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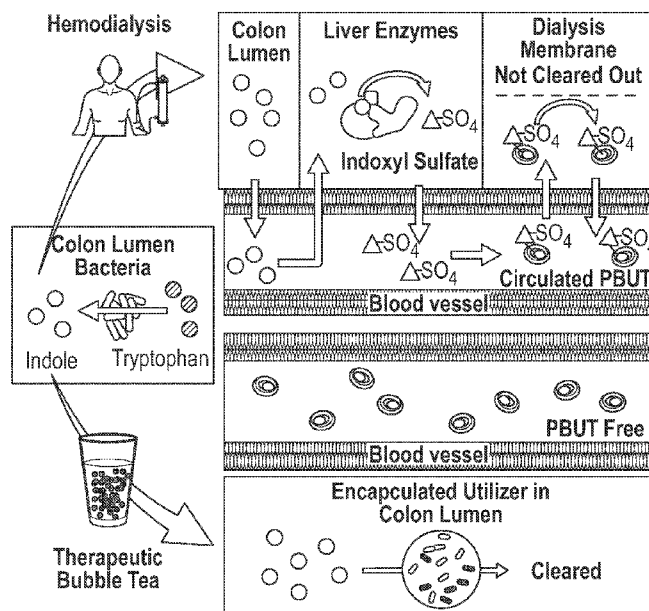


FIG. 1

(57) Abstract: Compositions, kits, and methods for removal of one or more toxins and/or one or more toxin precursors from a subject's digestive system. A composition for ingestion by the subject includes hydrogel particles that include agents encapsulated therein. The agents are configured to reduce local concentrations of one or more toxins and/or one or more toxin precursors from the subject's digestive system with ingestion of the composition. Compositions can include foods or beverages, such as bubble tea beverages, that are functional for removal of toxins and/or precursors from the digestive system.

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- *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

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TOXIN REMOVAL FROM THE GUT WITH GEL ENCAPSULATED MICROORGANISMS

CROSS-REFERENCE(S) TO RELATED APPLICATION(S)

[0001] This application claims priority to, and the benefit of, U.S. Provisional Patent Application No. 63/508,451, filed June 15, 2023; U.S. Provisional Patent Application No. 63/642,086, filed May 03, 2024; U.S. Provisional Patent Application No. 63/651,198, filed May 23, 2024; the contents of which are hereby incorporated by reference in their entirety for all purposes.

STATEMENT OF GOVERNMENT LICENSE RIGHTS

[0002] This invention was made with government support under DE-SC0020356 awarded by the Department of Energy, 1R01DK130815-01 awarded by the National Institutes of Health, and HR0011-17-2-0064 awarded by the Defense Advanced Research Projects Agency. The government has certain rights in the invention.

BACKGROUND

[0003] Chronic kidney disease (CKD) is a public health problem, affecting 850 million people worldwide, and is the 10th leading cause of death in the U.S. Loss of kidney function is a health condition that increases the risk of cardiovascular disease, the leading cause of death in the U.S. CKD is commonly associated with bone and mineral disorders (referred to as CKD-MBD) which is a systemic disorder of mineral and bone metabolism. CKD-MBD results in increased risks for fractures, osteoporosis, vascular calcification and cardiovascular disease. Hyperphosphatemia is considered a contributing factor to CKD-MBD, and is caused primarily by an inability of kidney excretory function to maintain phosphate homeostasis. CKD-MBD is also associated with hormonal abnormalities, including hyperparathyroidism, diminished calcitriol synthesis, and increased circulating FGF-23 concentrations, each of which can contribute to the observed increased morbidity and mortality risks. Hyperphosphatemia in individuals with CKD is commonly treated with prescribed dietary phosphate binders (such as calcium carbonate, calcium acetate, lanthanum carbonate and sevelamer hydrochloride). However, these agents only bind a modest portion of dietary phosphate, are associated frequently with gastrointestinal side effects, change the human microbiome, and require the intake of

many pills per meal. New and improved phosphate mitigation strategies are needed to prevent and treat hyperphosphatemia effectively.

[0004] Dialysis is the leading therapy when kidneys lose their capacity to remove toxins from the blood, however, it fails to fully fulfill the function of healthy kidneys. Small solutes (such as urea and creatinine) are removed from the blood with a dialysis treatment. However, dialysis is a costly treatment that decreases the life quality of a patient significantly, and there are no effective alternatives on the market. In addition, dialysis is not effective at clearing protein-bound molecules that accumulate in the blood and lead to a myriad of problems such as cardiovascular disease and death. For example, protein-bound uremic toxins (PBUTs) such as indoxyl sulfate and para-cresol sulfate, are not effectively removed by dialysis because they bind tightly to plasma albumin and remain on the blood side of the dialysis membranes. The build-up of PBUTs in the plasma has repeatedly been related to cardiovascular morbidity, and mortality in patients with chronic kidney disease (CKD) and end stage renal disease (ESKD).

[0005] CKD is commonly associated with bone and mineral disorders (referred to as CKD-MBD) which is a systemic disorder of mineral and bone metabolism. CKD-MBD results in increased risks for fractures, osteoporosis, vascular calcification and cardiovascular disease. Hyperphosphatemia is considered a contributing factor to CKD-MBD, and is caused primarily by an inability of kidney excretory function to maintain phosphate homeostasis. CKD-MBD is also associated with hormonal abnormalities, including hyperparathyroidism, diminished calcitriol synthesis, and increased circulating FGF-23 concentrations, each of which can contribute to the observed increased morbidity and mortality risks. Hyperphosphatemia in individuals with CKD is commonly treated with prescribed dietary phosphate binders (such as calcium carbonate, calcium acetate, lanthanum carbonate and sevelamer hydrochloride). However, these agents only bind a modest portion of dietary phosphate, are associated frequently with gastrointestinal side effects, change the human microbiome, and require the intake of many pills per meal. New and improved phosphate mitigation strategies are needed to prevent and treat hyperphosphatemia effectively.

[0006] Dialysis is the leading therapy when kidneys lose their capacity to remove toxins from the blood, however, it fails to fully fulfill the function of healthy kidneys. Small solutes (represented by urea and creatinine) are removed from the blood with a dialysis treatment. However, dialysis is a costly treatment that decreases the life quality

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[0007] Accordingly, there is a need for improved approaches for removal of toxins from the body that accumulate due to kidney diseases and disorders. The present disclosure addresses these and other long-felt and unmet needs in the art.

SUMMARY

[0008] This summary is provided to introduce a selection of concepts in a simplified form that are further described below in the Detailed Description. This summary is not intended to identify key features of the claimed subject matter, nor is it intended to be used as an aid in determining the scope of the claimed subject matter.

[0009] In an aspect, the disclosure provides a composition for ingestion by a subject and removal of one or more toxins and/or one or more toxin precursors from the subject's digestive system, the composition comprising: hydrogel particles comprising agents encapsulated within the hydrogel particles, wherein the agents are configured to reduce local concentrations of the one or more toxins and/or the one or more toxin precursors from the subject's digestive system.

[0010] In embodiments, the composition is formulated as an ingestible bubble tea composition and the hydrogel particles are configured as gel beads of the bubble tea composition.

[0011] In embodiments, the composition is formulated as an ingestible bubble tea composition that comprises larger digestible boba particles that contain smaller hydrogel particles therein that comprise at least a portion of the agents therein.

[0012] In embodiments, the larger digestible boba particles further contain one or more probiotic organisms and/or one or more prebiotic substances.

[0013] In embodiments, the larger digestible boba particles are comprised of one or more colloids configured to protect the one or more probiotic organisms, the one or more prebiotic substances, and the smaller hydrogel particles from stomach acid.

[0014] In embodiments, the one or more colloids comprises a gum, a thickener, xanthan gum, carrageenan, one or more mannans, one or more glucans, agar, or any combination thereof.

[0015] In embodiments, the hydrogel particles are configured for: removal of multiple different toxins, and/or removal of multiple different toxin precursors, from the subject's digestive system.

[0016] In embodiments, at least a portion of the agents comprises phosphate-accumulating organisms (PAOs), wherein the PAOs are denitrifying PAOs (dPAOs) and/or are configured for enhanced biological phosphate removal (EBPR PAOs).

[0017] In embodiments, at least a portion of the agents comprises a heterotrophic denitrifier, an autotrophic denitrifier, one or more organisms configured for aerobic removal of one or more aromatics, or any combination thereof.

[0018] In embodiments, at least a portion of the agents comprises Comamox bacteria, ammonium oxidizing bacteria, ammonium oxidizing archaea, nitrite oxidizing bacteria, autotrophic denitrifiers, Anammox bacteria, iron oxidizers, heterotrophic denitrifiers, denitrifying PAOs, or any combination thereof.

[0019] In embodiments, at least a portion of the agents comprises fermenting bacteria configured to convert fibrous material to short chain fatty acids (SCFAs).

[0020] In embodiments, at least a portion of the hydrogel particles comprises a fibrous material selected from the group consisting of: a cellulosic material, a lignocellulosic particle, a chia seed, bagasse, flax, hemp, jute, ramie, a quinoa seed, kenaf, and any combination thereof.

[0021] In embodiments, at least a portion of the hydrogel particles comprises a metal catalyst selected from the group consisting of: iron, copper, manganese, or any combination thereof, to function as an electron carrier or electron donor/acceptor.

[0022] In embodiments, a PBUT degrader is included as at least one agent and is selected from the group consisting of: *Shewanella oneidensis*, *Geobacter sulfurreducens*, *Desulfobacula indolicum*, *Thauera aminoaromatica*, *Geobacter metallireducans*, *Pseudomonas putida*, and any combination thereof.

[0023] In embodiments, a PAO is included as at least one agent and is selected from the group consisting of: *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Lactobacillus acidophilus*, *Lactobacillus coryniformis*, *Lactobacillus brevis*, *Bifidobacterium longum*, *Bifidobacterium adolescentis*, *Bifidobacterium breve*, and *Lactiplantibacillus paraplantarum*, *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, *Lactobacillus delbrueckii*, *Tetrasphaera japonica*, *Tetrasphaera elongata*, *Tetrasphaera australiensis*, *Tetrasphaera vanveenii*, *Tetrasphaera veronensis*, *Microthunatus phosphovoratus*, and any combination thereof.

[0024] In embodiments, hydrogel particles of the portion of the hydrogel particles comprise oxygen precursors and enzymes configured for conversion of the oxygen precursors into at least oxygen (O₂) for use of the O₂ as an electron acceptor by one or more PAOs, one or more PBUT degraders, and/or one or more nitrifiers.

[0025] In embodiments, the oxygen precursors are hydrogen peroxide (H₂O₂) molecules, and the enzymes are catalase enzymes.

[0026] In embodiments, the H₂O₂ molecules are encapsulated within inner cores of the hydrogel particles for release from the inner cores and reaction with the catalase enzymes.

[0027] In embodiments, at least a portion of the hydrogel particles comprises nitrate, nitrite, or both, for use as electron acceptors by one or more PAOs, one or more PBUT degraders, and/or one or more nitrifiers.

[0028] In embodiments, at least a portion of the agents comprises aerobic and anaerobic bacteria configured for: conversion of urea and protein-bound uremic toxins (PBUTs) to nitrite; and conversion of nitrite to nitrogen (N₂) and/or carbon dioxide (CO₂) with use of organic carbon, hydrogen sulfide (H₂S), and/or ammonium as one or more electron donors.

[0029] In embodiments, at least a portion of the agents comprises aerobic ammonium oxidizing bacteria (AOB), aerobic ammonium oxidizing archaea (AOA), nitrite oxidizing bacteria (NOB), anaerobic ammonium oxidizing bacteria (Annamox), or any combination thereof.

[0030] In embodiments, at least a portion of the agents comprises a nitrifying *Nitrospira*, *Nitrobacter*, *Nitrosomonas*, *Nitrospinae*, *Nitosococcus*, *Nitrococcus*, *Nitrososphaera viennensis*, *Nitrosospira lacus*, *Nitrosomonas ureae*, *Nitrosomonas nitrosa*, *Nitrospira muscovinensis*, *Nitrososphaera viennensis*, *Nitrosotennuis aquarius*,

Nitrosopumilus maritimus, *Nitrosarchaeum koreense*, a bacterium configured for denitrification, *Clostridium spp.*, *Thiobacillus denitrificans*, *Micrococcus denitrificans*, a species of *Serratia*, *Pseudomonas*, *Achromobacter*, or any combination thereof.

[0031] In embodiments, at least a portion of the agents comprises complete ammonia oxidizing (Comammox) bacteria, anaerobic ammonium oxidizing (Anammox) bacteria, autotrophic and heterotrophic prokaryotic and eukaryotic denitrifiers, or any combination thereof.

[0032] In embodiments, at least a portion of the Anammox bacteria comprises: *Candidatus Brocadia*, *Candidatus Kuenenia*, *Candidatus Anammoxoglobus*, *Candidatus Jettenia*, *Candidatus Scalindua*, or any combination thereof.

[0033] In embodiments, at least a portion of the agents comprises one or more SCFAs bacteria selected from the group consisting of: *Faecalibacterium prausnitzii*, *Eubacterium rectale*, *Roseburia inulinivorans*, *Roseburia intestinalis*, *Anaerostipes butyraticus*, *Anaerostipes caccae*, *Butyricicoccus pullicaecorum*, *Lactococcus lactis*, *Leuconostoc*, *Pediococcus*, *Bifidobacterium*, *Enterococcus*, *Lactocaseibacillus casei*, *Lactobacillaceae*, *Lachnospiraceae*, *Ruminococcus*, *Streptococcus thermophilus*, *Peptostreptococcaceae*, *Oenococcus oeni*, *Lactobacillus brevis*, *Limosilactobacillus fermentum*, *Lactobacillus helveticus*, *Lactobacillus delbrueckii subsp. bulgaricus*, *Propionibacterium freudenreichii*, *Bifidobacteriaceae*, *Propionibacterium*, *Hafnia*, *Gemella*, *Arthrobacter*, *Bifidobacterium animalis*, and any combination thereof.

[0034] In embodiments, at least a portion of the agents comprises a fungi selected from the group consisting of: *Saccharomyces*, *Aspergillus*, *Galactomyces*, *Geotrichum*, *Saprochaete*, *Candida*, *Malassezia*, a *Fusarium oxysporum* strain, *Cylindrocarpon tonkinense*, *Cladosporium*, and any combination thereof.

[0035] In embodiments, at least a portion of the agents comprises a methanogen selected from the group consisting of: *Methanobacteria*, *Ca. Methanofastidiosum*, *Methanosaeta*, *Methanobacterium*, *Methanosarcina*, *Methanococcus*, *Methanospirillum*, *Methanolinea*, and any combination thereof.

[0036] In embodiments, at least a portion of the agents comprises urease, urease-releasing microbes, or any combination thereof.

[0037] In embodiments, at least a portion of the agents comprises one or more ammonia binders configured for ion-exchange removal of ammonia.

[0038] In embodiments, the one or more ammonia binders comprises one or more zeolites configured for removal of one or more toxins and/or one or more toxin precursors.

[0039] In embodiments, at least a portion of the hydrogel particles comprises one or more phosphate binders.

[0040] In embodiments, the one or more phosphate binders comprises ferric oxyhydroxide.

[0041] In embodiments, at least a portion of the hydrogel particles comprises iron cross-linked alginate.

[0042] In embodiments, at least a portion of the hydrogel particles comprises iron cross-linked alginate and ferric oxyhydroxide.

[0043] In embodiments, at least a portion of the hydrogel particles comprises iron cross-linked alginate or iron cross-linked alginate and ferric oxyhydroxide with a coating of chitosan which minimizes release of iron and thus avoids associated side effects.

[0044] In embodiments, at least a portion of the hydrogel particles comprises a net electrostatic charge at cores of the hydrogel particles such that the hydrogel particles are pH responsive.

[0045] In embodiments, the composition further comprises a vitamin, a cofactor, zinc, iron, calcium, bismuth subsalicylate, iron hydroxide, or any combination thereof.

[0046] In embodiments, at least a portion of the hydrogel particles is active at an alkaline pH and comprises one or more shell layers configured to: prevent the release of cells entrapped near a surface of the hydrogel particles, and provide a pH-responsive barrier to diffusion.

[0047] In embodiments, at least a portion of the hydrogel particles is active at an acidic pH and comprises one or more phosphate binders and swells at acidic pH levels and contracts at neutral pH levels.

[0048] In embodiments, at least a portion of the hydrogel particles comprises a coating that protects the hydrogel particles from degradation in the stomach of the subject for delivery of protected hydrogel particles to the small intestine, the colon, or both, for removal of one or more toxins and/or one or more toxin precursors from the small intestine, the colon, or both.

[0049] In embodiments, at least a portion of the hydrogel particles are comprised of a plurality of different-sized hydrogel particles for reduced diffusion constraints with

smaller hydrogel particles and increased diffusion constraints with larger hydrogel particles.

[0050] In embodiments, at least a portion of the hydrogel particles are comprised of a plurality of different-sized hydrogel particles for a greater anaerobic volume fraction with larger sizes and a greater aerobic volume fraction with smaller sizes.

[0051] In embodiments, the composition further comprises one or more palatable agents for increased palatability of the composition.

[0052] In an aspect, the disclosure provides a kit comprising a composition and an instructional material configured for direction of use of the kit in a method of treating or preventing onset or progression of one or more diseases or disorders.

[0053] In embodiments, the one or more diseases or disorders comprises kidney disease or chronic kidney disease.

[0054] In an aspect, the disclosure provides a method of treating or preventing onset or progression of one or more diseases or disorders, the method comprising administering a composition to a subject in need thereof.

