct use of 0.06 mM H₂O₂ which was close to the steady molar concentration released by 2 g wet CaO₂-2 beads (Figure 8a) can only remove 15.70% of algae within 4 days. The algae grew back quickly after H₂O₂ was gradually
15 consumed, demonstrating that adding H₂O₂ is not a persistent method to remove algae. In contrast, wet CaO₂-2 beads of 1-4 g all exhibit excellent algae removal due to their continuous H₂O₂ release ability (Figure 1b), which were much superior to H₂O₂ solution and Ca(OH)₂ beads. In 6 h, about 80%, 93%, 98% and 99% of initial algae were removed with the CaO₂-2 beads dosage increasing from 1 g to 4 g. Within 24 h, almost 99.99% of algae
20 cell number was removed and the inhibition could last for quite a long time. The algae treated by 1 g beads started to regrow after 60 d. The algae did not grow back until 90th d with initially adding 2 g CaO₂-2 beads while dosage of 3 g or 4 g beads could inhibit algae growth for over 170 d (Figure 9). The cyanobacteria removal by H₂O₂ chemical with a concentration around 0.23 mM could last up to 21 d, which was much less than the duration by the CaO₂-
25 2 beads. Furthermore, even after such a long time for over 170 d, the basic structure of the beads was almost unchanged and still very stable. In all, the CaO₂-2 beads are very efficient in algae removal.

Besides the algae removal, the simultaneous adsorption of phosphorus by the CaO₂-2 beads
30 was also obtained (Figure 8b). The phosphorus removal ratio increased from 33% to 70% with higher dosage. Therefore, the long-time inhibition of algae regrowth might be attributed

- 37 -

to two reasons: 1) the continuous release of H_2O_2 by CaO_2 -2 beads kept providing oxidation pressure to algae; 2) the nutrient of phosphorus was adsorbed and then fast algal proliferation was inhibited. The pH of algae suspension just slightly changed after the addition of CaO_2 -2 beads (Figure 8c), meaning that the water circumstances could be kept quite stable even
5 with added beads.

To further evaluate the ability of CaO_2 -2 beads to remove algae of different concentrations, four initial algae cell numbers ranging from $(4.5\text{-}5.1) \times 10^6 \text{ cells}\cdot\text{mL}^{-1}$ to $(2.7\text{-}3.1) \times 10^7 \text{ cells}\cdot\text{mL}^{-1}$ were investigated (Figure 8d). Algae with different initial concentrations were all
10 completely removed in 2 days. It obviously indicates that CaO_2 -2 beads possess excellent performance for algae removal and can be used in many algae-laden scenarios with different concentrations.

Realistic Algae-contaminated Water Test. To figure out the feasibility of practical
15 application of CaO_2 -2 beads, realistic algae-laden water scenarios were taken and investigated in flasks and tanks. Even though the nitrogen (0.63 mg/L) and phosphorus ($< 0.05 \text{ mg/L}$) in this water was quite low (Table 5), the algae still grew very well probably due to the persistent release of nutrients from sediments. As shown in Figure 10a, for flask-scale experiments conducted in a lab incubator, algae could be totally removed within 1 d by wet
20 CaO_2 -2 beads with a dosage of 2 g or 4 g/ (50 mL) and the water pH changed slightly after the removal of algae.

Table 5. Water quality parameters of sampled algae-contaminated reservoir water.

Parameters	Values
pH	8.20~9.10
DOC (mg/L)	6.31
TC (mg/L)	16.40~22.71
IC (mg/L)	10.09~16.40
TN (mg/L)	1.52
$\text{NH}_4^+\text{-N}$ (mg/L) ^a	0.63
$\text{PO}_4\text{-P}$ (mg/L) ^b	< 0.05

- 38 -

SUV ₂₅₄ (L/(mg·m)) ^c	1.43~1.67
DO (mg/L) ^d	8.14~8.30
OD _{680nm} ^e	0.04

^aNH₄⁺-N was measured by ion chromatograph quipped with a cation system (Shimadzu, Co. Ltd., Japan).