[0055] In embodiments, the one or more diseases or disorders comprises kidney disease or chronic kidney disease.

[0056] In an aspect, the disclosure provides a use of a composition in the preparation of a medicament for administration to a subject for treating or preventing onset or progression of one or more diseases or disorders in the subject.

DESCRIPTION OF THE DRAWINGS

[0057] The foregoing aspects and many of the attendant advantages of this disclosure will become more readily appreciated as the same become better understood by reference to the following detailed description, when taken in conjunction with the accompanying drawings.

[0058] FIG. 1 shows how protein-bound uremic toxins (PBUTs), such as indoxyl sulfate and para-cresol sulfate, are not effectively removed by dialysis because they bind tightly to plasma albumin and remain on the blood side of the dialysis membranes. The disclosure provides approaches that use encapsulated bacteria to provide the metabolisms needed to degrade uremic toxins, such as p-cresol and indole, before they can be absorbed into the blood and become PBUTs, according to aspects of the disclosure.

[0059] FIG. 2 shows an example overview of the composition of the hydrogel particles, containing or at least partially comprising engineered particles, according to aspects of the disclosure.

[0060] FIG. 3 shows an example overview of the principle of technology used for the therapeutic bubble tea, wherein the toxins shown in the left column are removed by the possible mechanisms shown in the middle column via pH responsive gels shown in the right column to protect cargo throughout trip of the gut, according to aspects of the disclosure.

[0061] FIG. 4 shows an example result showing an overview of a multifunctional hydrogel bead, wherein the hydrogel encapsulates both a source of oxygen and microbes to degrade urea, phosphate, and aromatics to then be excreted intact from the patient, and that for anaerobic microbes, the oxygen supplying core is removed, according to aspects of the disclosure.

[0062] FIG. 5 shows phosphate removal through chemical binder and phosphate accumulating organisms encapsulated in hydrogel, according to aspects of the disclosure.

[0063] FIG. 6 shows anaerobic (left) and aerobic PAO (right) metabolism in enhanced biological phosphate removal (EBPR) of phosphate-accumulating organisms (PAOs), according to aspects of the disclosure.

[0064] FIG. 7 shows a schematic of a hydrogel bead encasing p-cresol oxidizing iron reducing bacteria with oxidized Fe(III) oxide particles, wherein the bacteria can consume and oxidize the p-cresol that diffuses into the hydrogel bead and can use the electrons to reduce the oxidized iron particles to the more reduced state of Fe(II) oxide particles; the Fe(II)oxide end-product and the bacteria remain within the hydrogel as it passes through the digestive system, according to aspects of the disclosure.

[0065] FIG. 8 shows a conceptual overview of an example proposed solution to lower urea and uremic toxins precursors from the gut, according to aspects of the disclosure. The stable hydrogel can encapsulate both a source of oxygen and bacteria to degrade urea, p-cresol, and indole, then be excreted intact from the patient. For anaerobic bacteria, the oxygen supplying core may not be used.

[0066] FIG. 9 shows metabolisms of different nitrifiers, according to aspects of the disclosure.

[0067] FIG. 10 shows an example of how Comammox and anaerobic ammonium oxidizing bacteria (Anammox) can be concomitantly grown in hydrogels for ammonia or urea removal to N_2 , according to aspects of the disclosure.

[0068] FIG. 11 shows an example diagram indicating that entrapping hydrogen peroxide reservoirs in microcapsules within the inner core of hydrogel particles enables controlled diffusion outward toward aerobic organisms, according to aspects of the disclosure.

[0069] FIG. 12 shows an example of oxygen transport via H_2O_2 in hydrogels, according to aspects of the disclosure. Small beads are fully oxygenated while bigger beads allow anaerobic processes to occur.

[0070] FIGs 13A-13B show an example overview of (FIG. 13A) fiber and fermented bacteria encapsulated within hydrogel to deliver valuable short chain fatty acids for gut health; and (FIG. 13B) fiber and fermented bacteria, coupled with denitrifiers, to eliminate the residual of nitrate being used for toxin removal process, according to aspects of the disclosure.

[0071] FIG. 14 shows an example result showing that the level of phosphate uptake by *Lactobacillus casei* encapsulated in hydrogels in response to pH changes from 3 to 6.5, according to aspects of the disclosure.

[0072] FIG. 15 shows an example result showing the level of phosphate removal by *Tetrasphaera japonica* under aerobic condition, according to aspects of the disclosure.

[0073] FIG. 16 shows an example result showing an image of *Lactobacillus Casei*-encapsulated hydrogel under a stereomicroscope, according to aspects of the disclosure.

[0074] FIG. 17 shows an example result showing a fluorescence *in situ* hybridization image of *Lactobacillus Casei*-encapsulated hydrogel indicating that *Lactobacillus Casei* (shown in white) was well-distributed throughout the hydrogel, according to aspects of the disclosure.

[0075] FIG. 18 shows an example result from comparison of different chemical ammonium adsorbents, according to aspects of the disclosure. 100 mg mass of adsorbents in 50 mL equal to 2 g /L adsorbents, Ammonium 26 mg/L , 37°C, 110 RPM, pH 5.6. Natural Zeolite, KW8 cation exchange resin, MN500 modified Zeolite. Maximum ammonium adsorption (binding capacity) for the natural Zeolite was measured as 9.7 mg NH_4^+ /g of Zeolite.

[0076] FIG. 19 shows an example pretreatment of Zeolite with sodium chloride, according to aspects of the disclosure. NaZeolite increased Ammonium removal efficiency of Zeolite (15-20 % during the time), suggesting that Na exchange plays a role ammonium removal efficiency; 100 mg mass of adsorbents in 50 mL (2 g/L) Ammonium 50 mg/L , 37°C, 110 RPM, pH:5.6.

[0077] FIG. 20 shows results evaluating the effect of pH on ammonium adsorption for NaZeolite and KW8 ion exchange resin, according to aspects of the disclosure. KW8 shows minimal adsorption at pH 3 while Zeolite adsorption activity is increasing. The optimum adsorption for both ammonium binders occurs between pH 5 and 6.

[0078] FIG. 21 shows an example result for phosphate absorption efficiency of three alginates of different molecular weights that were cross-linked by AlCl_3 or FeCl_3 , according to aspects of the disclosure.

[0079] FIG. 22 shows an example schematic of the preparation of chitosan-coated iron alginate gel beads, according to aspects of the disclosure.

[0080] FIG. 23 shows an example effect of chitosan coating on the release of iron from FeCl_3 alginate beads, and that the chitosans of all three molecular weights tested substantially reduced the loss of iron from the gel beads at pH 3, according to aspects of the disclosure.

[0081] FIG. 24 shows an example effect of pH on phosphate removal efficiency by iron oxyhydroxide (FeOOH) powder, according to aspects of the disclosure.

[0082] FIG. 25 shows an example schematic of the preparation of chitosan and carrageenan double coated iron alginate gel beads (top) and the images of some of the alginate gel beads (bottom), according to aspects of the disclosure.

[0083] FIG. 26 shows an example comparison of the overall phosphate removal of three versions of iron alginate beads made of 3% alginate (SA3) cross-linked by FeCl_3 in an assay that mimicked the trip of material through the GI tract. Inclusion of FeOOH in the iron alginate beads enhances phosphate absorption by ~48% compared to iron alginate alone, according to aspects of the disclosure.

[0084] FIG. 27 shows an example of urea nitrogen removal in an ammonia oxidizing bacteria-Anammox bead bioreactor on hydrogen peroxide. Dashed: H_2O_2 total removed; straight line: urea ammonia oxidized with H_2O_2 . No external oxygen was

added, but all O₂ was derived from O₂ produced from catalase derived hydrolysis of H₂O₂ to O₂ and H₂O, according to aspects of the disclosure.

[0085] FIG. 28 shows an example result showing the ammonia-oxidizing bacteria performed urea hydrolysis to form ammonium followed by its oxidation to nitrite, according to aspects of the disclosure.

[0086] FIG. 29 shows an example result showing simultaneous nitrification and denitrification by the use of a combination of denitrifying strains to create cross-feeding networks in the hydrogels, according to aspects of the disclosure. The denitrifiers simultaneously reduced nitrate and nitrite as they were produced during the nitrification process.

[0087] FIG. 30 shows an example result showing the aerobic zone volume per unit mass of hydrogel beads for varying hydrogel bead sizes, according to aspects of the disclosure.

[0088] FIG. 31 shows an example of *Desulfobacula indolicum* encapsulated in PEGDMA hydrogel beads, with use of sulfate as electron acceptor by degrading indole, a uremic toxin precursor, according to aspects of the disclosure.

[0089] FIG. 32 shows an example image of a PVA/SA hydrogel bead containing *T. aminoaromatica* (left) and a cross-section light (middle) and SYBR Green-stained fluorescence (right) images of a hydrogel bead containing *T. aminoaromatica*, according to embodiments of the disclosure.

[0090] FIG. 33 shows an example result showing that the cells in the planktonic culture displayed a lag phase when presented with p-cresol, whereas the lag was avoided by encapsulating the *T. aminoaromatica* cells in hydrogel particles, according to aspects of the disclosure.

[0091] FIG. 34 shows an example of microbial sink of p-cresol, a uremic toxin precursor, by a hydrogel encapsulated *Thauera aminoaromatica* S2 (open circles) compared to that of a culture not encapsulated (filled circles), according to aspects of the disclosure.

[0092] FIG. 35 shows an example image of the non-magnetic, insoluble Fe(III)oxide before the inoculation of *Geobacter metallireducans* (left) and the image of the magnetic, insoluble Fe(II) oxide after the inoculation and growth of *Geobacter metallireducans* (right), according to aspects of the disclosure.

[0093] FIG. 36 shows an example experimental setup for the Ferrozine assay of reduced iron (Fe(II)) in inoculated or uninoculated tubes of Fe(III) oxides, according to aspects of the disclosure.

[0094] FIG. 37 shows an example result showing a fluorescent *in situ* hybridization image of a hydrogen with oxygen penetrated from the core, wherein aerobic bacteria (such as nitrifies) thrived in the aerobic zone within the core, and a denitrifying and hence anaerobic strain of bacteria inhabited the anaerobic zone at the peripheral of the hydrogel that was exposed to the anaerobic bulk liquid, according to aspects of the disclosure.

[0095] FIG. 38A shows an example experimental result, according to aspects, of the disclosure, showing that *Nitrospira inopinata* encapsulated in hydrogel beads from left to right in order: *Nitrospira inopinata* alone, *N. inopinata* with platinum powder in gel beads, *N. inopinata* with platinum powder and H₂O₂ microcapsules (MCs) in gel beads. Initially and at earlier times, only about 20% of the beads were floating. After 30 or more hours, 80% or more were floating. This suggests the MCs are functional.

[0096] FIG. 38B shows an example result showing a close-up view of the beads containing H₂O₂ microcapsules. Beads are floating due to oxygen release from the MCs. The small white particles are MCs.

[0097] FIG. 39 shows an example result showing that acetic acid, one type of short-chain fatty acid, was produced from the fibrous Kenaf material (representative of any other fibrous or cellulosic material such as but not limited to Chia seeds, Bagasse, flax, hemp, jute, ramie, quinoa seeds, and kenaf) encapsulated in hydrogels, according to aspects of the disclosure.

[0098] FIG. 40 shows an example result showing encapsulation of Fungi and fiber in a hydrogel to support carbon and nitrogen cycling across bacteria and fungi.

[0099] FIG. 41 shows an example result showing that hydrogel enclosed hydrogenotrophic methanogen producing methane from CO₂ and H₂.

DETAILED DESCRIPTION

[0100] The impact of the human gut microbiome on human health is extensive, and the intersection of diet and the gut microbiota is a driver of host well-being, including kidney health. Altering the production or enabling removal of these toxins from the gut, *e.g.*, by chemical binders or by targeted changes to the human gut microbiome, is needed,

since PBUTs culminating in the blood of CKD patients are derived from gut microbiota. An imbalanced intestinal microbial community composition, having quantitative and qualitative alterations in microbial abundances and variations in metabolic activities of the gut microbiota, can be observed during CKD.

[0101] The equilibrium of gas concentrations in the intestine is a delicate system, as one gas affects another's production, which can be a factor in the fermentation process of carbon substrates, such as PBUT. Fermentation produces H_2 as a byproduct during carbon conversion. Too high H_2 concentrations can lead to an inhibition of the digestion process of organic carbon to CH_4 , which can influence the net human gut microbial community composition and its function as well as toxin removal. For example, CH_4 is produced from H_2 and CO_2 during methanogenesis, which depends on processes such as acetogenesis and acidogenesis which can get inhibited by its own fermentation product H_2 . Hydrogenotrophic methanogens can lower the H_2 concentrations hence sustaining carbon turnover. Therefore, adding methanogens to a composition of the disclosure, such as a bubble tea composition, can help maintain a balanced community and carbon conversions within the hydrogel and gut communities, and enable a smooth conversion of carbon cycling and all other cycles (S, N, metal) that can depend on it.

[0102] Another sensitive gaseous component is H_2S , which can be produced because of microbial sulfur cycling that can unlock toxin removal if coupled to carbon (*e.g.*, PBUT such as sulfate reduction coupled to carbon oxidation) or nitrogen (*e.g.*, removal of nitrate through sulfide driven autotrophic denitrification released during urea/ammonia conversion) cycling but at the same time S-cycling can generate H_2S that can be inhibitory to microbes and hence uremic toxin removal. For example, Anammox, AOB and other N-converting microbes - that together can convert urea and ammonia to harmless nitrogen gas - are inhibited by too high H_2S concentrations. A good countermeasure to remove this gas is to include at least some H_2S scavenging agents within the hydrogel. Sulfide oxidizing microbes, such as autotrophic denitrifiers, can be beneficial if included in a bubble tea composition of the disclosure; this can also remove nitrate (by product of urea conversion if nitrification and denitrification are coupled). In addition, H_2S readily reacts with iron hydroxides or oxides to form iron sulfide, and in addition, bismuth subsalicylate is known to lower H_2S as well. Therefore, adding bismuth subsalicylate and iron hydroxide into a hydrogel of a composition of the disclosure can help bind H_2S to lower any potential toxic effects from sulfur cycling microbes.

[0103] In various aspects, the disclosure provides novel, active therapies for treating and preventing CKD and loss of kidney function, that can be formulated into a drinkable format. The disclosed approaches target uremic toxins (*e.g.*, nitrogenous waste, aromatics, and phosphate) that have adverse health effects, not only for the kidney, but also for the cardiovascular, gastrointestinal, and neurological systems. The disclosed innovative bubble tea-inspired solutions integrate bio-, chemical-, and civil- engineering technologies into a targeted solution that safely and effectively removes uremic toxins from within the gut before these toxins enter the bloodstream. These approaches can serve as the cornerstone of a radically improved treatment strategy, that leverages the gut - the largest semi-permeable membrane in the human body - as the site for highly efficient uremic toxin removal. The technology disclosed herein represents a novel, transformative, patient-centered approach to eliminate systemic metabolic and uremic toxins linked to onset and progression of CKD, cognitive dysfunction, and cardiovascular disease, among other diseases and disorders.

[0104] The disclosed approaches do not rely on attempting to change the gut microbiome directly, but instead utilize encapsulated bacteria to provide the metabolisms needed to degrade uremic toxins, such as p-cresol and indole, before they can be absorbed into the blood and become PBUTs (FIG. 1). The encapsulated bacteria can perform their chosen metabolic functions in the gut without being washed out by the growth of other bacteria and are then simply excreted with the feces. Hydrogel encapsulation provides a way of protecting the enclosed bacteria from the numerous bacteria present in the gut, allows the amount of introduced bacteria to be controlled, and protects the gut microbiome from the introduced microbes. It also enables more complex biochemical strategies to be implemented, such as cross-feeding between different strains and even supporting localized aerobic microbial metabolisms if a slow-release oxygen source is included in the gel particles.

[0105] Described herein are compositions of a therapeutic bubble tea (hydrogel particles comprising agents deployed in the GI tract which, alone or in combination, bind, catalyze, remove, *etc.* target toxins (toxins and/or toxin precursors) to replace lost functions of normally processing healthy kidneys) which are proposed to manage levels of potentially toxic products in the body of persons suffering from at least some degree of kidney failure. In at least some cases, the disclosed approaches can be used to delay the need for conventional dialysis treatment, or can be used as an adjunct therapy to reduce

the frequency of dialysis sessions, and in at least some instances, as an alternative to such dialysis sessions. In contrast to conventional treatments, which attempt to remove toxins from the blood by dialysis, the solution presented herein prevents the formation of toxins at their origin.

[0106] The disclosed solutions include a novel microbe enriched “bubble tea” containing one or more, *e.g.*, several, types of hydrogel particles which prevent the absorption of excess phosphate and expand the capacity of the gut to degrade the uremic toxin (UT) precursors (indole and p-cresol), and thereby prevent sulfonation to toxic forms in the liver, and reduce the amount of urea in the blood. The bubble components carrying biological agents can be engineered to remain tight in the stomach, hence protecting the hydrogel entrapped microbes from low pH, and then swell at neutral pH in the small intestine and colon, allowing UTs to diffuse into the hydrogel where the UT degrading microorganisms can deactivate them. Finally, the hydrogel particles of the bubble tea can be excreted intact without altering the natural gut microbiome. These approaches can be implemented in bioengineering and environmental engineering, including but not limited to drug delivery and wastewater treatment.