^bPO₄-P and total phosphorus (TP) was measured by phosphorus test vials (TNT 843, HACH, USA).

5 ^cSpecific UV absorbance at 254 nm (SUV_{254nm}), obtained by dividing UV absorbance at 254 nm by DOC (mg/L) and then times 100.

^dDissolved oxygen, measured by a DO meter (HQ430D, HACH, USA).

^eOD_{680nm} was measured by a spectrometer (Jasco International Co., Ltd, V-660, Japan).

10 In a larger scale experiment conducted outdoor under sunlight with a tank filled with 60 L realistic water, 75% of algae could be removed within 1 d (Figure 10c), which was consistent with the disappearance of the absorbance peak at 680 nm (Figure 10d). Meanwhile, the water became much cleaner after being exposed to CaO₂-2 beads (Figures 1d and 11) and thus water solution absorbance in the range of 400-800 nm decreased obviously. This might be
15 caused by the removal of algae cells and the precipitation of their dead bodies. As shown in Figures 10e and 10f, the ratio of algae number compared to added florescent beads after 26 d was about 31% of the control. In fact, the low concentration of algae could be kept for over 67 d and the structure of the beads was still stable even after such a long time, which made
20 the beads easy to recover. The DOC and pH of the treated sample were also almost unchanged in the tank experiments compared to the control. Therefore, the realistic experimental results demonstrated that CaO₂-2 beads are promising in treating realistic algae-laden water without destroying the water circumstances.

Realistic Phosphate and/or Nitrogen species-contaminated Water Test. The
25 performance of beads in phosphate and nitrogen species removal was tested by using six kinds of realistic wastewater (RW) taken from different locations. The six different kinds of wastewaters were name as RW1, RW2, RW3, RW4, RW5, and RW6. Among the samples,

- 39 -

RW1, RW2 and RW3 were taken from a prawn-culture place, in which RW1 was the water in biological treatment field while RW2 and RW3 were waters for prawn growth at different stages; RW4 was the wastewater from a RO treatment tank in NUS; RW5 and RW6 were water samples from two different agricultural sites. As shown in Table 6, the prepared beads are very effective in PO₄-P removal in samples of RW3, RW4, RW5 and RW6 with 3.2 g wet beads. For sample of RW1, the initial PO₄-P concentrations were extremely high; thus, the beads' dosage was not enough to keep a high PO₄-P removal performance. For RW2, the initial PO₄-P was very low, it is difficult to be further removed due to the low PO₄-P ion reactivity. Clearly, Table 6 has demonstrated that the CaO₂ beads are effective for PO₄-P removal.

Table 6. Initial concentrations of waste in wastewater.

	RW1	RW2	RW3	RW4	RW5	RW6
PO ₄ -P (mg/L)	127.92	0.04	0.53	8.23	1.70	47.95
DOC (mg/L)	48.88	30.26	6.438	14.42	289	79.95
TN (mg/L)	10.9	0.5381	11.91	16.98	23.05	169.4
Nitrate (mg/L)			44.42	45.28	68.87	913.19
Ammonia (mg/L)			0.07	3.37	3.25	1.27

Table 7. Phosphate (PO₄-P) removal percentage from six types of realistic wastewaters.

Sample volume: 50 mL. Beads were used as their wet state.

Dose (g)	Time (h)	RW1	RW2	RW3	RW4	RW5	RW6
1.6	6	11.67%	38.05%	41.60%	71.10%	60.60%	6.90%
	36	7.46%	53.44%	46.54%	82.69%	77.35%	9.30%
3.2	6	19.37%	45.74%	86.32%	96.72%	70.41%	56.81%

- 40 -

36	13.48%	41.90%	88.64%	98.78%	86.40%	73.98%
----	--------	--------	--------	--------	--------	--------

Furthermore, we also tested the nitrate and ammonia removal from RW3-RW6 samples by beads. As shown in Table 8, nitrate and ammonia can be also partially removed. The nitrogen removal percentage ranged from 8% to 43% depending on the bead dosage and initial
 5 nitrogen species concentrations. In comparison, Fenton process has weak ammonia oxidation ability.