Primary Uremic Toxins

[0107] Phosphate toxicity: Dialysis is generally not effective at clearing phosphate that accumulates in the blood, which leads to a myriad of problems. This is at least one reason why patients need to limit phosphate intake through following strict diets and/or by using phosphate binding medication. Hyperphosphatemia is commonly associated with bone and mineral disorders (referred to as CKD-MBD) which is a systemic disorder of mineral and bone metabolism. CKD-MBD results in increased risks for fractures, osteoporosis, vascular calcification, and cardiovascular disease. Hyperphosphatemia is considered one of the most critical contributing factors to CKD-MBD, and is caused primarily by an inability of kidney excretory function to maintain phosphate homeostasis. CKD-MBD is also associated with hormonal abnormalities, including hyperparathyroidism, diminished calcitriol synthesis, and increased circulating FGF-23 concentrations, each of which can contribute to the observed increased morbidity and mortality risks. Hyperphosphatemia in individuals with CKD is commonly treated with prescribed dietary phosphate binders (such as calcium carbonate, calcium acetate, lanthanum carbonate, and sevelamer hydrochloride). However, these agents only bind a

modest portion of dietary phosphate, are associated frequently with gastrointestinal side effects and changes to the human gut microbiome, and require intake of many pills per meal. New phosphate mitigation strategies are urgently needed to prevent and treat hyperphosphatemia.

[0108] Aromatic Uremic Toxins: Aromatic PBUTs, such as indoxyl sulfate and para-cresol sulfate, are not effectively removed by dialysis because they bind tightly to plasma albumin and remain on the blood side of the dialysis membranes. The build-up of PBUTs in the plasma has repeatedly been related to cardiovascular morbidity and mortality in patients with CKD. PBUTs are byproducts of gut microbial metabolism; indole originates in the gut from the fermentation of the aromatic amino acid tryptophan and is sulfonated in the liver to form the PBUT indoxyl sulfate. Likewise, phenolics such as p-cresol are produced in the gut by fermentation of the amino acid tyrosine before being sulfonated to its toxic protein-binding form in the liver.

[0109] Nitrogenous Waste Products – Urea and Creatinine. While urea and creatinine are small solutes and can be removed by dialysis, slowing their accumulation in the blood, for instance by degrading them in gut using one or more approaches of the disclosure, can extend the time between dialysis treatment for patients and can help avoid dialysis entirely for early-stage patients.

COMPOSITIONS

[0110] Hydrogel Engineering. A hydrogel includes any three-dimensional network of hydrophilic polymers which can hold a comparatively large amount of water while maintaining structural integrity due to chemical or physical cross-linking of individual polymer chains. Hydrogels have been successfully implemented to immobilize and grow aerobic and anaerobic microbes, as they provide a format for gentle cell entrapment. The gels also provide a matrix similar to a naturally occurring biofilm, in which microbial cells are protected in a matrix of extracellular polymeric substances, promoting microbial interactions on a micrometer scale without community washout. The Boba in “bubble tea” drinks are one example form of hydrogel made of starch, which is digestible. Many other natural and synthetic substances, both digestible and non-digestible, can be used to make hydrogels with different useful properties. For example, methods can be implemented that use combinations of natural carbohydrate colloids to

encapsulate probiotic microbes so that they survive passage through the stomach and are delivered in live active form to the intestines or colon.

[0111] Some gut centered hydrogel applications exist, but their use remains sparse and is at the research stage. Ingestion of microbes can be implemented, for example, as *Lactobacillus* enriched probiotic yogurt products. However, it is currently unknown how changes in the human microbiome impact the long-term health of a patient, and the removal of an introduced strain may be practically unfeasible if it starts colonizing the colon. Delivering microbes, chemical binders, and enzymes in mobile hydrogels that add functionality to the gut without releasing the bacteria into the GI tract provides a patient-friendly therapeutic in the form of a bubble tea drink that minimizes the physically and mentally draining dialysis treatment. Hydrogels used for this purpose can meet several specifications: the ability to host live cells at high density, the ability to prevent the escape of the cells, and the ability to protect the cells from the harsh acidic conditions of the stomach.

[0112] In embodiments, hydrogel particles contain or are at least partially comprised of engineered particles, such as shown by way of a non-limiting example at FIG. 2. Engineered particles can have further compound structures, for example, particles containing smaller particles, and at least some particles can have one or more enteric coatings. At least some particles can contain small molecules acting as binding moieties for various target toxins. The hydrogel and its contents can be protected from the low pH of the stomach with an enteric coating, allowing them to be fully functional in the large intestine. In at least some embodiments, the agent, *e.g.*, bacteria or adsorbent, is in the core and the shell of the particle, or alternatively, only in the core. In embodiments, the shell of the particle allows diffusion of uremic toxins into the core. In embodiments, the core comprising the agent, *e.g.*, bacteria or adsorbent, comprises indigestible hydrogel. In embodiments, the shell comprises a polymer or another material that acts as an enteric coating and/or protects the particle core and the agent immobilized within the core from the acidic environment of the stomach.

[0113] **Multifunctional Therapeutic Bubble Tea.** In various aspects, the disclosure provides an easy to use, comprehensive approach to managing effects of kidney disease. While single toxin degradation can be a viable therapeutic depending on use case, an additional strategy of the disclosure includes use of a combination of hydrogel beads that target a number of removal strategies simultaneously (FIG. 3). This

innovation offers a “mix and match” toolbox for biological, chemical, and enzymatic approaches to remove uremic toxins such as phosphate, urea, creatinine, and aromatics materials that absorb phosphate that are incorporated into edible “bubbles” for inclusion in a bubble tea composition. Hydrogel microbeads can include various microbes that absorb phosphate or degrade aromatic uremic toxin precursors or the simple nitrogenous compounds urea and creatinine, and can also be incorporated into the therapeutic “bubbles” (FIG. 4). By using a combination of these or other agents, at least some phosphate can be absorbed in the stomach by chemical binders, while additional phosphate can continue to be absorbed in the small intestines by phosphate-accumulating microbes as a meal passes through the digestive tract. Other microbes encapsulated in a different subset of microbeads can become active in the lower intestines and colon to degrade aromatic toxin precursors as they are produced by the gut bacteria. Another subset of microbeads contains microbes which can degrade nitrogenous wastes and produce electron acceptors which the toxin-degrading microbes in other microbeads can utilize to perform their intended biochemical functions.

Methods for Removing Specific Uremic Toxins and Toxin Precursors

[0114] Chemical phosphate binders. Phosphate binders can lower the amount of phosphate in the digestive system by forming complexes that may not be absorbed by the gut. Chemical removal of phosphate from the GI tract provides relief for hyperphosphatemia and uremia as the commonly prescribed phosphate binder sevelamer simultaneously improved plasma p-cresol levels. There are three major types of phosphate binders: calcium-containing binders; aluminum binders; and new non-calcium-based binders such as sevelamer and lanthanum carbonate. Even though calcium-based salts are the most extensively used and the lowest cost option, recent research indicates that they may increase the risk of hypercalcemia and vascular calcium accumulation. Aluminum-containing binders are the most efficient and cost-effective phosphate binders to treat hyperphosphatemia, but they are no longer widely used due to their linkage to neurodegenerative diseases. Sevelamer hydrochloride has a substantial pill burden and high costs, preventing its widespread usage.

[0115] Lanthanum carbonate is a non-aluminum, calcium-free phosphate binder that is efficient in reducing phosphate levels in dialysis patients. Nevertheless, it is costly, and it is causing some complications -- amongst the most often reported adverse effects

are nausea and diarrhea. The commercially available phosphate binders can only bind 200 mg of the entire daily phosphate intake (1,400 to 2,500 mg). As a result, phosphate binders account for a significant amount of the pill load in patients with end-stage renal disease, potentially affecting patient adherence but have, overall, only limited capacity to lower plasma phosphate concentrations.

[0116] Ferric oxyhydroxide is one of the most promising new generations of phosphate binders in medicine, which one can immobilize in a hydrogel bead that swells at low pH, hence allowing active transport of phosphate into the gel, in various embodiments. Highest anion (phosphate) uptake can be expected in the acidic stomach (where phosphate absorption is most critical to prevent hyperphosphatemia), because under these conditions, the hydrogel and adsorbent surfaces are protonated and become more positively charged, hence considerably increasing the amount of negatively charged phosphate anions absorbed. One of the newest phosphate binders now undergoing trials is iron based, sucroferric oxyhydroxide. This largely insoluble iron compound is administered as chewable tablets which contain ferric oxyhydroxide, sucrose, and starch. It is effective in reducing serum phosphate, and it releases minimal iron in the gut. The side effects include mild diarrhea, which usually subsides quickly. It does not result in changes to serum iron parameters to the extent that ferric citrate does, and is not expected to have much effect on the gut microbiota for the same reason. Therefore, one can use this phosphate binder encapsulated in a hydrogel bead in a new manner that further minimizes iron release, in embodiments. Ferric oxyhydroxide can be immobilized in alginate beads that are cross-linked using Fe^{3+} and the beads coated with chitosan, which forms strong associations with alginate and is an iron chelator, in embodiments. Highest anion (phosphate) uptake can be expected in the acidic stomach, where phosphate absorption is a factor to prevent hyperphosphatemia, because under these conditions, the hydrogel and chitosan are protonated (FIG. 5). Using microbial phosphate absorbers active in the intestines in combination with these chemical phosphate absorbers active in the stomach can provide continuous removal of phosphate during digestion.

[0117] Microbial Phosphate absorbers - PAOs to address Hyperphosphatemia. In embodiments, preventing and treating hyperphosphatemia can be achieved by harnessing the power of phosphate-accumulating organisms (PAOs) to remove phosphate from the digestive system, as an agent encapsulated in a hydrogel particle or bead that is safely ingested via a 'therapeutic bubble tea' by a patient (FIG. 5).

The bacteria add functionality to the gut (P-absorption) without altering the human microbiome, as the beads are excreted intact. While research has already looked into utilizing PAO as a prebiotic to manage hyperphosphatemia in CKD, the PAO employed herein differ in metabolism. Many bacteria (even *E. coli*) can accumulate phosphate as internal storage component (polyp) to maintain a pool of P for growth under conditions where carbon availability is high while P is depleted. This type of polyP accumulation typically leads to just a small percentage of P in the cell. A fermenter, such as *Lactobacillus casei*, stores phosphate at high pH and releases phosphate at low pH to neutralize the local pH surrounding the cell. The fermenter can absorb phosphate in the intestines after passing through the stomach where the low pH primes the cells for phosphate uptake. Therefore, fermenters can be encapsulated, including, but not limited to, *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Lactobacillus acidophilus*, *Lactobacillus coryniformis*, *Lactobacillus brevis*, *Bifidobacterium longum*, *Bifidobacterium adolescentis*, *Bifidobacterium breve*, *Lactiplantibacillus paraplantarum*, or any combination thereof, in hydrogels of compositions of the disclosure for functionality in the intestine, in embodiments.

[0118] An efficient phosphate storage metabolism exists in some bacteria, which relies on oxygen availability. This metabolism, called enhanced biological phosphate removal (EBPR), is used in wastewater treatment and it can be utilized in the gut if a slow-release oxygen source is included in the gel particles encapsulating the EBPR organisms. The biochemical pathway of the EBPR process is a special adaptation of bacteria: the phosphate-accumulating organisms (PAOs) release ortho-phosphate from internally stored polyphosphate (polyP) to generate energy (as ATP) and also taking up volatile fatty acids (VFA) under anaerobic conditions (*i.e.*, in the absence of an electron acceptor like oxygen or nitrate) and convert it to energy-rich intracellular poly-hydroxyalkanoates (PHA) while glycogen is metabolized by glycolysis to produce ATP and NADH (FIG. 6). Under aerobic conditions, the PAOs use oxygen as an electron acceptor. Nitrate and nitrite are also electron acceptors enabling denitrification. PAOs are thus actually denitrifying PAOs (dPAOs). Orthophosphate is taken up from the mixed liquor to replenish poly-P reserves in the cell. PHA is used as an energy source for the replenishment of glycogen reserves, generation of ATP, and production of new biomass (FIG. 6). The uptake of P is occurring in the aerobic phase due to the generation of new biomass. Hydrogels of the disclosure can include encapsulated PAOs including, but not

limited to: *Tetrasphaera japonica*, *Tetrasphaera elongata*, *Tetrasphaera australiensis*, *Tetrasphaera vanveenii*, *Tetrasphaera veronensis*, *Microthamnion phosphovorus*, or any combination thereof, to sequester phosphate in the intestine, in embodiments.

[0119] At least some embodiments of the PAO, in compositions of the disclosure, use the mechanism of enhanced biological phosphate removal (EBPR). EBPR-based PAOs use polyP to conserve energy via the generation of ATP, enabling the PAOs to accumulate large quantities of P in cell under aerobic conditions. While the gut is primarily anaerobic, the provision of some oxygen via the epithelial cells into the gut is a reported phenomenon and, as a result, many gut microorganisms are facultative aerobes using or tolerating oxygen. By entrapping hydrogen peroxide in the bead core, targeted oxygen delivery to the bubble tea microbial community (but not the gut community) removes large amounts of phosphate from the gut. Another approach is to utilize pH driven poly-P storage. This includes a probiotic PAO, such as *Lactobacillus casei* (*L. casei*), which is capable of storing higher amounts of PolyP; without wishing to be bound by any particular theory, this may be for utilizing phosphate uptake and release as a mechanism to maintain internal pH when external pH is fluctuating. In various embodiments, hydrogels can implement one or more pH responsive PAOs (including, but not limited to, *Lactobacillus*) to withstand low pH in stomach, and to then accumulate PolyP at higher pH such as is present in the small intestine.

[0120] **Urease and chemical ammonia binders.** In addition to ammonia conversion by natural gut microbiota, encapsulated urease enzyme and urease-releasing microbes can convert urea to ammonia which can be further removed by microbes and chemical binders. Ammonia binders, such as zeolites, have been widely documented to exhibit excellent and targeted ammonia removal capacity from wastewater, which is of comparable complexity to gut fluids and offers promise as a potential adsorbent material for CKD patients. The basic principle of ammonia binders is replacing ammonia or ammonium with similarly charged ions from the mobile phase of a chemical binder or ion exchange material. Ion-exchange has recently been used for ammonia removal from an aqueous solution owing to its high removal efficiency and ease of use. In embodiments, a combination of urease (enzymes) and zeolites can be implemented to provide a simple means of removing urea and ammonia (without microbes). Zeolites can remove renal uremic toxins without directly adhering to serum albumin, and they can be used clinically as components of potent hemostatic and gastroprotective drugs. Therefore, naturally-

occurring and/or non-naturally-occurring zeolites can be used, in embodiments of the disclosure, for ammonium removal, as they have some advantages in comparison with cation exchange materials like organic resins, such as their low-cost, excellent selectivity, release of non-toxic exchangeable cations (*e.g.*, Na⁺ and Mg²⁺), and fast reaction rates, which can be helpful if applied in a gut setting where digestion times are short.

[0121] Consumption of indole and para-cresol in gut milieu by encapsulated microbes. The sulfate reducer *Desulfobacterium indolicum* is capable of utilizing indole anaerobically as its sole source of carbon and energy, and is known to produce anthranilic acid, an intermediate that is degraded under anaerobic conditions and is not known to be toxic at low levels in the gut. The anaerobic degradation of p-cresol to CO₂ can occur in several *Desulfobacterium* species with sulfate as electron acceptor. Sulfate reduction yields sulfides, a compound with pleiotropic properties in the gut, *i.e.*, beneficial at low concentrations, but toxic at elevated concentrations.

[0122] The disclosure provides for anaerobic indole and para-cresol degradation in pure cultures of *Desulfobacula phenolica*, *Thauera aminoaromatica*, and *Geobacter metallireducans*, under sulfate reducing, nitrate reducing, and iron reducing conditions, respectively. Other metal converting microbes can include *Shewanella oneidensis*, and *Geobacter sulfurreducens*, which can couple iron and carbon cycling to sulfur cycling by producing H₂S that can be used for denitrification. Autotrophic denitrification can use sulfide (S₂⁻), elemental sulfur (S₀), and thiosulfate (S₂O₃²⁻) as electron donors that can be encapsulated in the hydrogel or provided from within the gut. It is shown herein that encapsulated *D. phenolica* and *T. aminoaromatica* removed para-cresol and indole, and three indole degrading *E. coli* can be isolated from human feces. Notably, para-cresol degradation by *T. aminoaromatica* was not inhibited in the presence of acetate. This is relevant in light of the complex carbon substrate environment in the human gut and the likelihood of catabolite repression (of p-cresol metabolism) by simpler organic substrates, as is often observed in microbial metabolism. The electron acceptors, sulfate and nitrate, can be found in the gut environment and can be supplemented to the encapsulated microbes if necessary using slowly metabolized macromolecular forms or enteric delivery formulations, similar to those used for pharmaceuticals. Fine particulate iron oxide can be supplied to the iron reducing bacteria by co-encapsulating iron oxide in the gel beads as can be done with the ferric oxyhydroxide used for chemical absorption of phosphate.

[0123] Fe as electron acceptor for bacteria that p-cresol degrading bacteria.

The insoluble Fe (III) oxide, as well as the p-cresol oxidizing Fe reducing bacterium/Fe reducing bacterial consortia, can be encapsulated in a hydrogel (FIG. 7) so that no external electron acceptor needs to be supplied. The bacteria can uptake, consume and degrade p-cresol with concomitant reduction of the iron oxide from the +III state to the +II oxidation state. The encapsulated bacterial-iron oxide cargo will continue through the digestive passage, ultimately being eliminated with fecal matter. In embodiments, any of diverse iron-reducing bacteria capable of consuming p-cresol, like *Geotalea daltonii*, *Desulfitobacterium aromaticivorans*, and *Geobacter toluenoxydans*, can be used.

[0124] Aerobic UT and PBUT removal. Many aerobes can remove ammonia, indole, and p-cresol, allowing a wider range for species selection of uremic O₂ toxin degraders. Delivering oxygen into the gut presents a viable format for pairing a cocktail of aerobic and anaerobic bacteria to treat uremic toxins in the colon (FIG. 8 and FIG. 4). In embodiments, aerobic nitrifiers can be combined to produce nitrite from urea, and then nitrite is reduced by heterotrophic or autotrophic denitrifiers utilizing organic carbon (like indole or p-cresol), H₂S, or ammonium as electron donor to harmless nitrogen gas, carbon dioxide or other beneficial / probiotic products. Such a partnership allows simultaneously lowering urea and PBUTs, improving the health outcomes for kidney disease patients.

[0125] Biological N removal. FIG. 9 depicts metabolisms possessed by various identified nitrifiers. Nitrifying bacteria, such as ammonia-oxidizing bacteria (AOB) that can oxidize ammonia to nitrite, which is then oxidized to nitrate by nitrite-oxidizing bacteria (NOB), can be used in conjunction with denitrifying bacteria for nitrogen removal as inert N₂ gas (FIG. 9). At least some members of the nitrite-oxidizing genus *Nitrospira* are capable of complete ammonia oxidation (Comammox), converting ammonia to nitrate. In embodiments, aerobic ammonium oxidizing bacteria and/or Archaea (AOB, AOA), and anaerobic ammonium oxidizing bacteria (Anammox) such as *Brocadia*, *Kuenenia*, *Anammoxoglobus*, *Jettenia*, and/or *Scalindua*, are leveraged in the hydrogel bead to allow for urea and ammonia conversion in the otherwise oxygen limited gut (FIG. 10). The system can be based on the principle that Comammox produces nitrate as a product of ammonia oxidation, with nitrite as an intermediate (FIG. 9). The intermediate nitrite can be consumed by Anammox along with ammonium yielding full N removal (FIG. 9).

[0126] Some gut microbes can convert creatinine into urea, which is fed to Comammox and AOB. The ability of these and other bacteria, such as ordinary heterotrophs as well as strains such as *Nitrososphaera viennensis* (AOA), *Nitrospira lacus* (AOB), *Nitrosomonas ureae* (AOB), *Nitrosomonas nitrosa* (AOB), and even nitrite oxidizers such as *Nitrospira muscovinensis* to hydrolyze urea to ammonia as well as to decompose hydrogen peroxide to water and oxygen through the catalase enzyme, can be leveraged (FIG. 9 and FIG. 11). The biotically produced oxygen can be used by Comammox for ammonia oxidation followed by denitrification to N₂ via Anammox. The advantage of Anammox based systems is that it is using 60% less oxygen than conventional nitrification-denitrification based systems which would hence minimize the amount of peroxide addition to the gut needed. Combining these concepts in an ingestible bubble tea format can enable urea conversion in the otherwise oxygen limited gut (FIG. 10).

[0127] Nitrification-denitrifying consortia can also serve as a potential solution to lower overall uremia in CKD patients by integrating different types of indole and para-cresol denitrifying strains, which can help remove concerns over a possible health effect of nitrite toxicity. The use of Comammox can be coupled with phosphate removal by using few available strains of denitrifying PAOs (dPAOs).

[0128] **Enabling Aerobic Metabolisms in the Gut through Microcapsule and Hydrogel Engineering Strategies.** Delivery of oxygen to the hydrogel bead microbial community in an anaerobic gut environment can enable several advantageous microbial metabolisms to be harnessed for removing uremic toxins. The intestinal niche is believed to be devoid of oxygen. However, both aerobic and facultative anaerobic bacteria are present in the gut. The microbiota from newborns suggests that the initial community of aerobic and facultative anaerobic bacteria consumes oxygen in the intestine, allowing the population of obligate anaerobes to develop. The mechanism responsible for maintaining the anaerobic environment of the gut lumen is unclear, in part owing to the difficulty in quantifying intestinal oxygen levels. An encapsulated consortium of bacteria, delivered orally, can break down urea (and creatine) in the gut before they diffuse through the intestinal mucosal barrier into blood. Three aerobic cultures of microbiota, isolated from fecal matter, can be used; urea was converted to ammonia via *Escherichia*, creatinine converted to ammonia via *Bacillus*, and ammonia converted to amino acids via *Enterobacter*. Urea blood levels were lowered without detrimental effects. All these

bacterial consortia used in the urea breakdown experiment were aerobic organisms indicating that they can have used oxygen and that the therapy can be oxygen limited in the gut.

[0129] The disclosure also provides avenues for oxygen delivery into the gut, in the form of hydrogen peroxide. Many gut bacteria have the catalase enzyme, which converts H_2O_2 to oxygen, and supplementing catalase to the gut has been tested in hydrogel encapsulated form as an oral treatment for inflammatory bowel diseases and combined with other encapsulation technologies for targeted drug delivery to the colon. Hydrogen peroxide delivery into the human gut has also been proposed for enhancing cell growth to aid the attachment of synthetic epithelial linings, and hydrogen peroxide has shown to be a viable format for oxygen delivery to bacteria in bioreactor systems. The release of hydrogen peroxide can be demonstrated by entrapping the hydrogen peroxide in poly(methyl methacrylate) (PMMA) microcapsules, whereby the hydrogen peroxide diffuses out of the microcapsule. PMMA is already approved by at least one health regulatory authority for certain surgical applications, and demonstrates shelf-life stability under refrigeration.

[0130] Entrapping hydrogen peroxide reservoirs (*e.g.*, microcapsules) and catalase in the inner core of hydrogel particles of a therapeutic bubble tea enables controlled diffusion outward toward aerobic organisms (FIG. 11). The aerobic microbial breakdown of urea, indole, or p-cresol permits consumption of oxygen before it reaches the natural anaerobic gut microbiota. This platform enables the lowering of UT and PBUTs with the reduced possibility of side-effects. When the H_2O_2 -loaded gel microbeads with encapsulated aerobic microbes are ingested, the aerobic organisms can operate in the low oxygen environment of the gut to consume toxins or amend the function of the microbiome in a targeted way. The co-encapsulated catalase ensures a high concentration of cells remain active by facilitating a direct conversion of H_2O_2 to O_2 , hence keeping the oxidative stress of H_2O_2 low. The catalase enhances the biological processing capacity by protecting the biology from high peroxide concentrations, and also enabling high oxygen supply levels to ensure the biology does not become oxygen-limited which maximizes UT degradation. The technique can allow aerobic uptake of phosphate via PAOs as well as oxidation of ammonia to nitrate followed by denitrification of the nitrate to N_2 . One benefit of a targeted oxygen delivery in hydrogels

is for oxygen to be metabolized only by those organisms enclosed in the beads (*i.e.*, PAOs), hence maintaining the otherwise oxygen deplete conditions in the gut.

[0131] *Lactobacillus*, one of the PAOs used in embodiments, is a facultative bacterium and helps scavenge oxygen to maintain an anaerobic gut milieu. As a microscale example, PMMA microbubbles can contain about 4.5% H_2O_2 , in which 1 mL of microbubbles can have about 0.0014 mol H_2O_2 which can generate 0.0014 mol of oxygen. This amount of oxygen can oxidize 0.8 mmol of ammonia. Approximately more than 1000 microbubbles can fill a volume of a 250 μm diameter hydrogel. In the meantime, the typical intake of phosphate through a typical diet is 1,500 – 2,500 mg per day, from which 300 – 1300 mg of phosphate needs to be removed in a CKD patient. In the presence of oxygen, approximately 10 – 30 mL of hydrogel beads can effectively remove all incoming phosphate.

[0132] In embodiments, the use of an abiotic catalyst, embedded in the same hydrogel, ensures the rapid breakdown of diffused hydrogen peroxide to oxygen, thereby protecting the entrapped biology also within the hydrogel. FIG. 2 shows an overview of an example of such a concept. In embodiments wherein a stable hydrogel is used for encapsulation, the abiotic catalyst can be excreted intact, preventing the release of the catalyst into the gut. In embodiments, the hydrogel can contain an abiotic catalyst, microorganisms, and hydrogen peroxide microcapsules. When ingested, aerobic organisms operate in the low oxygen environment of the gut to consume toxins or amend the function of the microbiome in a targeted way. The abiotic catalyst ensures a high concentration of cells remain active by facilitating a direct conversion of H_2O_2 to O_2 , hence keeping H_2O_2 levels low. Abiotic catalysts enhance the biological processing capacity by protecting the biology from the toxic effects of peroxides, and also enabling high oxygen supply levels to ensure the biology does not become oxygen limited by barriers, such as the rate of dissolution of gases, competing consumption of oxygen within a highly active environment, or lack of access to other oxygen supply sources. Premature release of O_2 from the system may prevent the use of these systems for *in situ* toxin removal, and thus, in at least some embodiments, hydrogels or hydrogen peroxide microcapsules can be combined with other or existing technologies for enteric targeted drug delivery to the colon.

[0133] Influence of hydrogel bead size on aerobic and anaerobic processes. Within aggregates, transport phenomena of substrates results in resource gradients with

increasing depth, allowing species with different ecophysiological characteristics to survive and coexist. Their co-occurrence can be explained by different substrate profiles within a biofilm / aggregate (high or low due to consumption or production of substrates), which impact the competition and spatial organization of microbes. Small and large aggregates play different functional roles in nitrogen removal by selecting for different microbial species. If small and large spheres are subjected to the same bulk oxygen concentrations, the oxygen penetration depths can be similar, leading to a higher aerobic volume fraction in smaller hydrogels that can be more favorable to aerobic organisms, such as ammonia oxidizers or ordinary heterotrophs. Conversely, denitrifying microbes can grow in bigger hydrogels in which a larger anoxic core can exist. As such, size fractions can be selected for functionally distinct microbial communities. Here, H_2O_2 , which is converted to oxygen, can diffuse out of microbubbles in a hydrogel. Small hydrogels can be fully oxygenated while larger bubbles can develop an oxygenated core in which anaerobic conditions will exist at the bead periphery, hence allowing simultaneous nitrification and denitrification to occur in one bead (FIG. 12). Alternatively, the smaller beads can be fully oxygenated (FIG. 12, left) in which case some oxygen might leak into the gut environment, where natural gut native microbes may oxidize toxins with their naturally existing genetic inventory for toxin degradation. This can support the bead community in their toxin degradation efforts or beads can also function as a vehicle for electron acceptor delivery in the otherwise electron acceptor-limited gut.

[0134] Enabling Short chain fatty acid production and nitrate/nitrite cleanup in the gut through hydrogel encapsulating fibers strategies. Fibers in the intestine are fermented by the gut microbiome to generate short chain fatty acids (SCFAs) such as acetate, propionate, and butyrate, in embodiments. SCFAs maintain gut barrier integrity through direct action on enterocytes and regulate inflammatory and immune responses, blood pressure, energy metabolism, and lipid and glucose homeostasis through indirect mechanisms. SCFAs, particularly butyrate, are substrates for maintaining colonic epithelium, with butyrate preferred as the oxidative fuel by colonocytes. Butyrate exhibits a dual role: inducing proliferation in normal colonocytes while promoting terminal differentiation and apoptosis in neoplastic cells, known as the “butyrate paradox” or “Warburg effect”. SCFA production lowers luminal pH, inhibiting pathogens and enhancing nutrient absorption; for example, acetate can be used for bifidobacteria to

inhibit enteropathogens. SCFAs play a role in controlling metabolic syndrome by reducing obesity and insulin resistance, primarily through butyrate's effect on down-regulating peroxisome proliferator-activated receptor gamma (PPAR γ), shifting from lipid synthesis to lipid oxidation. Butyrate and propionate induce the differentiation of T-regulatory cells, which helps control intestinal inflammation and maintain gut barrier integrity, reducing the risk of inflammatory bowel disease and CRC, in embodiments.

[0135] Hydrogel-based methods, as employed herein to remove toxins, can involve the external addition of nitrate/nitrite as an electron acceptor. However, the residual nitrate/nitrite can have adverse effects on human health. Short-chain fatty acids can serve as electron donors for denitrifiers to reduce nitrate/nitrite to nitrogen gas, in embodiments, thereby eliminating side effects and closing the loop for toxin removal.

[0136] Fiber is not often incorporated in modern diets, particularly in the age of fast food. Therefore, fiber can be interrogated with active fermented bacteria in a hydrogel that can enhance the SCFAs production, in embodiments. Fermentative bacteria (FIG. 13) and fungi can be encapsulated in a hydrogel to enhance SCFAs production, incorporating various prokaryotic strains, including but not limited to: *Faecalibacterium prausnitzii*, *Eubacterium rectale*, *Roseburia inulinivorans*, *Roseburia intestinalis*, *Anaerostipes butyraticus*, *Anaerostipes caccae*, *Butyricicoccus pullicaecorum*, or any combination thereof, as well as eukaryotic strains including but not limited to *Saccharomyces*, *Aspergillus*, *Galactomyces*, *Geotrichum*, *Saprochaete*, *Candida*, *Malassezia*, *Cladosporium*, or any combination thereof. In embodiments, fiber and beneficial fermented bacteria can be a lone pair in a hydrogel to boost SCFAs production, thereby improving gut health, inhibiting pathogens, regulating intestinal inflammation, maintaining gut barrier integrity, and bolstering anti-inflammatory responses, reducing obesity, and mitigating insulin resistance (FIG. 13A). In embodiments, fiber and beneficial fermented bacteria can be paired with denitrifiers and/or other toxin removers, such as *Thauera aminoaeromatica*, under nitrate reducing conditions (FIG. 13B). This pairing can facilitate the elimination of any remaining nitrate once *Thauera aminoaeromatica* completes the reduction of para-cresol and indole. Any denitrification by products can be converted by bacteria, including but not limited to *Clostridium* spp., *Thiobacillus denitrificans*, *Micrococcus denitrificans*, at least some species of *Serratia*, *Pseudomonas*, *Achromobacter*, or any combination thereof, as well as fungi, including but not limited to *Fusarium oxysporum* strain(s), *Cylindrocarpon tonkinense*, or both.

[0137] Accordingly, in various aspects, the disclosure provides innovative approaches for removal of toxins and/or toxin precursors from a subject's digestive system, for prevention or treatment of health problems that can be characterized, at least in part, by the presence of such toxins and/or toxin precursors within the digestive system. Non-limiting examples of health conditions capable of being prevented and/or treated by aspects of the disclosure include chronic kidney disease (CKD), CKD associated with bone and mineral disorders (CKD-MBD), kidney disease (renal disease; RD), end-stage kidney disease (end-stage renal disease; ESRD), hyperphosphatemia, and others.

[0138] Compositions of the disclosure can be formulated as foods, beverages, or other functional food products, and are configured to deliver one or more agents or biologically-active ingredients, optionally with one or more suitable carriers or delivery mediums, to the subject's digestive system upon ingestion. In non-limiting examples of the disclosure, a composition can be formulated as a bubble tea composition or bubble tea beverage that is suitable for consumption. Once the composition is ingested and one or more agents of the composition are localized to the digestive system, the one or more agents can bind, catabolize, absorb, or otherwise remove one or more toxins and/or toxin precursors from localized portions of the subject's digestive system for prevention and/or treatment of one or more health conditions, diseases, and/or disorders. In at least some aspects, the composition can include a plurality of carriers or delivery mediums that change, or are differentially activated, at different locations within the subject's digestive system. This can be achieved based on one or more biological conditions at those locations, such that one or more toxins and/or toxin precursors, or subsets thereof, are removed at those locations.

[0139] The disclosure also provides methods of using the compositions for prevention and/or treatment of one or more health conditions, diseases, and/or disorders, kits containing one or more compositions and/or one or more ingredients of compositions, and uses of compositions and/or ingredients in methods of producing medically relevant and/or biologically relevant foods, beverages, additives, and/or medicines or medicaments for the prevention and/or treatment of one or more health conditions, diseases, and/or disorders.

[0140] Accordingly, in various aspects, the disclosure provides compositions that are suitable for ingestion by a subject which, upon ingestion, deliver one or more

biologically active agents to the digestive system of the subject. The one or more agents remove one or more toxins and/or one or more toxin precursors from the subject's digestive system, and reduce the local concentration of these compounds at one or more positions along a length of the digestive system. The one or more agents can be configured to: process one or more toxins and/or one or more toxin precursors to form one or more safe or less toxic byproducts (*e.g.*, through one or more anabolic and/or catabolic biological processes); bind one or more intact, processed, and/or at least partially processed toxins or toxin precursors to physically remove these elements from the subject, or any combination thereof. By removing the toxins and/or toxin precursors from the digestive system, the systemic concentration of these compounds is lowered and their impact on the subject's health is altered or reduced for improved health and well-being of the subject.

[0141] In various embodiments, a composition comprises hydrogel particles that comprise agents encapsulated within the hydrogel particles. In embodiments, hydrogel particles can be shaped as hydrogel beads (*e.g.*, spherical), *e.g.*, of a bubble tea beverage, and can include an inner core and a matrix, and optionally, an outer shell and/or other layers or features. In embodiments, an inner core can be configured to deliver one or more electron acceptors to a matrix of a hydrogel particle, whereupon one or more agents of the matrix (*e.g.*, one or more microorganisms) utilize the one or more electron acceptors in a biological process of removal of one or more uremic toxins and/or uremic toxin precursors from the subject's digestive system.