Table 8. Nitrate or ammonia removal from wastewater samples of RW3-RW6. Sample volume: 50 mL. Beads were used as their wet state. Units for concentrations in table: mg/L.

10

	Dosage (g)	Time (h)	RW3	RW4	RW5	RW6
Nitrate	0	0	44.42	45.28	68.87	913.19
		6	42.94	43.45	64.39	870.61
	1.6	36	41.81	42.16	57.46	844.02
		6	43.2	43.49	60.77	876.06
	3.2	36	41.84	42.09	56.7	846.4
Ammonia / ammonium	0	0	0.07	3.37	3.25	1.27
	3.2	36	0.04	2.61	2.56	0.89

At least 30% of ammonia (or ammonium) can be removed from leachate water (rich in organic carbon, with ammonia of 1.27 mg/L) with about 3.6 g of beads in about 50 mL of leachate water after about 36 hr. At least about 21% of ammonia (or ammonium) can be
 15 removed from agricultural water using 3.6 g beads in 50 mL after 36 hr. At least about 28% of ammonia can be removed from wastewater [mixture of fish and vegetable growth] using 1.6-3.2 g of beads in 50 mL, after 6 h.

TN was decreased as compared to the Fenton process which showed no change. Dissolved
 20 oxygen carbon (DOC) increased when beads were used. Alginate could be slowly dissolved into water.

Table 9. DOC and TN content in wastewater treatment

- 41 -

	Amount	RW1	RW2	RW3	RW4	RW5	RW6
DOC (mg/L)	0	48.88	30.26	6.438	14.42	289	79.95
	1.6 g	109.3	68.87	64.73	75.63	290.1	111
	3.2 g	162.7	94.93	126.3	112.7	304	118.8
TN (mg/L)	0	10.9	0.5381	11.91	16.98	23.05	169.4
	1.6 g	2.362	0.779	9.472	13.54	19.89	159.9
	3.2 g	1.312	0.7477	8.184	11.53	19.51	153.5
Fenton process							
TOC (mg/L)	before	48.88	30.26	6.438	14.42	289	79.95
	after	69.85	26.34	17.97	42.26	174	83.88
TN (mg/L)	before	10.9	0.5381	11.91	16.98	23.05	169.4
	after	10.74	0.9067	12.13	16.37	22.84	169.9

Mechanism Analysis. As shown in Figure 12, CaO_2 may be directly encapsulated in alginate beads (loop form) or cross-linked (linear form) with alginate due to the possible two structures of CaO_2 . On one hand, this CaO_2 -encapsulated bead shows an excellent performance for $\text{PO}_4\text{-P}$ removal through pathway (a). The $\text{PO}_4\text{-P}$ might be adsorbed in the beads or cross-linked with alginate due to the precipitation role of Ca^{2+} . About 97.2% of $\text{PO}_4\text{-P}$ could be efficiently removed by precipitation with Ca^{2+} within 20 min in a wide pH from 3.0-10.0, of which the range is much superior to the optimal pH range (commonly 3.0-7.0) of the extensively studied La-based materials. The adsorbed phosphorus can also be effectively recovered by elution with HCl solution.

On the other hand, the existence of CaO_2 particles ($0.44 \text{ mmol} \cdot \text{g}^{-1} \text{ -O-O-}$) in beads enabled a long-lasting period for simulated algae removal, which benefited from the steady and long-lasting H_2O_2 release forming a persistent oxidation pressure (Pathway (b)).

CONCLUSIONS

- 42 -

Simultaneous removal of phosphorus nutrient while controlling algae growth is a strategy for long-term algae bloom control from source which was previously not attempted. Although lots of methods have been developed to remove $\text{PO}_4\text{-P}$ or algae separately, there are few reported materials that can handle them simultaneously due to the need of both adsorption affinity to phosphorus and oxidation ability to algae in one material, which is challenging. The eco-friendly CaO_2 -encapsulated alginate beads were successfully in-situ synthesized in this work and has been demonstrated to be efficient for simultaneously removing phosphorus and algae. The $\text{PO}_4\text{-P}$ could be removed via a precipitation pathway while algae can be killed by the slowly-released H_2O_2 .

10

Besides the simulated algae-laden water, the synthesized beads were also effective for realistic algae-contaminated scenario. Due to the high LC_{50} values (2 g L^{-1})³⁰ of CaO_2 and the editable and biodegradable ability of alginate, the CaO_2 -alginate beads possess an eco-friendly property. Even though the evaluation of the possible effect of using the beads on the other organisms are needed, the CaO_2 -encapsulated beads have performed a promising and prosperous application in simultaneously removing phosphorus and algae in remediating realistic waterbodies.

15

Chemicals. All chemicals were analytically pure and used without further purification.

20

Hydrogen peroxide (H_2O_2 , 30wt.% in H_2O) was offered by Merck KGaA, Germany. Alginate acid sodium salt (~250 cp), NaOH , CaCl_2 , hydrochloric acid (HCl , 32wt.% in H_2O), sulfuric acid (H_2SO_4 , 95.0-98.0%), horseradish peroxidase (POD), N, N-diethylphenylenediamine (DPD), K_2CrO_4 , phosphoric acid (H_3PO_4 , $\geq 85\text{wt.}\%$ in H_2O), 1, 5-diphenylcarbazine (DPC), acetone, KMnO_4 , KH_2PO_4 , NaCl , NaNO_3 , Na_2CO_3 , Na_2SO_4 , NaHCO_3 , L-ascorbic acid, ammonium molybdate, potassium antimony(III) tartrate hydrate, and cyanobacterial BG-11 freshwater solution (C3061-500 mL) were purchased from Sigma Aldrich. Fluorescence beads ($2.0 \mu\text{m}$, yellow-green fluorescent, 2% solids) were obtained from Thermo Fisher.

25

30

The stock solution with a concentration of 100 mg/L phosphorus was prepared by KH_2PO_4 with an adjusted pH around 7.0. The ultrapure water ($18.25 \text{ M}\Omega\cdot\text{cm}$) from the water

- 43 -

purification system (Milli-Q-Gard® 2, Millipore Corporation) was used throughout the experimental process. BG-11 solution was 50 times diluted when used as algae culture medium. Diluted HCl and NaOH of 0.1 M was used for pH adjustment.

- 5 **In-situ Synthesis of CaO₂-Functionalized Beads.** The process is shown in Figure 13. Two kinds of solutions A and B were used to prepare different alginate beads (Table 1). Solution A with a total volume of 50 mL was composed of alginate acid sodium salt (2% w/v) and different concentrations of NaOH. Solution B with a volume of 100 mL was composed of 0.1 M CaCl₂ and different concentrations of H₂O₂. Alginate beads could be immediately
10 formed due to the cross-link effect by dropwise adding solution A with a syringe into solution B placed on a magnetic stirrer (400 rpm) at the room temperature. CaO₂ could be in-situ formed via eqs. (4)-(6) and encapsulated by beads. The formed beads were crosslinked for 4 h and stored in a cold room around 4°C after being washed with ultrapure water thoroughly. Five kinds of alginate beads of CaCl₂, Ca(OH)₂, CaO₂-0, CaO₂-1, and
15 CaO₂-2 were obtained with different chemical compositions (Table 1). The diameter of synthesized beads was around 3 mm and 99% of the bead's component was water.

- Phosphorus Removal and Desorption.** All solutions were adjusted to the desired pH before experiments. Wet beads were used and experiments were conducted in bottles with a volume
20 of 100 mL on a shaker with a speed of 200 rpm under ambient conditions. Samples were taken at fixed intervals and filtered through 0.45 µm syringe filters to analyze phosphorus concentration. Adsorption isotherm studies were conducted at pH 7.0 with different initial phosphorus concentration. pH 3.0, 7.0 and 10.0 were chosen to evaluate the pH impact.
- 25 Desorption experiments were conducted to reveal the phosphate adsorption mechanisms and recovery efficiency. Solutions of 0.5 M NaHCO₃, 0.5 M NaOH and 0.5 M HCl were chosen as eluents.