[0142] In embodiments, one or more agents can be comprised of one or more biological agents, such as one or more microorganisms, one or more bacteria, one or more archaea, or any combination thereof, configured for removal of toxins and/or toxin precursors. In embodiments, at least a portion of the agents comprises phosphate-accumulating organisms (PAOs), and in embodiments, the PAOs can be denitrifying PAOs (dPAOs) and/or can be configured for enhanced biological phosphate removal (EBPR PAOs). In embodiments, at least a portion of the agents comprises a heterotrophic denitrifier, an autotrophic denitrifier, one or more organisms configured for aerobic removal of one or more aromatics, or any combination thereof. In embodiments, at least a portion of the agents comprises Commamox bacteria, ammonium oxidizing bacteria, ammonium oxidizing archaea, Anammox bacteria, or any combination thereof.

[0143] Biological agents, such as microorganisms, can be isolated from natural sources, isolated, and then suitably altered by, for example, *in vitro* directed evolution, mutagenesis, and/or another genetic modification technique, for use in a composition as needed, or can be artificially synthesized by way of an *in vitro* artificial cell synthesis process that takes place outside of a typical biological context. In this regard, in at least some embodiments, one or more biological pathways for survival, growth, reproduction, toxin removal, toxin precursor removal, or any combination thereof, can be included in an artificially-synthesized cell by way of one or more *in vitro* artificial cell synthesis techniques. As is generally known in the art of artificial cell synthesis, artificial cells can be produced by a top-down approach, whereby a living cell is used as a starting point and its genome is reduced or simplified to create a “minimal cell” with only the genes needed for survival and the desired biological pathways, or can be produced by a bottom-up approach, whereby an artificial cell is built from scratch by assembling basic components, such as cell-free extracts and synthetic molecules such as lipid vesicles.

[0144] In embodiments, compositions can be formulated as ingestible bubble tea compositions. In such embodiments, the hydrogel particles are configured as gel beads of the bubble tea composition and are food safe and ingestible. Examples of “bubble tea” compositions can include, but are not limited to, pearl milk tea, bubble milk tea, tapioca milk tea, boba tea, boba, and the like. In embodiments, a bubble tea composition can be comprised of any hydrocolloids or natural hydrocolloids approved for food use, such as chewy tapioca balls, grass jelly, aloe vera, red bean, popping boba, or the like. Bubble tea compositions of the disclosure can comprise one or more palatable agents for increased palatability of the composition. Non-limiting examples of palatable agents can include one or more flavor compounds, one or more sweeteners, one or more sugars, juices, milk or one or more milk ingredients, and the like, as is known in the culinary or functional food arts.

[0145] In embodiments, hydrogel particles are configured for removal of multiple different toxins, and/or removal of multiple different toxin precursors, from the subject’s digestive system. The different toxins can be removed at one or more locations along the subject’s digestive system, in various embodiments. In embodiments, the different toxins and/or toxin precursors can be removed by one type of hydrogel particle, and the one type of hydrogel particle can be characterized at least in part by the presence of one or more agents, contained within the one type of hydrogel particle, that are configured for removal

of the multiple different toxins and/or toxin precursors. In other embodiments, the different toxins and/or toxin precursors can be removed by two or more types of hydrogel particle, and the two or more types of hydrogel particle can be characterized at least in part by the presence of one or two or more agents, contained within the two or more types of hydrogel particle, that are configured for removal of the multiple different toxins and/or toxin precursors.

[0146] In embodiments, at least some hydrogel particles comprise one or more oxygen precursors and one or more enzymes or catalysts configured for conversion of the one or more oxygen precursors into at least oxygen (O_2) for use of the O_2 as an electron acceptor by one or more oxygen-consuming microorganisms, *e.g.*, PAOs, of the hydrogel particles and/or composition. In embodiments, the oxygen precursors are hydrogen peroxide (H_2O_2) molecules, and the enzymes are catalase enzymes. In at least some embodiments, H_2O_2 molecules are encapsulated within inner cores of the hydrogel particles for release from the inner cores and reaction with the catalase enzymes. In embodiments, at least a portion of the hydrogel particles comprises nitrate, nitrite, or both, for use as electron acceptors by the PAOs, however, other electron acceptors can be used without departing from the scope and spirit of the disclosure.

[0147] In embodiments, at least a portion of the agents comprises aerobic and anaerobic bacteria configured for: conversion of urea and protein-bound uremic toxins (PBUTs) to nitrite, and conversion of nitrite to nitrogen (N_2) and/or carbon dioxide (CO_2) with use of organic carbon, hydrogen sulfide (H_2S), and/or ammonium as one or more electron donors. In at least some embodiments, at least a portion of the agents comprises aerobic ammonium oxidizing bacteria (AOB), aerobic ammonium oxidizing archaea (AOA), anaerobic ammonium oxidizing bacteria (Anammox) (FIG. 10), or any combination thereof. In at least some embodiments, at least a portion of the agents comprises *Nitrososphaera viennensis*, *Nitrospira lacus*, *Nitrosomonas ureae*, *Nitrosomonas nitrosa*, *Nitrospira muscovicinensis*, or any combination thereof.

[0148] In embodiments, at least a portion of the agents comprises complete ammonia oxidizing (Comammox) bacteria, anaerobic ammonium oxidizing (Anammox) bacteria, or any combination thereof. In embodiments, at least a portion of the Anammox bacteria comprises: *Candidatus Brocadia*, *Candidatus Kuenenia*, *Candidatus Anammoxoglobus*, *Candidatus Jettenia*, *Candidatus Scalindua*, or any combination thereof.

[0149] In embodiments, at least a portion of the agents comprises urease, urease-releasing microbes, or any combination thereof. In embodiments, at least a portion of the agents comprises one or more ammonia binders configured for ion-exchange removal of ammonia. In at least some embodiments, the one or more ammonia binders comprises one or more zeolites configured for removal of one or more uremic toxins and/or one or more uremic toxin precursors. In at least some embodiments, the one or more zeolites comprises calcium, iron, copper chloride, or any combination thereof.

[0150] In embodiments, at least a portion of the hydrogel particles comprises one or more phosphate binders. In at least some embodiments, the one or more phosphate binders comprises ferric oxyhydroxide, iron alginate, or any combination thereof.

[0151] In embodiments, at least a portion of the hydrogel particles comprises a net electrostatic charge at cores of the hydrogel particles such that the hydrogel particles are pH responsive. In at least some embodiments, at least a portion of the hydrogel particles is active at a neutral to alkaline pH and comprises one or more shell layers configured to: prevent the release of cells entrapped near a surface of the hydrogel particles, and provide a pH-responsive barrier to diffusion. In at least some embodiments, at least a portion of the hydrogel particles is active at an acidic pH and comprises one or more phosphate binders and swells at acidic pH levels and contracts at neutral pH levels. In at least some embodiments, at least a portion of the hydrogel particles comprises a coating that protects the hydrogel particles from degradation in the stomach of the subject for delivery of protected hydrogel particles to the small intestine, the colon, or both, for removal of one or more uremic toxins and/or one or more uremic toxin precursors from the small intestine, the colon, or both (FIG. 2).

KITS

[0152] In another aspect, the disclosure provides kits for use by subjects in the treatment and/or prevention of one or more diseases or disorders. In embodiments, a kit comprises a composition and an instructional material to direct use of the kit in a method of treating or preventing onset or progression of one or more diseases or disorders. In embodiments, the one or more diseases or disorders comprises kidney disease or chronic kidney disease. The kits can be provided such that the composition therein is “ready for use” or ready for consumption by a subject, or alternatively, can be provided such that the composition can be readily prepared for use or consumption, for example, by adding

water or a liquid phase of a bubble tea composition. In this regard, in at least some embodiments, a kit can comprise all or a portion of a bubble tea composition, for example. In at least some such embodiments, a kit can comprise or can include only essentially hydrogel beads including one or more agents therein, for example, such that the hydrogel beads are ready for suspension and formulation into a bubble tea composition for consumption once hydrated.

METHODS

[0153] In an aspect, the disclosure provides a method of treating or preventing onset or progression of one or more diseases or disorders, the method comprising administering a composition of the disclosure to a subject in need thereof. In at least some embodiments, the one or more diseases or disorders treatable and/or preventable by the method includes kidney disease or chronic kidney disease. Methods can be performed by subjects of their own accord, or at the direction of a healthcare provider, for example.

MEDICINAL USES

[0154] In another aspect, the disclosure provides for various uses of compositions of the disclosure in the preparation of medicaments for administration to subjects for treating or preventing onset or progression of one or more diseases or disorders in the subject. Uses can include preparation of experimental medicaments as well as preparation of medicaments that have been approved by a regulatory body.

DEFINITIONS

[0155] As used herein, the terms “bubble tea,” “bubble tea composition,” and the like, refer generally to an ingestible suspension, colloid, solution, or other liquid, that contains one or more hydrogel particles, and/or one or more other ingestible matrices, that carry one or more agents configured for toxin and/or toxin precursor removal therein.

[0156] As used herein, the term “type,” as used with reference to a “type” of hydrogel particle, refers to a set of structures and/or functions of the referenced hydrogel particle that can serve to differentiate it from one or more other types of hydrogel particles of a composition. A composition, for example, a bubble tea composition, can include one type of hydrogel particle or, alternatively, can include two or more types of hydrogel particles.

[0157] As used herein, “precursor,” as used with reference to an “oxygen precursor,” refers to one or more chemicals which, when chemically processed by one or more biological or biochemical reactions, produces molecular oxygen (O₂) as a product or intermediate. For example, catalase can process hydrogen peroxide (H₂O₂) to produce molecular oxygen, and in this instance, H₂O₂ is the oxygen precursor; however, other oxygen precursors can be utilized instead of, or in addition to, H₂O₂ according to various embodiments.

[0158] As used herein, “biological agent” refers to an agent, configured for toxin removal and/or toxin-precursor removal, that has one or more biological characteristics or origins. A biological agent can refer to a cell, a microorganism, a bacteria, an archaea, or the like. Likewise, a biological agent can refer to an artificial cell, an artificial microorganism, an artificial bacteria, an artificial archaea, or the like. In this regard, a biological agent can refer to a derivative of a naturally-occurring organism, wherein the derivative is modified in a meaningful way, relative to the naturally-occurring organism, for added advantage with respect to the composition. For example, a genome of a naturally-occurring bacteria can be modified to include genes or genomic elements needed for essential biological pathways, *e.g.*, survival of the derived bacteria and removal of one or more toxins and/or toxin precursors, and/or can be modified to exclude genes or genomic elements that are toxic or undesirable to humans or animals. Similarly, the genome can be modified to include genes or genomic elements that increase safety or tolerability of the biological agent with ingestion of the composition, increase speed or efficiency of toxin and/or toxin precursor removal, increase survival of the biological agent, increase compatibility of the biological agent with one or more other biological agents or organisms, increase compatibility of the biological agent with the composition or a matrix carrying the biological agent (*e.g.*, hydrogel), and the like.

[0159] The complete disclosure of all patents, patent applications, and publications, and electronically available material cited herein are incorporated by reference in their entirety. Supplementary materials referenced in publications (such as supplementary tables, supplementary figures, supplementary materials and methods, and/or supplementary experimental data) are likewise incorporated by reference in their entirety. In the event that any inconsistency exists between the disclosure of the present application and the disclosure(s) of any document incorporated herein by reference, the disclosure of the present application shall govern. The foregoing detailed description and

examples have been given for clarity of understanding only. No unnecessary limitations are to be understood therefrom.

[0160] The description of embodiments of the disclosure is not intended to be exhaustive or to limit the disclosure to the precise form disclosed. While the specific embodiments of, and examples for, the disclosure are described herein for illustrative purposes, various equivalent modifications are possible within the scope of the disclosure.

[0161] Specific elements of any foregoing embodiments can be combined or substituted for elements in other embodiments. Moreover, the inclusion of specific elements in at least some of these embodiments may be optional, wherein further embodiments may include one or more embodiments that specifically exclude one or more of these specific elements. Furthermore, while advantages associated with certain embodiments of the disclosure have been described in the context of these embodiments, other embodiments may also exhibit such advantages, and not all embodiments need necessarily exhibit such advantages to fall within the scope of the disclosure.

[0162] As used herein and unless otherwise indicated, the terms “a” and “an” are taken to mean “one”, “at least one,” or “one or more”. Unless otherwise required by context, singular terms used herein shall include pluralities and plural terms shall include the singular.

[0163] Unless the context clearly requires otherwise, throughout the description and the claims, the words ‘comprise’, ‘comprising’, and the like are to be construed in an inclusive sense as opposed to an exclusive or exhaustive sense; that is to say, in the sense of “including, but not limited to”. Words using the singular or plural number also include the plural and singular number, respectively. Additionally, the words “herein,” “above,” and “below” and words of similar import, when used in this application, shall refer to this application as a whole and not to any particular portions of the application.

[0164] Unless otherwise indicated, all numbers expressing quantities of components, molecular weights, and so forth used in the specification and claims are to be understood as being modified in all instances by the term “about.” Accordingly, unless otherwise indicated to the contrary, the numerical parameters set forth in the specification and claims are approximations that may vary depending upon the desired properties sought to be obtained. At the very least, and not as an attempt to limit the doctrine of equivalents to the scope of the claims, each numerical parameter should at least be

construed in light of the number of reported significant digits and by applying ordinary rounding techniques.

[0165] Notwithstanding that numerical ranges and parameters are approximations, the numerical values set forth in the specific examples are reported as precisely as possible. All numerical values, however, inherently contain a range necessarily resulting from the standard deviation found in their respective testing measurements.

[0166] All headings are for the convenience of the reader and should not be used to limit the meaning of the text that follows the heading, unless so specified.

[0167] All of the references cited herein are incorporated by reference. Aspects of the disclosure can be modified, if necessary, to employ the systems, functions, and concepts of the above references and application to provide yet further embodiments of the disclosure. These and other changes can be made to the disclosure in light of the detailed description.

[0168] It will be appreciated that, although specific embodiments have been described herein for purposes of illustration, various modifications may be made without deviating from the scope and spirit of the disclosure.

TABLES

[0169] Table 1. Aluminum leaching from Zeolite at varying pH. Complete dealumination occurred when the pH was 2 in 30 min, showing that the zeolite mineral starts dissolving at pH 2. Al leaching was negligible in other pHs after 30 minutes, with very small amounts measure after 3 hours.

	pH 2 (mg/L)	pH 3 (mg/L)	pH 5 (mg/L)	pH 8 (mg/L)
NaZeolite 30 min.	100.00	----	----	----
Zeolite 30 min.	94.60	----	----	----
NaZeolite 3 h	132.6	0.014	0.005	0.004
Zeolite 3 h	130.6	0.012	0.009	0.002
Aluminum leaching from Zeolite at varying pH. Complete dealumination occurred when the pH was 2 in 30 min., that shows the zeolite mineral starts dissolving at pH of 2. Al leaching was negligible in other pHs after 30 min., with very small amounts measured after 3 hrs.				

[0170] Table 2 Aluminum release from aluminum cross-linked alginate beads at various pH's. Phosphate is absorbed efficiently by aluminium alginate hydrogel beads at low pH and at pH 6, similar to conditions in the stomach and upper intestines during meal digestion. Release of phosphorus and iron was also measured. Aluminium release is reduced compared to conventional tablet forms.

Item	Al (ppm)	P (ppm)	Fe (ppm)
PH3 , 25°C	8.00	0.12	0.19
PH6, 25°C	0.08	1.22	0.00
PH9, 25°C	0.08	2.62	0.01
PH6, 35 °C	0.01	0.63	0.02
PH6, 45 °C	0.16	0.65	0.04

EXAMPLES

Biological Removal of Phosphate

EXAMPLE 1. Ability of *Lactobacillus casei* encapsulated in hydrogel to absorb phosphate under gut-like conditions.

[0171] This example demonstrates, among other benefits, the ability of compositions of the disclosure to function under conditions within the GI tract. In the digestive system, most of ortho-phosphate is absorbed in the small intestine, which is also

where *Lactobacillus casei* can take up phosphate as it has the highest growth rate around pH 6 to 7, which lies the typical pH range of the small intestine. As a result, data were collected in which a pH switch mimicked the changes of pH exposure from the stomach to the small intestine. The results here showed that *L. casei* removed phosphate when the pH alternated between low pH (3-4) and close to neutral pH. It was found that *L. casei* can uptake phosphate at pH 6 (typical pH in the small intestine) and form polyP, hence yielding a net extraction from the liquid (which is equivalent to the gut fluids). The strategy of using *L. casei* enhances phosphate absorption in the small intestine. The beads avoid the escape of the bacteria, which can otherwise associate with the gut linings where phosphate can escape into the gut lumen. Instead, the polyP containing *L. casei* can be retained in the hydrogel, which can be excreted intact by the patient, hence lowering the net phosphate serum levels.

[0172] Examples are included of two types of bacteria that can absorb phosphate (FIG. 14, FIG. 15). *Lactobacillus casei* absorbs phosphate and stores it as polyphosphate (polyP) in response to pH changes. Other lactic acid bacteria that can be used include, but are not limited to, *Lactobacillus rhamnosus*, *Lactobacillus acidophilus*, *Lactobacillus coryniformis*, *Lactobacillus brevis*, *Bifidobacterium longum*, *Bifidobacterium adolescentis*, *Bifidobacterium breve*, *Lactiplantibacillus paraplantarum*, *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, and *Lactobacillus delbrueckii*.