- Figure 14 shows the removal of phosphate by wet CaO₂-2 beads. [wet beads] = 40 g/L, [PO₄-
30 P] = 10, 25 and 50 mg/L.

- 44 -

Algae Culture and Removal in Lab and Realistic Scenarios. HABs removal experiments were conducted duplicate using lab-cultured cyanobacteria suspensions and realistic algae-contaminated reservoir water. In the lab experiments, cyanobacteria, *Microcystis aeruginosa*, was cultivated with autoclaved BG-11 medium in 2 L glass bottles under ambient conditions in an incubator equipped with fluorescent lamps (GPX-350BP, Yangzhou Huike Electronics Co., Ltd.). The bottles were covered by air breathing membranes. The light-dark cycle was set as 18 h/6 h. Optical density at 680 nm (OD_{680}) measured by a spectrophotometer (Shimazu, Japan, UV 1800) with a wavelength of 1 cm was used to prepare algae suspensions with consistent live cell numbers. The cell number quantified with microscope was $(4.5-5.1) \times 10^6 \text{ cells} \cdot \text{mL}^{-1}$ when OD_{680} was 0.1.

Microcystis aeruginosa removal experiments were conducted duplicate in 100 mL conical flasks. The autoclaved flasks were sterilized in biosafety cabinet (BSC) under UV irradiance for 40 min before use. Then, algae suspensions of 50 mL were prepared by diluting algae stock into desired OD_{680} . Algae suspensions with added beads or chemicals were placed in the incubator. Air-breathing membranes were covered on the top of flasks.

For realistic algae-contaminated water treatment by wet beads, both flasks and tank experiments were conducted using surface water sampled on a sunny noon in Singapore. Some parameters of the reservoir water are provided in Table 5. The raw water sample was well mixed each time before use. Experiments conducted in flasks were carried out in the incubator while experiments in tanks (Figure 15) were finished on a balcony of a building in National University of Singapore. Pumps and diffusers were used to aerate air for algae growth. Beads were held by mesh bags and the bags were hung on the top layer of the water (Figure 15).

Phosphorus Concentration Analysis and Adsorption Kinetics Fitting. The concentration of phosphorus was measured by the molybdate method. The reagent was prepared by mixing 300 mL of 50% v/v H_2SO_4 solution, 100 mL of 13% w/v ammonium molybdate tetrahydrate solution and 100 mL of 0.56% w/v potassium antimony(III) tartrate hydrate. Then 10 mL diluted phosphorus sample was mixed with 0.2 mL 10% w/v L-ascorbic acid solution and

- 45 -

0.4 mL reagent. After being incubated for 20 min, the absorbance of the solution with a blue color was measured at 700 nm by a spectrophotometer (HACH DR3900, USA). The detection limit for phosphorus was $0.01 \text{ mg}\cdot\text{L}^{-1}$. Different fitting models including pseudo-first-order, pseudo-second-order and intraparticle diffusion model were used to figure out the $\text{PO}_4\text{-P}$ removal process.

The release of Ca^{2+} in this process was measured by ion chromatography-optical emission spectrometer (ICP-OES, Optima 3000DV, Perkin Elmer). Calibration was prepared before each measurement.

10

Algae Cell Number Measurement. To measure the algae cell number, the sample suspension of 1 mL was first filtered through $60 \text{ }\mu\text{m}$ filter to remove the solids. For cyanobacteria measurement, the fluorescent beads of $10 \text{ }\mu\text{L}$ with 100 times dilution ($4.73\times 10^5 \text{ counts}\cdot\text{mL}^{-1}$) were added while for algae cell change in raw water, fluorescent beads of $10 \text{ }\mu\text{L}$ with 500 times dilution were used. The cell number could be determined by a flow cytometer (Cytoflex S, Beckman Coulter, USA) according to the ratio between algae cell numbers and known added fluorescent beads.

H_2O_2 Release and Detection. To evaluate the H_2O_2 release by synthesized beads, two different initial pHs of 6.0 and 9.0 were adopted. The experiments were carried out in plastic tubes placed on a shaker at a rate of 200 rpm. The quantitative measurements of H_2O_2 were obtained by a colorimetric method using DPD and POD at 551 nm. The water samples were first filtered through a $0.22 \text{ }\mu\text{m}$ syringe filter. Then one-time diluted sample solution was used for analyzing H_2O_2 concentration. H_2O_2 could oxidize DPD to pink DPD^{*+} catalyzed by POD. The absorbance was measured within $45 \pm 5 \text{ s}$.

Verification and Quantification of Total Peroxo Groups (-O-O-). Since peroxo groups in H_2O_2 have the ability to reduce Cr(VI) under acidic conditions, Cr(VI) reduction was studied to confirm the presence of -O-O-. The experiments were conducted in tubes on the orbital shaker at a rate of 200 rpm. Cr(VI) was measured by a colorimetric method. A

30

- 46 -

complex with pink color could be formed between Cr(VI) and DPC. The absorbance was measured after 15 minutes at a wavelength of 540 nm.

For the determination of total -O-O- in beads, chemical reduction of KMnO_4 by beads was done. Unused CaO_2 -2 beads of 1.0 g were added into 10 mL of 1.0 M H_3PO_4 solution and then shaken on the shaker for 4 h to dissociate CaO_2 to release -O-O-. KMnO_4 (0.1 mM) dissolved in 1.0 M H_2SO_4 solution was used to quantify the -O-O- amount according to pink color disappearance due to Eq. 7. The amount of -O-O- was calculated according to the consumption volume of KMnO_4 solution. Meanwhile, the UV-vis spectra were measured from 400 to 650 nm with a quartz cuvette.

Characterization. Different beads were dried in an oven at 105°C before being tested for characterization analysis. The crystal structure of different beads was obtained by the X-ray diffractometer (XRD, D/Max-3A, Rigaku.Co) at a voltage of 40 kV and a current of 30 mA with Cu $\text{K}\alpha$ radiation. To analyze the morphology structure and composition, a scanning electronic microscopy (SEM, ZEISS Merlin) equipped with an energy dispersive X-ray spectroscopy (EDS) was used. Chemical elemental compositions of beads were studied by the X-ray photoelectron spectroscopy (XPS) instrument (Kratos AXIS Ultra, Kratos Analytical Ltd.) with monochromatic Al $\text{K}\alpha$ radiation at voltage of 15 kV and current of 5 mA.

It will be appreciated that many further modifications and permutations of various aspects of the described embodiments are possible. Accordingly, the described aspects are intended to embrace all such alterations, modifications, and variations that fall within the spirit and scope of the appended claims.

Throughout this specification and the claims which follow, unless the context requires otherwise, the word “comprise”, and variations such as “comprises” and “comprising”, will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

- 47 -

Throughout this specification and the claims which follow, unless the context requires otherwise, the phrase "consisting essentially of", and variations such as "consists essentially of" will be understood to indicate that the recited element(s) is/are essential i.e. necessary elements of the invention. The phrase allows for the presence of other non-recited elements
5 which do not materially affect the characteristics of the invention but excludes additional unspecified elements which would affect the basic and novel characteristics of the method defined.

The reference in this specification to any prior publication (or information derived from it),
10 or to any matter which is known, is not, and should not be taken as an acknowledgment or admission or any form of suggestion that that prior publication (or information derived from it) or known matter forms part of the common general knowledge in the field of endeavour to which this specification relates.