[0173] The enhanced biological phosphate remover (EBPR) organism *Tetrasphaera japonica* stores phosphate at aerobic conditions for later use in metabolism along with the polymeric carbon source poly lactic acid. The EBPR metabolism requires oxygen which can be supplied by micro-encapsulated hydrogen peroxide included in the gel beads. The PAOs encapsulated in hydrogels to sequester phosphate in the intestine in this example include, but are not limited to, *Tetrasphaera japonica*, *Tetrasphaera elongata*, *Tetrasphaera australiensis*, *Tetrasphaera vanveenii*, *Tetrasphaera veronensis*, and *Microtholunatus phosphovorus*.

[0174] Stereomicroscopic images revealed the spherical shape of hydrogel encapsulating *Lactobacillus Casei* (FIG. 16), and fluorescent *in situ* hybridization showcased the uniform distribution of *Lactobacillus Casei* throughout the cross-section (FIG. 17).

EXAMPLE 2. Chemical Ammonium Binders.

[0175] Comparison of solid ammonium binders Zeolite and KW8 ion exchange resin. 100mg of solid ammonium binder is suspended in 26mg/L ammonium solution at pH 5.6, 37°C, with stirring at 110 rpm. FIG. 18 shows a comparison of ammonium adsorption capacity for different chemical ammonium adsorbents: Natural Zeolite, KW8 cation exchange resin, and MN500 modified Zeolite.

[0176] Enhancement of ammonium binding to Zeolite by pretreatment with sodium ions. Five samples were compared, natural Zeolite, Zeolite pre-treated with sodium (NaCl), Zeolite pre-treated with potassium (KCl), MN500 modified Zeolite, and KW8 ion exchange resin. 100mg of solid ammonium binder is suspended in 50mg/L ammonium solution at pH 5.6, 37°C, with stirring at 110 rpm. NaZeolite increased Ammonium removal efficiency of Zeolite (15-20 % during the time), and as such, Na exchange plays a role in determining the ammonium removal efficiency (FIG. 19).

[0177] Determining the pH optimum for ammonium binding by these adsorbents. 100mg of solid ammonium binder is suspended in 50mg/L ammonium solution at several pH values between 2 and 8, 37°C, with stirring at 110 rpm. Na Zeolite is found to be effective at pH as low as 3, but both adsorbents show optimum efficiency between pH 5 and 6 (FIG. 20).

[0178] pH-dependent leaching of aluminum ions from the Zeolite structure. It can be advantageous to avoid aluminum compounds in oral therapeutic agents, and this test can be beneficial in evaluating aluminum compounds in this scenario. It is found that Zeolite should not be exposed to pH 2 or lower (Table 1). This can be achieved by an enteric coating or embedding in a gel matrix that buffers pH or binds aluminum ions.

EXAMPLE 3. Chemical Phosphate Binders.

[0179] Aluminum leaching from aluminum cross-linked alginate. Aluminum alginate is an efficient absorber for phosphate. It is found that leaching of aluminum from Al-alginate gels is reduced compared to a tablet form of aluminum, which has been used as phosphate absorbing treatments previously (Table 2).

[0180] Comparison of aluminum and iron cross-linked alginates as phosphate absorbers. Ten of each type of alginate beads were tested at pH 5.5 for phosphate absorption in 2 hours. The iron cross-linked alginate was as good or better than aluminum alginate as phosphate absorber (FIG. 21).

[0181] Improving iron alginate phosphate absorber by reducing iron leaching with gel coatings. Iron alginate can be a desirable phosphate absorber because

it is efficient, and iron has low toxicity and is acceptable in oral dosage forms. One phosphate absorbing compound is ferric oxyhydroxide. The only side effects it causes can be believed to be due to the small amount of iron that leaches out of this solid substance as it passes through the GI tract. Hydrogel beads made of iron alginate can be coated with chitosan, which forms complexes with iron, to substantially reduce iron leakage from the gel beads (FIG. 22, FIG. 23). This technique can also be used with ferric oxyhydroxide, to further improve the therapeutic outlook for this promising phosphate absorber. Three chitosans of differing MWs were tested as coating on iron alginate beads. Phosphate removal efficiency was slightly reduced while iron loss was reduced about 8-fold (FIG. 23).

[0182] Two enhancements to iron alginate gel beads as phosphate absorbers – incorporation of iron oxyhydroxide (FeOOH) and kappa-carageenan coating. 200mg of FeOOH was stirred in 50mL of 130mg/L phosphate solution of several pH values (FIG. 24). FeOOH is highly effective even at pH 2. This makes it a good complement to FeCl₃ alginate. Effect of addition of kappa carrageenan coating on iron alginate beads and of incorporation of FeOOH into iron alginate beads were compared in a cumulative phosphate absorption assay solution that mimics the trip through the GI tract using 20 beads in 10mL of simulated gastric fluid containing 130mg/L phosphate (FIG. 25). The assay includes 3 pHs found in different parts of the GI tract and the predicted residence times in these regions as material transits the GI tract (FIG. 26).

EXAMPLE 4. Biological Removal of Ammonium.

[0183] The combination of ammonia oxidizing bacteria (AOB) with Anammox removes nitrogen in the form of ammonium or urea with minimal oxygen consumption (FIG. 27). Oxygen can be supplied in liquid form by hydrogen peroxide (H₂O₂), since many bacteria contain the catalase enzyme, which converts H₂O₂ to oxygen and water. There are uremic bound toxins (produced in the liver that can diffuse back into the gut from the blood) and uremic toxin precursors that are produced in the gut and which diffuse from the gut into the blood, which are prevented through consumption of a bubble tea composition, such that their formation in the liver is prevented. To eliminate any residual nitrite and nitrate resulting from urea/ammonia or p-cresol (or any other representative uremic bound toxin or toxin precursors, such as indole/tryptophan and indoxyl sulfate, respectively) removal, a combination of denitrifying strains is utilized to create cross-feeding networks for simultaneous nitrification and denitrification. In this

process, denitrifiers reduce nitrate and nitrite, which can be produced from nitrification (a secondary process from urea removal as shown in FIG. 28), or can simply be residual external electron acceptors used in p-cresol removal (FIG. 29). The denitrifiers that can be employed include, but are not limited to, strains *Clostridium spp.*, *Thiobacillus denitrificans*, *Micrococcus denitrificans*, and some species of *Serratia*, *Pseudomonas*, *Achromobacter*, or any combination thereof.

[0184] Influence of hydrogel bead size on aerobic and anaerobic processes. Small hydrogel beads allow oxygen produced by H_2O_2 to diffuse out from the core. Due to their smaller size, hydrogel beads have a higher aerobic zone volume per unit mass compared to larger hydrogel beads. In larger beads, oxygen penetration is limited, creating an anaerobic layer at the outer edge while the core remains fully oxygenated (FIG. 30).

EXAMPLE 5. Biological removal of aromatic uremic toxin precursors.

[0185] Examples are included for bacteria degrading the aromatic uremic toxin precursors indole and p-cresol. *Desulfobacula indolicum* is one of the few pure cultures known to degrade indole. It is an anaerobic sulfur-reducing bacterium. FIG. 31 shows *D. indolicum* encapsulated in PEGDMA hydrogel beads degrading indole in synthetic medium. *T. aminoaromatica* cells can be grown readily on acetate-containing medium and then harvested and concentrated into PVA/SA gel beads (FIG. 32). FIG. 33 shows that the cells embedded in the hydrogel are more resistant to the growth inhibition caused by p-cresol at concentrations above 1mM. FIG. 34 shows an example of microbial sink of p-cresol by a hydrogel encapsulated *Thauera aminoaromatica* S2 significantly faster as compared to that of a culture not encapsulated.

[0186] *T. aminoaromatica* S2 encapsulated in PVA-SA hydrogels. The encapsulation of *T. aminoaromatica* S2 was performed with hydrogel formula, polyvinyl alcohol-sodium alginate (PVA-SA). High biomass concentrations were obtained by cultivation in media supplemented with acetate and NO_3^- as the electron acceptor. The 2.1×10^9 cell suspension was encapsulated in the PVA-SA hydrogel. After encapsulation, the hydrogels were transferred into the media supplemented with 0.3 mM p-cresol and 15 mM NO_3^- . The hydrogels showed complete removal of p-cresol within 4 days at 30 °C. On day 6 of the incubation period, the bacteria cells were observed in the bulk liquid of the culture, and these cells accounted for 0.17 % of the initial cells being encapsulated. In order to verify degradation activity by encapsulated hydrogels (and not the suspended

culture), a comparative analysis was performed, in which free cells present in the original culture were separated into another culture and incubated with media supplemented with p-cresol. The p-cresol degradation was compared with the culture with the presence of both hydrogels and the free cells. Both cultures showed comparable levels of cells on the starting day ($2.1 - 2.5 \times 10^5$ cells/mL bulk liquid). After addition of 0.1 mM p-cresol and 14 mM NO_3^- to both cultures, the p-cresol degradation rates of the hydrogel cultures was approximately four times higher than the culture of free cells only.

[0187] Performance in the environment mimicking colon condition. The temperature in human colon is $\sim 37^\circ\text{C}$ and the pH varies from 5-7, and neither is optimal to *T. aminoaromatica* S2. The proximal region presents lower pH (~ 5) and the distal region presents neutral pH (~ 7). In order to remediate anticipated stresses to *T. aminoaromatica* p-cresol degradation in the colon environments, an additional step of p-cresol incubation to biomass cells, was performed.

[0188] At pH 7 condition, the encapsulation of 1.1×10^9 cell material into the media supplemented with 0.6 mM p-cresol and 4 mM NO_3^- showed complete removal of p-cresol within 24 hours. Doubling cell biomass resulted in the complete removal of 0.5 - 0.9 mM p-cresol within 16 hours or 6 hours at maximum. With the maximum removal rate, the patient with target removal of 0.75 mmol per day can need to consume 14 mL of hydrogels. On hour 24 of the incubation period, the free cells were measurable in the bulk liquid (0.17 % of the initial cells). According to the comparative test, the culture with hydrogel completely removed 0.4 p-cresol within 24 hours, whereas the culture without hydrogel did not show the decrease of p-cresol in the same incubation period.

[0189] Another culture, *Geobacter metallireducens*, which can also degrade acetate, benzoate, or p-cresol using iron III (ferric ion) as electron acceptor, has also been tested. This bacterium can also use solid iron III oxide as the ferric source. This offers the advantage of being able to provide a solid electron acceptor co-immobilized in gel beads with the bacteria, providing a way of supplying the culture and the required electron acceptor together in a streamlined package. FIG. 35 shows that the reddish and non-magnetic ferric oxide was converted to the black and magnetic ferrous oxide as the culture consumes acetate in the present experiments. It can be the case that *G. metallireducens* can also couple p-cresol oxidation to Fe oxide similar to its oxidation of p-cresol to soluble Fe-citrate.

[0190] Some of the ferric iron produced by the *G. metallireducens* was solubilized and can be detected in the culture medium using the ferrozine assay, as shown in FIG. 36.

[0191] **Provision of slow-release oxygen to microbes encapsulated in hydrogel beads.** This provision of slow-release oxygen enables the use of mixed aerobic and anaerobic metabolisms described above, such as dPAOs performing EBPR and denitrifiers coupled with nitrate/nitrite/NO/N₂O-reducing organisms. The technique involves small, 10-to-100-micron diameter polymeric microcapsules (MCs) loaded with hydrogen peroxide (H₂O₂) in the hydrogel beads along with the oxygen utilizing organisms. In a layered gel bead, the core can be the aerobic zone that harbors aerobic organisms, while the outer layer hosts the anaerobic organisms (FIG. 37). An example is provided of an oxygen-requiring nitrifier, *Nitrospira inopinata*, encapsulated in gel beads along with H₂O₂ MCs and oxidizing ammonium to nitrite in an anerobic culture bottle, in which the headspace is filled with nitrogen gas and the culture medium is purged of oxygen. FIG. 38 showed the formation of oxygen gas bubbles from the H₂O₂ MCs within hydrogel beads.

[0192] **Solid carbon substrates co-encapsulated with bacteria to produce short chain fatty acids.** Metabolizable solids, such as cellulose and lignocellulosic plant matter, co-encapsulated in a hydrogel with bacterial cells can provide a convenient way of supplying carbon sources that can be converted to SCFAs by encapsulated bacteria; these can include, but are not limited to: *Faecalibacterium prausnitzii*, *Eubacterium rectale*, *Roseburia inulinivorans*, *Roseburia intestinalis*, *Anaerostipes butyraticus*, *Anaerostipes caccae*, *Butyricicoccus pullicaecorum*, *Lactococcus lactis*, *Leuconostoc*, *Pediococcus*, *Bifidobacterium*, *Enterococcus*, *Lacticaseibacillus casei*, *Lactobacillaceae*, *Lachnospiraceae*, *Ruminococcus*, *Streptococcus thermophilus*, *Peptostreptococcaceae*, *Oenococcus oeni*, *Lactobacillus brevis*, *Limosilactobacillus fermentum*, *Lactobacillus helveticus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Propionibacterium freudenreichii*, *Bifidobacteriaceae*, *Propionibacterium*, *Hafnia*, *Gemella*, *Arthrobacter*, *Bifidobacterium animalis*, or any combination thereof. This makes it possible to combine strains and form cross-feeding networks as described above to, for instance, encapsulate denitrifying microbes (such as *Thiobacillus denitrificans*, *Micrococcus denitrificans*, and some species of *Serratia*, *Pseudomonas*, and *Achromobacter*) to consume nitrate left unused by co-encapsulated p-cresol degrader, such as for example, *Thauera*

aminoaromatica, as it degrades p-cresol. An example is provided of a culture of denitrifiers co-encapsulated with lignocellulosic Kenaf converting the solid Kenaf particles to the SCFA acetic acid (FIG. 39). Along with this, FIG. 40 shows an example of fungi encapsulating with kenaf fibers to produce SCFAs. In addition, methanogens can lower the otherwise inhibitory H₂ as well, as can utilize acetate produced during fermentation (FIG. 41).

[0193] Methanogens. The methanogen can include at least one species selected from: *Methanobacteria*, *Ca. Methanofastidiosum*, *Methanosaeta*, *Methanobacterium*, *Methanosarcina*, *Methanococcus*, *Methanospirillum*, *Methanolinea*, or any combination thereof.

NON-LIMITING EMBODIMENTS

[0194] While general features of the disclosure are described and shown and particular features of the disclosure are set forth in the claims, the following non-limiting embodiments relate to features, and combinations of features, that are explicitly envisioned as being part of the disclosure. The following non-limiting Embodiments contain elements that are modular and can be combined with each other in any number, order, or combination to form a new non-limiting Embodiment, which can itself be further combined with other non-limiting Embodiments.

[0195] Embodiment 1. A composition for ingestion by a subject and removal of one or more toxins and/or one or more toxin precursors from the subject's digestive system, the composition comprising: hydrogel particles comprising agents encapsulated within the hydrogel particles, wherein the agents are configured to reduce local concentrations of the one or more toxins and/or the one or more toxin precursors from the subject's digestive system.

[0196] Embodiment 2. The composition of Embodiment 1 or any other Embodiment, wherein the composition is formulated as an ingestible bubble tea composition and the hydrogel particles are configured as gel beads of the bubble tea composition.

[0197] Embodiment 3. The composition of any one of Embodiments 1-2 or any other Embodiment, wherein the composition is formulated as an ingestible bubble tea composition that comprises larger digestible boba particles that contain smaller hydrogel particles therein that comprise at least a portion of the agents therein.

[0198] Embodiment 4. The composition of Embodiment 3 or any other Embodiment, wherein the larger digestible boba particles further contain one or more probiotic organisms and/or one or more prebiotic substances.

[0199] Embodiment 5. The composition of Embodiment 4 or any other Embodiment, wherein the larger digestible boba particles are comprised of one or more colloids configured to protect the one or more probiotic organisms, the one or more prebiotic substances, and the smaller hydrogel particles from stomach acid.

[0200] Embodiment 6. The composition of Embodiment 5 or any other Embodiment, wherein the one or more colloids comprises a gum, a thickener, xanthan gum, carrageenan, one or more mannans, one or more glucans, agar, or any combination thereof.

[0201] Embodiment 7. The composition of any one of Embodiments 1-6 or any other Embodiment, wherein the hydrogel particles are configured for: removal of multiple different toxins, and/or removal of multiple different toxin precursors, from the subject's digestive system.

[0202] Embodiment 8. The composition of any one of Embodiments 1-7 or any other Embodiment, wherein at least a portion of the agents comprises phosphate-accumulating organisms (PAOs), wherein the PAOs are denitrifying PAOs (dPAOs) and/or are configured for enhanced biological phosphate removal (EBPR PAOs).

[0203] Embodiment 9. The composition of any one of Embodiments 1-8 or any other Embodiment, wherein at least a portion of the agents comprises a heterotrophic denitrifier, an autotrophic denitrifier, one or more organisms configured for aerobic removal of one or more aromatics, or any combination thereof.

[0204] Embodiment 10. The composition of any one of Embodiments 1-9 or any other Embodiment, wherein at least a portion of the agents comprises Comammox bacteria, ammonium oxidizing bacteria, ammonium oxidizing archaea, nitrite oxidizing bacteria, autotrophic denitrifiers, Anammox bacteria, iron oxidizers, heterotrophic denitrifiers, denitrifying PAOs, or any combination thereof.

[0205] Embodiment 11. The composition of any one of Embodiments 1-10 or any other Embodiment, wherein at least a portion of the agents comprises fermenting bacteria configured to convert fibrous material to short chain fatty acids (SCFAs).