- 48 -

Claims

1. A functionalised bead having a core and a surface, comprising:
 - a) peroxide moieties within the core of the bead; and
 - 5 b) peroxide particles adhered to the surface of the bead;wherein the core comprises anionic and hydrophilic polymers crosslinked with multivalent cations;
wherein the functionalized bead has an average diameter of about 0.2 mm to about 10 mm;
and
- 10 wherein the peroxide particles have an average diameter of about 10 nm to about 900 nm.
2. The functionalised bead according to claim 1, wherein the peroxide moieties within the core of the bead comprises peroxide particles.
- 15 3. The functionalised bead according to claims 1 or 2, wherein the concentration of the peroxide moieties and peroxide particles in the functionalised bead is about $0.3 \text{ mmol} \cdot \text{g}^{-1}$ to about $0.6 \text{ mmol} \cdot \text{g}^{-1}$.
4. The functionalised bead according to any one of claims 1 to 3, wherein the
- 20 multivalent cations are selected from a Group 2 metal, or transition metal.
5. The functionalised bead according to any one of claims 1 to 4, wherein the functionalised bead is further functionalised with alkaline moieties within the core or the surface of the bead.
- 25 6. The functionalised bead according to any one of claims 1 to 5, wherein the anionic and hydrophilic polymers is a polysaccharide.
7. The functionalised bead according to any one of claims 1 to 6, wherein the bead is at
- 30 least 97% hydrated with an aqueous medium.

- 49 -

8. The functionalised bead according to any one of claims 1 to 7, wherein H_2O_2 is releasable from the functionalised bead when dispersed in an aqueous medium, the H_2O_2 is releasable at a rate of about 0.03 mM/g/d to about 0.1 mM/g/d.
- 5 9. The functionalised bead according to any one of claims 1 to 8, wherein a pH of about 6.0 to about 9.0 is maintainable when the functionalised bead is dispersed in an aqueous medium.
- 10 10. The functionalised bead according to any one of claims 1 to 9, wherein the multivalent cation has a dissolution of less than about $1.4 \text{ mg} \cdot \text{L}^{-1}$.
- 15 11. The functionalised bead according to any one of claims 1 to 10, wherein the functionalised bead is for use in inhibiting algae growth, removing algae, disinfecting an aqueous medium, oxidizing organic compounds in an aqueous medium, oxidizing or removing low-valent inorganic compounds in an aqueous medium, or a combination thereof.
12. A method of removing phosphate phosphorus ($\text{PO}_4\text{-P}$) and/or algae from an aqueous medium, comprising:
- 20 a) dispersing the functionalised bead according to any one of claims 1 to 10 in the aqueous medium for at least 20 min in order to allow the $\text{PO}_4\text{-P}$ to bind onto the surface of the functionalised bead, and/or dispersing the functionalised bead in the aqueous medium for at least 4 h in order to release the peroxide from the core of the functionalised bead or from the peroxide particles into the aqueous medium for removing the algae.
- 25 13. The method according to claim 12, wherein the $\text{PO}_4\text{-P}$ binds onto the surface of the functionalised bead as a monolayer and is characterised by a Langmuir constant (k_L) for $\text{PO}_4\text{-P}$ adsorption of about $0.2 \text{ L} \cdot \text{mg}^{-1}$ to about $0.4 \text{ L} \cdot \text{mg}^{-1}$.
- 30 14. The method according to claim 12 or 13, wherein the functionalised bead has a pseudo second order rate constant (k_2) for $\text{PO}_4\text{-P}$ adsorption of about $0.15 \text{ g} \cdot \text{mg}^{-1} \cdot \text{min}^{-1}$ to about $2 \text{ g} \cdot \text{mg}^{-1} \cdot \text{min}^{-1}$.

- 50 -

15. The method according to any one of claims 12 to 14, having a $\text{PO}_4\text{-P}$ removal efficiency of more than about 95% within 40 min.
- 5 16. The method according to any one of claims 12 to 15, wherein the functionalised beads have a selectivity of more than 90% for $\text{PO}_4\text{-P}$ in the presence of at least one of Cl^- , NO_3^- , CO_3^{2-} , and SO_4^{2-} .
17. The method according to any one of claims 12 to 16, wherein the $\text{PO}_4\text{-P}$ bonded on
10 the surface of the functionalised bead is at least 80% recoverable when reacted with an acid.
18. The method according to any one of claims 12 to 17, wherein a pH of about 6.0 to about 9.0 is maintainable.
- 15 19. The method according to any one of claims 12 to 18, wherein the functionalised bead has an algacide and/or inhibition efficiency of more than about 80% after 6 h.
20. The method according to any one of claims 12 to 19, wherein at least about 5 mg/L of $\text{PO}_4\text{-P}$ is removed from the aqueous medium when the functionalised bead is characterised
20 by a loading of about 1 to 4 g/50-mL of aqueous medium.
21. The method according to any one of claims 12 to 20, wherein at least about 4×10^6 cells/mL of algae is removed from the aqueous medium when the functionalised bead is characterised by a loading of about 1 to 4 g/50-mL of aqueous medium.
25
22. The according to any one of claims 12 to 21, wherein the functionalised bead is dispersed at or near the top of the aqueous medium.
23. A method of inhibiting the accumulation of $\text{PO}_4\text{-P}$ and/or growth of algae in an
30 aqueous medium, comprising:

- 51 -

a) dispersing the functionalised bead according to any one of claims 1 to 10 in the aqueous medium for at least 20 min.

24. A method of fabricating a functionalised bead having a core and a surface,
5 comprising:

a) contacting an anionic and hydrophilic polymer solution with a solution comprising a multivalent cation and a peroxide in order to form the core of the bead; and

b) cross-linking the core of the bead of step a) under a predetermined condition in order to grow the core and form the surface of the bead;

10 wherein the core comprises anionic and hydrophilic polymers crosslinked with the multivalent cation;

wherein the peroxide is encapsulated within the core of the bead as peroxide moieties;

wherein peroxide is adhered to the surface of the bead as peroxide particles;

wherein the functionalized bead has an average diameter of about 0.2 mm to about 10 mm;

15 and

wherein the peroxide particles have an average diameter of about 10 nm to about 900 nm.

25. The method according to claim 24, wherein the polymer solution is added drop-wise into the solution under constant agitation.

20

26. The method according to claim 24 or 25, wherein the polymer solution is a sodium alginate solution.

27. The method according to any one of claims 24 to 26, wherein the polymer solution
25 has a concentration of about 2% w/v.

28. The method according to any one of claims 24 to 27, wherein the polymer solution further comprises an alkaline.

30 29. The method according to claim 28, wherein the alkaline in the polymer solution is about 0.1 M to about 0.5 M.

- 52 -

30. The method according to any one of claims 24 to 29, wherein the multivalent cation in the solution is selected from Ca^{2+} , Zn^{2+} , Mg^{2+} , Cu^{2+} , Ce^{3+} , Fe^{3+} , La^{3+} or a combination thereof at about 0.05 M to about 0.5 M.

5

31. The method according to any one of claims 24 to 30, wherein the peroxide in the solution comprises a metal salt and hydrogen peroxide (H_2O_2).

32. The method according to claim 31, wherein the hydrogen peroxide at about 0.05 M
10 to about 0.5 M.

33. The method according to any one of claims 24 to 32, wherein the core of the beads is cross-linked for at least 4 h.

15 34. A method of removing nitrate and/or ammonia in an aqueous medium, comprising:
a) dispersing the functionalised bead according to any one of claims 1 to 10 in the aqueous medium for at least 4 h in order to allow the nitrate and/or ammonia to bind onto the surface of the functionalised bead.

20 35. The method according to claim 34, wherein at least about 20% of ammonia is removed from the aqueous medium when the functionalised bead is characterised by a loading of about 1 to 4 g/50-mL of aqueous medium.

36. The method according to any one of 34 or 35, wherein at least about 5% of nitrate is
25 removed from the aqueous medium when the functionalised bead is characterised by a loading of about 1 to 4 g/50-mL of aqueous medium.