[0206] Embodiment 12. The composition of any one of Embodiments 1-11 or any other Embodiment, wherein at least a portion of the hydrogel particles comprises a

fibrous material selected from the group consisting of: a cellulosic material, a lignocellulosic particle, a chia seed, bagasse, flax, hemp, jute, ramie, a quinoa seed, kenaf, and any combination thereof.

[0207] Embodiment 13. The composition of any one of Embodiments 1-12 or any other Embodiment, wherein at least a portion of the hydrogel particles comprises a metal catalyst selected from the group consisting of: iron, copper, manganese, or any combination thereof, to function as an electron carrier or electron donor/acceptor.

[0208] Embodiment 14. The composition of any one of Embodiments 1-13 or any other Embodiment, wherein a PBUT degrader is included as at least one agent and is selected from the group consisting of: *Shewanella oneidensis*, *Geobacter sulfurreducens*, *Desulfobacula indolicum*, *Thauera aminoaromatica*, *Geobacter metallireducans*, *Pseudomonas putida*, and any combination thereof.

[0209] Embodiment 15. The composition of any one of Embodiments 1-14 or any other Embodiment, wherein a PAO is included as at least one agent and is selected from the group consisting of: *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Lactobacillus acidophilus*, *Lactobacillus coryniformis*, *Lactobacillus brevis*, *Bifidobacterium longum*, *Bifidobacterium adolescentis*, *Bifidobacterium breve*, and *Lactiplantibacillus paraplantarum*, *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, *Lactobacillus delbrueckii*, *Tetrasphaera japonica*, *Tetrasphaera elongata*, *Tetrasphaera australiensis*, *Tetrasphaera vanveenii*, *Tetrasphaera veronensis*, *Microthumatus phosphovorus*, and any combination thereof.

[0210] Embodiment 16. The composition of any one of Embodiments 1-15 or any other Embodiment, wherein hydrogel particles of the portion of the hydrogel particles comprise oxygen precursors and enzymes configured for conversion of the oxygen precursors into at least oxygen (O₂) for use of the O₂ as an electron acceptor by one or more PAOs, one or more PBUT degraders, and/or one or more nitrifiers.

[0211] Embodiment 17. The composition of any one of Embodiments 1-16 or any other Embodiment, wherein the oxygen precursors are hydrogen peroxide (H₂O₂) molecules, and the enzymes are catalase enzymes.

[0212] Embodiment 18. The composition of Embodiment 17 or any other Embodiment, wherein the H₂O₂ molecules are encapsulated within inner cores of the hydrogel particles for release from the inner cores and reaction with the catalase enzymes.

[0213] Embodiment 19. The composition of any one of Embodiments 1-18 or any other Embodiment, wherein at least a portion of the hydrogel particles comprises nitrate, nitrite, or both, for use as electron acceptors by one or more PAOs, one or more PBUT degraders, and/or one or more nitrifiers.

[0214] Embodiment 20. The composition of any one of Embodiments 1-19 or any other Embodiment, wherein at least a portion of the agents comprises aerobic and anaerobic bacteria configured for: conversion of urea and protein-bound uremic toxins (PBUTs) to nitrite; and conversion of nitrite to nitrogen (N₂) and/or carbon dioxide (CO₂) with use of organic carbon, hydrogen sulfide (H₂S), and/or ammonium as one or more electron donors.

[0215] Embodiment 21. The composition of any one of Embodiments 1-20 or any other Embodiment, wherein at least a portion of the agents comprises aerobic ammonium oxidizing bacteria (AOB), aerobic ammonium oxidizing archaea (AOA), nitrite oxidizing bacteria (NOB), anaerobic ammonium oxidizing bacteria (Annamox), or any combination thereof.

[0216] Embodiment 22. The composition of any one of Embodiments 1-21 or any other Embodiment, wherein at least a portion of the agents comprises a nitrifying *Nitrospira*, *Nitrobacter*, *Nitrosomonas*, *Nitrospinae*, *Nitosococcus*, *Nitrococcus*, *Nitrososphaera viennensis*, *Nitrosospira lacus*, *Nitrosomonas ureae*, *Nitrosomonas nitrosa*, *Nitrospira muscovinensis*, *Nitrososphaera viennensis*, *Nitrosotenuis aquarius*, *Nitrosopumilus maritimus*, *Nitrosarchaeum koreense*, a bacterium configured for denitrification, *Clostridium* spp., *Thiobacillus denitrificans*, *Micrococcus denitrificans*, a species of *Serratia*, *Pseudomonas*, *Achromobacter*, or any combination thereof.

[0217] Embodiment 23. The composition of any one of Embodiments 1-22 or any other Embodiment, wherein at least a portion of the agents comprises complete ammonia oxidizing (Comammox) bacteria, anaerobic ammonium oxidizing (Anammox) bacteria, autotrophic and heterotrophic prokaryotic and eukaryotic denitrifiers, or any combination thereof.

[0218] Embodiment 24. The composition of any one of Embodiments 1-23 or any other Embodiment, wherein at least a portion of the Anammox bacteria comprises: *Candidatus Brocadia*, *Candidatus Kuenenia*, *Candidatus Anammoxoglobus*, *Candidatus Jettenia*, *Candidatus Scalindua*, or any combination thereof.

[0219] Embodiment 25. The composition of any one of Embodiments 1-24 or any other Embodiment, wherein at least a portion of the agents comprises one or more SCFAs bacteria selected from the group consisting of: *Faecalibacterium prausnitzii*, *Eubacterium rectale*, *Roseburia inulinivorans*, *Roseburia intestinalis*, *Anaerostipes butyraticus*, *Anaerostipes caccae*, *Butyricicoccus pullicaecorum*, *Lactococcus lactis*, *Leuconostoc*, *Pediococcus*, *Bifidobacterium*, *Enterococcus*, *Lactocaseibacillus casei*, *Lactobacillaceae*, *Lachnospiraceae*, *Ruminococcus*, *Streptococcus thermophilus*, *Peptostreptococcaceae*, *Oenococcus oeni*, *Lactobacillus brevis*, *Limosilactobacillus fermentum*, *Lactobacillus helveticus*, *Lactobacillus delbrueckii subsp. bulgaricus*, *Propionibacterium freudenreichii*, *Bifidobacteriaceae*, *Propionibacterium*, *Hafnia*, *Gemella*, *Arthrobacter*, *Bifidobacterium animalis*, and any combination thereof.

[0220] Embodiment 26. The composition of any one of Embodiments 1-25 or any other Embodiment, wherein at least a portion of the agents comprises a fungi selected from the group consisting of: *Saccharomyces*, *Aspergillus*, *Galactomyces*, *Geotrichum*, *Saprochaete*, *Candida*, *Malassezia*, a *Fusarium oxysporum* strain, *Cylindrocarpon tonkinense*, *Cladosporium*, and any combination thereof.

[0221] Embodiment 27. The composition of any one of Embodiments 1-26 or any other Embodiment, wherein at least a portion of the agents comprises a methanogen selected from the group consisting of: *Methanobacteria*, *Ca. Methanofastidiosum*, *Methanosaeta*, *Methanobacterium*, *Methanosarcina*, *Methanococcus*, *Methanospirillum*, *Methanolinea*, and any combination thereof.

[0222] Embodiment 28. The composition of any one of Embodiments 1-27 or any other Embodiment, wherein at least a portion of the agents comprises urease, urease-releasing microbes, or any combination thereof.

[0223] Embodiment 29. The composition of any one of Embodiments 1-28 or any other Embodiment, wherein at least a portion of the agents comprises one or more ammonia binders configured for ion-exchange removal of ammonia.

[0224] Embodiment 30. The composition of Embodiment 29 or any other Embodiment, wherein the one or more ammonia binders comprises one or more zeolites configured for removal of one or more toxins and/or one or more toxin precursors.

[0225] Embodiment 31. The composition of any one of Embodiments 1-30 or any other Embodiment, wherein at least a portion of the hydrogel particles comprises one or more phosphate binders.

[0226] Embodiment 32. The composition of Embodiment 31 or any other Embodiment, wherein the one or more phosphate binders comprises ferric oxyhydroxide.

[0227] Embodiment 33. The composition of any one of Embodiments 1-32 or any other Embodiment, wherein at least a portion of the hydrogel particles comprises iron cross-linked alginate.

[0228] Embodiment 34. The composition of any one of Embodiments 1-33 or any other Embodiment, wherein at least a portion of the hydrogel particles comprises iron cross-linked alginate and ferric oxyhydroxide.

[0229] Embodiment 35. The composition of any one of Embodiments 1-34 or any other Embodiment, wherein at least a portion of the hydrogel particles comprises iron cross-linked alginate or iron cross-linked alginate and ferric oxyhydroxide with a coating of chitosan which minimizes release of iron and thus avoids associated side effects.

[0230] Embodiment 36. The composition of any one of Embodiments 1-35 or any other Embodiment, wherein at least a portion of the hydrogel particles comprises a net electrostatic charge at cores of the hydrogel particles such that the hydrogel particles are pH responsive.

[0231] Embodiment 37. The composition of any one of Embodiments 1-36 or any other Embodiment, further comprising a vitamin, a cofactor, zinc, iron, calcium, bismuth subsalicylate, iron hydroxide, or any combination thereof.

[0232] Embodiment 38. The composition of any one of Embodiments 1-37 or any other Embodiment, wherein at least a portion of the hydrogel particles is active at an alkaline pH and comprises one or more shell layers configured to: prevent the release of cells entrapped near a surface of the hydrogel particles, and provide a pH-responsive barrier to diffusion.

[0233] Embodiment 39. The composition of any one of Embodiments 1-38 or any other Embodiment, wherein at least a portion of the hydrogel particles is active at an acidic pH and comprises one or more phosphate binders and swells at acidic pH levels and contracts at neutral pH levels.

[0234] Embodiment 40. The composition of any one of Embodiments 1-39 or any other Embodiment, wherein at least a portion of the hydrogel particles comprises a coating that protects the hydrogel particles from degradation in the stomach of the subject for delivery of protected hydrogel particles to the small intestine, the colon, or both, for

removal of one or more toxins and/or one or more toxin precursors from the small intestine, the colon, or both.

[0235] Embodiment 41. The composition of any one of Embodiments 1-40 or any other Embodiment, wherein a least a portion of the hydrogel particles are comprised of a plurality of different-sized hydrogel particles for reduced diffusion constraints with smaller hydrogel particles and increased diffusion constraints with larger hydrogel particles.

[0236] Embodiment 42. The composition of any one of Embodiments 1-41 or any other Embodiment, wherein a least a portion of the hydrogel particles are comprised of a plurality of different-sized hydrogel particles for a greater anaerobic volume fraction with larger sizes and a greater aerobic volume fraction with smaller sizes.

[0237] Embodiment 43. The composition of any one of Embodiments 1-42 or any other Embodiment, further comprising one or more palatable agents for increased palatability of the composition.

[0238] Embodiment 44. A kit, comprising the composition of any one of Embodiments 1-43 or any other Embodiment, and an instructional material configured for direction of use of the kit in a method of treating or preventing onset or progression of one or more diseases or disorders.

[0239] Embodiment 45. The kit of Embodiment 44 or any other Embodiment, wherein the one or more diseases or disorders comprises kidney disease or chronic kidney disease.

[0240] Embodiment 46. A method of treating or preventing onset or progression of one or more diseases or disorders, the method comprising administering the composition of any one of Embodiments 1-43 or any other Embodiment to a subject in need thereof.

[0241] Embodiment 47. The method of Embodiment 46 or any other Embodiment, wherein the one or more diseases or disorders comprises kidney disease or chronic kidney disease.

[0242] Embodiment 48. Use of the composition of any one of Embodiments 1-43 or any other Embodiment in the preparation of a medicament for administration to a subject for treating or preventing onset or progression of one or more diseases or disorders in the subject.

[0243] While illustrative embodiments have been illustrated and described, it will be appreciated that various changes can be made therein without departing from the spirit and scope of the disclosure.

CLAIMS

The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:

1. A composition for ingestion by a subject and removal of one or more toxins and/or one or more toxin precursors from the subject's digestive system, the composition comprising: hydrogel particles comprising agents encapsulated within the hydrogel particles, wherein the agents are configured to reduce local concentrations of the one or more toxins and/or the one or more toxin precursors from the subject's digestive system.

2. The composition of claim 1, wherein the composition is formulated as an ingestible bubble tea composition and the hydrogel particles are configured as gel beads of the bubble tea composition.

3. The composition of any one of claims 1-2, wherein the composition is formulated as an ingestible bubble tea composition that comprises larger digestible boba particles that contain smaller hydrogel particles therein that comprise at least a portion of the agents therein.

4. The composition of claim 3, wherein the larger digestible boba particles further contain one or more probiotic organisms and/or one or more prebiotic substances.

5. The composition of claim 4, wherein the larger digestible boba particles are comprised of one or more colloids configured to protect the one or more probiotic organisms, the one or more prebiotic substances, and the smaller hydrogel particles from stomach acid.

6. The composition of claim 5, wherein the one or more colloids comprises a gum, a thickener, xanthan gum, carrageenan, one or more mannans, one or more glucans, agar, or any combination thereof.

7. The composition of any one of claims 1-6, wherein the hydrogel particles are configured for: removal of multiple different toxins, and/or removal of multiple different toxin precursors, from the subject's digestive system.

8. The composition of any one of claims 1-7, wherein at least a portion of the agents comprises phosphate-accumulating organisms (PAOs), wherein the PAOs are denitrifying PAOs (dPAOs) and/or are configured for enhanced biological phosphate removal (EBPR PAOs).

9. The composition of any one of claims 1-8, wherein at least a portion of the agents comprises a heterotrophic denitrifier, an autotrophic denitrifier, one or more organisms configured for aerobic removal of one or more aromatics, or any combination thereof.

10. The composition of any one of claims 1-9, wherein at least a portion of the agents comprises Commamox bacteria, ammonium oxidizing bacteria, ammonium oxidizing archaea, nitrite oxidizing bacteria, autotrophic denitrifiers, Anammox bacteria, iron oxidizers, heterotrophic denitrifiers, denitrifying PAOs, or any combination thereof.

11. The composition of any one of claims 1-10, wherein at least a portion of the agents comprises fermenting bacteria configured to convert fibrous material to short chain fatty acids (SCFAs).

12. The composition of any one of claims 1-11, wherein at least a portion of the hydrogel particles comprises a fibrous material selected from the group consisting of: a cellulosic material, a lignocellulosic particle, a chia seed, bagasse, flax, hemp, jute, ramie, a quinoa seed, kenaf, and any combination thereof.

13. The composition of any one of claims 1-12, wherein at least a portion of the hydrogel particles comprises a metal catalyst selected from the group consisting of: iron, copper, manganese, or any combination thereof, to function as an electron carrier or electron donor/acceptor.

14. The composition of any one of claims 1-13, wherein a PBUT degrader is included as at least one agent and is selected from the group consisting of: *Shewanella oneidensis*, *Geobacter sulfurreducens*, *Desulfobacula indolicum*, *Thauera aminoaromatica*, *Geobacter metallireducans*, *Pseudomonas putida*, and any combination thereof.

15. The composition of any one of claims 1-14, wherein a PAO is included as at least one agent and is selected from the group consisting of: *Lactobacillus caseii*, *Lactobacillus rhamnosus*, *Lactobacillus acidophilus*, *Lactobacillus coryniformis*, *Lactobacillus brevis*, *Bifidobacterium longum*, *Bifidobacterium adolescentis*, *Bifidobacterium breve*, and *Lactiplantibacillus paraplantarum*, *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, *Lactobacillus delbrueckii*, *Tetrasphaera japonica*, *Tetrasphaera elongata*, *Tetrasphaera australiensis*, *Tetrasphaera vanveenii*, *Tetrasphaera veronensis*, *Microtholus phosphovoratus*, and any combination thereof.

16. The composition of any one of claims 1-15, wherein hydrogel particles of the portion of the hydrogel particles comprise oxygen precursors and enzymes configured for conversion of the oxygen precursors into at least oxygen (O₂) for use of the O₂ as an electron acceptor by one or more PAOs, one or more PBUT degraders, and/or one or more nitrifiers.

17. The composition of any one of claims 1-16, wherein the oxygen precursors are hydrogen peroxide (H₂O₂) molecules, and the enzymes are catalase enzymes.

18. The composition of claim 17, wherein the H₂O₂ molecules are encapsulated within inner cores of the hydrogel particles for release from the inner cores and reaction with the catalase enzymes.

19. The composition of any one of claims 1-18, wherein at least a portion of the hydrogel particles comprises nitrate, nitrite, or both, for use as electron acceptors by one or more PAOs, one or more PBUT degraders, and/or one or more nitrifiers.

20. The composition of any one of claims 1-19, wherein at least a portion of the agents comprises aerobic and anaerobic bacteria configured for:

conversion of urea and protein-bound uremic toxins (PBUTs) to nitrite; and

conversion of nitrite to nitrogen (N₂) and/or carbon dioxide (CO₂) with use of organic carbon, hydrogen sulfide (H₂S), and/or ammonium as one or more electron donors.

21. The composition of any one of claims 1-20, wherein at least a portion of the agents comprises aerobic ammonium oxidizing bacteria (AOB), aerobic ammonium oxidizing archaea (AOA), nitrite oxidizing bacteria (NOB), anaerobic ammonium oxidizing bacteria (Annamox), or any combination thereof.

22. The composition of any one of claims 1-21, wherein at least a portion of the agents comprises a nitrifying *Nitrospira*, *Nitrobacter*, *Nitrosomonas*, *Nitrospinae*, *Nitrosococcus*, *Nitrococcus*, *Nitrososphaera viennensis*, *Nitrospira lacus*, *Nitrosomonas ureae*, *Nitrosomonas nitrosa*, *Nitrospira muscovicinensis*, *Nitrososphaera viennensis*, *Nitrosotenuis aquarius*, *Nitrosopumilus maritimus*, *Nitrosarchaeum koreense*, a bacterium configured for denitrification, *Clostridium* spp., *Thiobacillus denitrificans*, *Micrococcus denitrificans*, a species of *Serratia*, *Pseudomonas*, *Achromobacter*, or any combination thereof.

23. The composition of any one of claims 1-22, wherein at least a portion of the agents comprises complete ammonia oxidizing (Comammox) bacteria, anaerobic

ammonium oxidizing (Anammox) bacteria, autotrophic and heterotrophic prokaryotic and eukaryotic denitrifiers, or any combination thereof.

24. The composition of any one of claims 1-23, wherein at least a portion of the Anammox bacteria comprises: *Candidatus Brocadia*, *Candidatus Kuenenia*, *Candidatus Anammoxoglobus*, *Candidatus Jettenia*, *Candidatus Scalindua*, or any combination thereof.

25. The composition of any one of claims 1-24, wherein at least a portion of the agents comprises one or more SCFAs bacteria selected from the group consisting of: *Faecalibacterium prausnitzii*, *Eubacterium rectale*, *Roseburia inulinivorans*, *Roseburia intestinalis*, *Anaerostipes butyraticus*, *Anaerostipes caccae*, *Butyricicoccus pullicaecorum*, *Lactococcus lactis*, *Leuconostoc*, *Pediococcus*, *Bifidobacterium*, *Enterococcus*, *Lacticaseibacillus casei*, *Lactobacillaceae*, *Lachnospiraceae*, *Ruminococcus*, *Streptococcus thermophilus*, *Peptostreptococcaceae*, *Oenococcus oeni*, *Lactobacillus brevis*, *Limosilactobacillus fermentum*, *Lactobacillus helveticus*, *Lactobacillus delbrueckii subsp. bulgaricus*, *Propionibacterium freudenreichii*, *Bifidobacteriaceae*, *Propionibacterium*, *Hafnia*, *Gemella*, *Arthrobacter*, *Bifidobacterium animalis*, and any combination thereof.

26. The composition of any one of claims 1-25, wherein at least a portion of the agents comprises a fungi selected from the group consisting of: *Saccharomyces*, *Aspergillus*, *Galactomyces*, *Geotrichum*, *Saprochaete*, *Candida*, *Malassezia*, a *Fusarium oxysporum* strain, *Cylindrocarpon tonkinense*, *Cladosporium*, and any combination thereof.

27. The composition of any one of claims 1-26, wherein at least a portion of the agents comprises a methanogen selected from the group consisting of: *Methanobacteria*, *Ca. Methanofastidiosum*, *Methanosaeta*, *Methanobacterium*, *Methanosarcina*, *Methanococcus*, *Methanospirillum*, *Methanolinea*, and any combination thereof.

28. The composition of any one of claims 1-27, wherein at least a portion of the agents comprises urease, urease-releasing microbes, or any combination thereof.

29. The composition of any one of claims 1-28, wherein at least a portion of the agents comprises one or more ammonia binders configured for ion-exchange removal of ammonia.

30. The composition of claim 29, wherein the one or more ammonia binders comprises one or more zeolites configured for removal of one or more toxins and/or one or more toxin precursors.

31. The composition of any one of claims 1-30, wherein at least a portion of the hydrogel particles comprises one or more phosphate binders.

32. The composition of claim 31, wherein the one or more phosphate binders comprises ferric oxyhydroxide.

33. The composition of any one of claims 1-32, wherein at least a portion of the hydrogel particles comprises iron cross-linked alginate.

34. The composition of any one of claims 1-33, wherein at least a portion of the hydrogel particles comprises iron cross-linked alginate and ferric oxyhydroxide.

35. The composition of any one of claims 1-34, wherein at least a portion of the hydrogel particles comprises iron cross-linked alginate or iron cross-linked alginate and ferric oxyhydroxide with a coating of chitosan which minimizes release of iron and thus avoids associated side effects.

36. The composition of any one of claims 1-35, wherein at least a portion of the hydrogel particles comprises a net electrostatic charge at cores of the hydrogel particles such that the hydrogel particles are pH responsive.

37. The composition of any one of claims 1-36, further comprising a vitamin, a cofactor, zinc, iron, calcium, bismuth subsalicylate, iron hydroxide, or any combination thereof.

38. The composition of any one of claims 1-37, wherein at least a portion of the hydrogel particles is active at an alkaline pH and comprises one or more shell layers configured to: prevent the release of cells entrapped near a surface of the hydrogel particles, and provide a pH-responsive barrier to diffusion.

39. The composition of any one of claims 1-38, wherein at least a portion of the hydrogel particles is active at an acidic pH and comprises one or more phosphate binders and swells at acidic pH levels and contracts at neutral pH levels.

40. The composition of any one of claims 1-39, wherein at least a portion of the hydrogel particles comprises a coating that protects the hydrogel particles from degradation in the stomach of the subject for delivery of protected hydrogel particles to the small intestine, the colon, or both, for removal of one or more toxins and/or one or more toxin precursors from the small intestine, the colon, or both.

41. The composition of any one of claims 1-40, wherein a least a portion of the hydrogel particles are comprised of a plurality of different-sized hydrogel particles for reduced diffusion constraints with smaller hydrogel particles and increased diffusion constraints with larger hydrogel particles.

42. The composition of any one of claims 1-41, wherein a least a portion of the hydrogel particles are comprised of a plurality of different-sized hydrogel particles for a greater anaerobic volume fraction with larger sizes and a greater aerobic volume fraction with smaller sizes.

43. The composition of any one of claims 1-42, further comprising one or more palatable agents for increased palatability of the composition.

44. A kit, comprising the composition of any one of claims 1-43, and an instructional material configured for direction of use of the kit in a method of treating or preventing onset or progression of one or more diseases or disorders.

45. The kit of claim 44, wherein the one or more diseases or disorders comprises kidney disease or chronic kidney disease.

46. A method of treating or preventing onset or progression of one or more diseases or disorders, the method comprising administering the composition of any one of claims 1-43 to a subject in need thereof.

47. The method of claim 46, wherein the one or more diseases or disorders comprises kidney disease or chronic kidney disease.

48. Use of the composition of any one of claims 1-43 in the preparation of a medicament for administration to a subject for treating or preventing onset or progression of one or more diseases or disorders in the subject.

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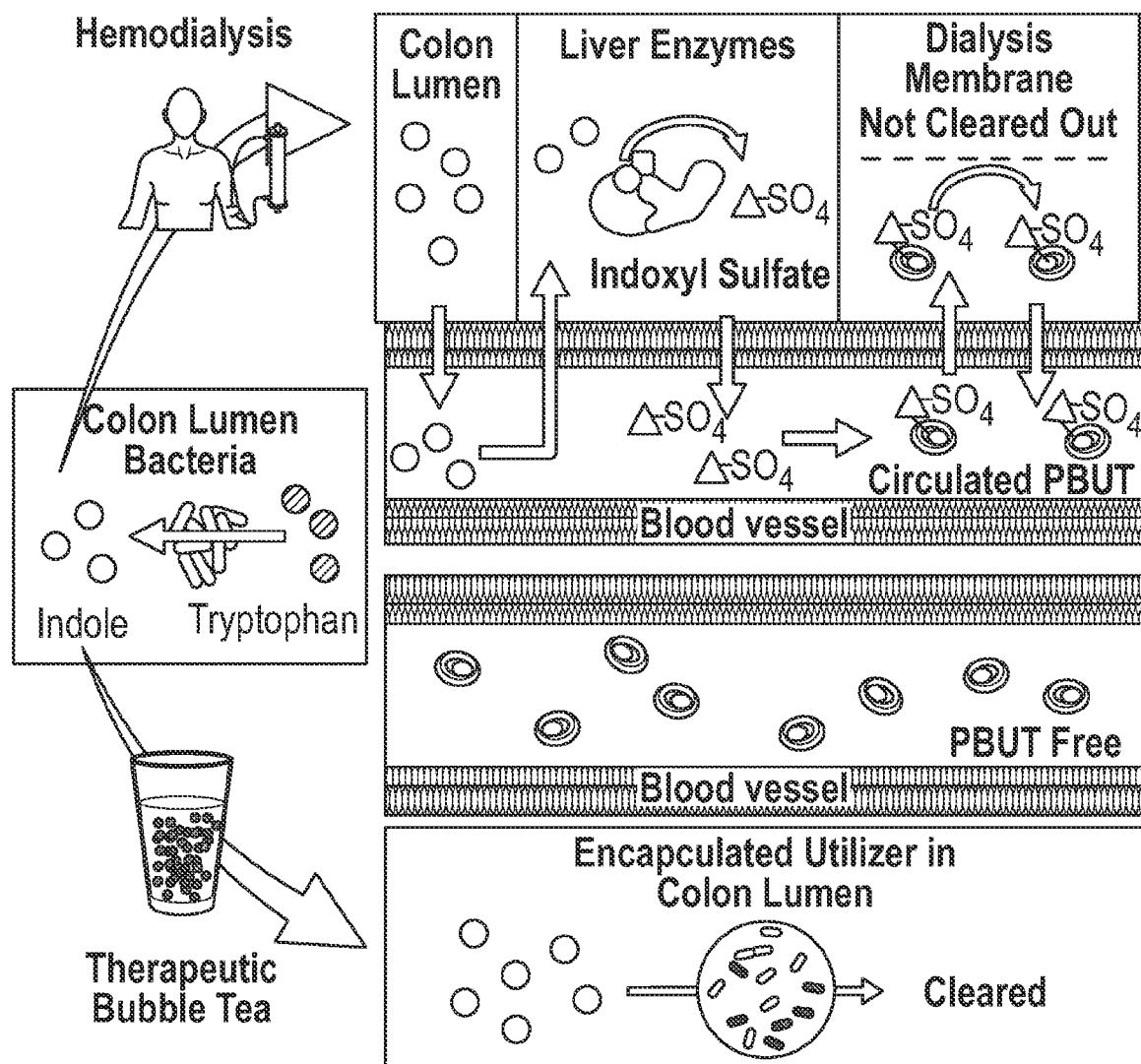


FIG. 1

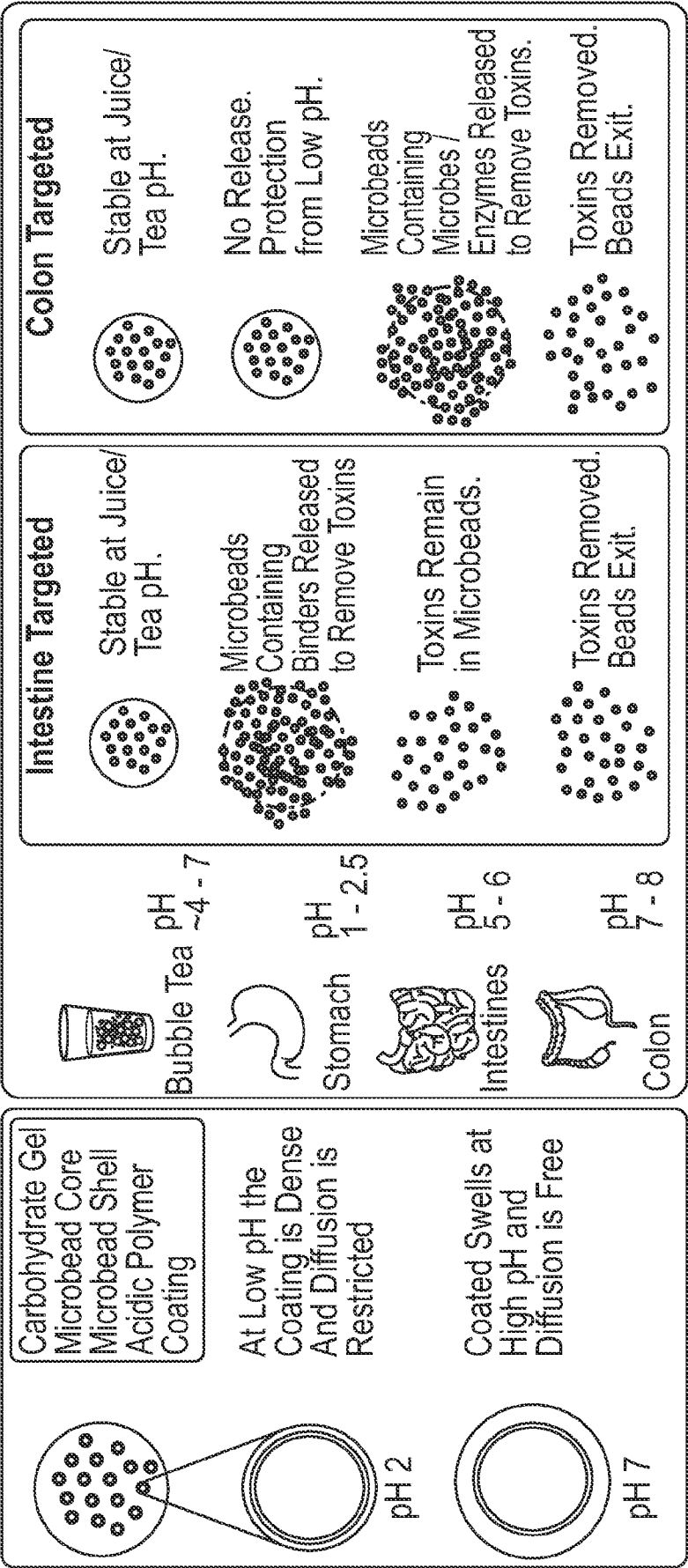


FIG. 2

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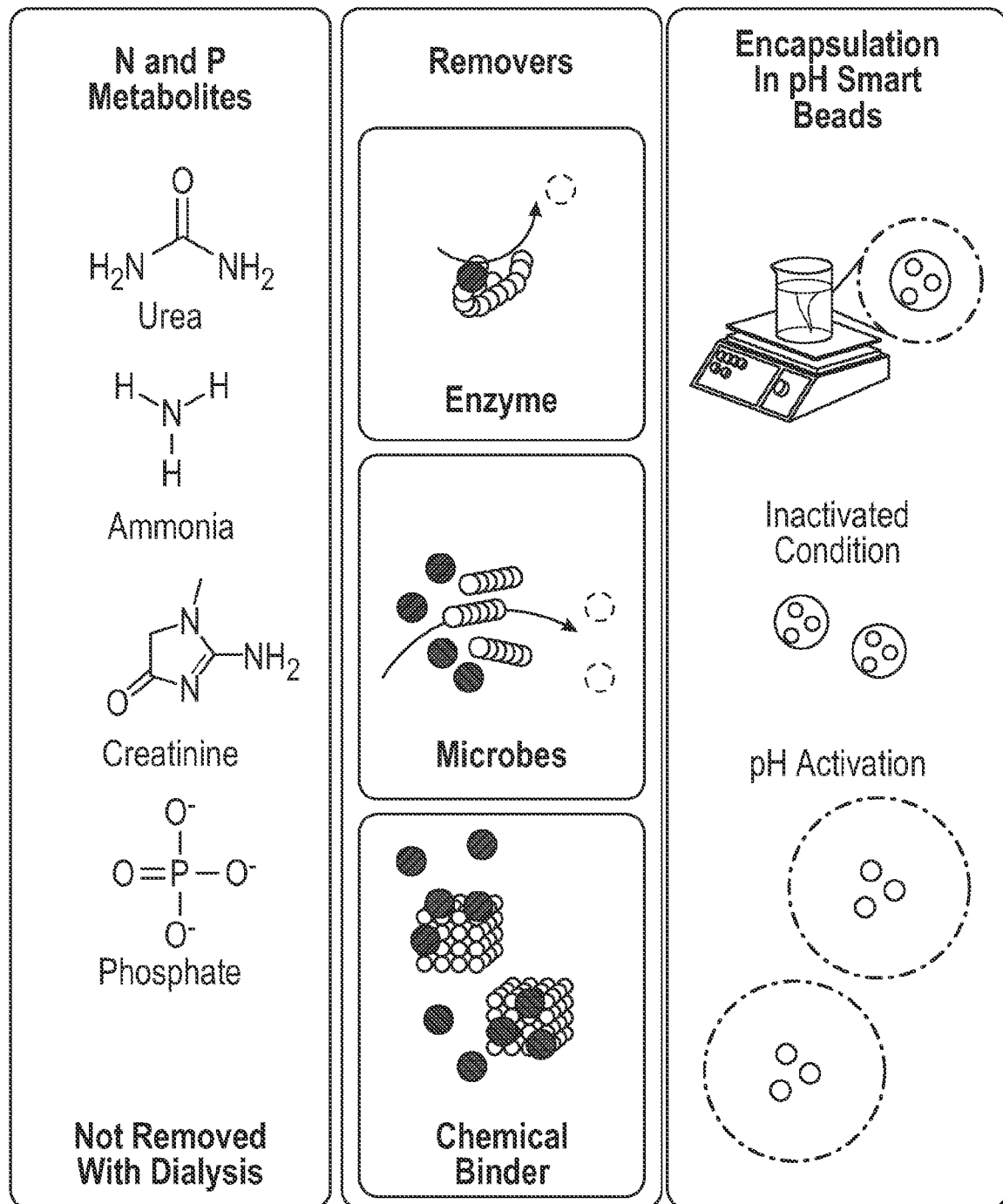


FIG. 3

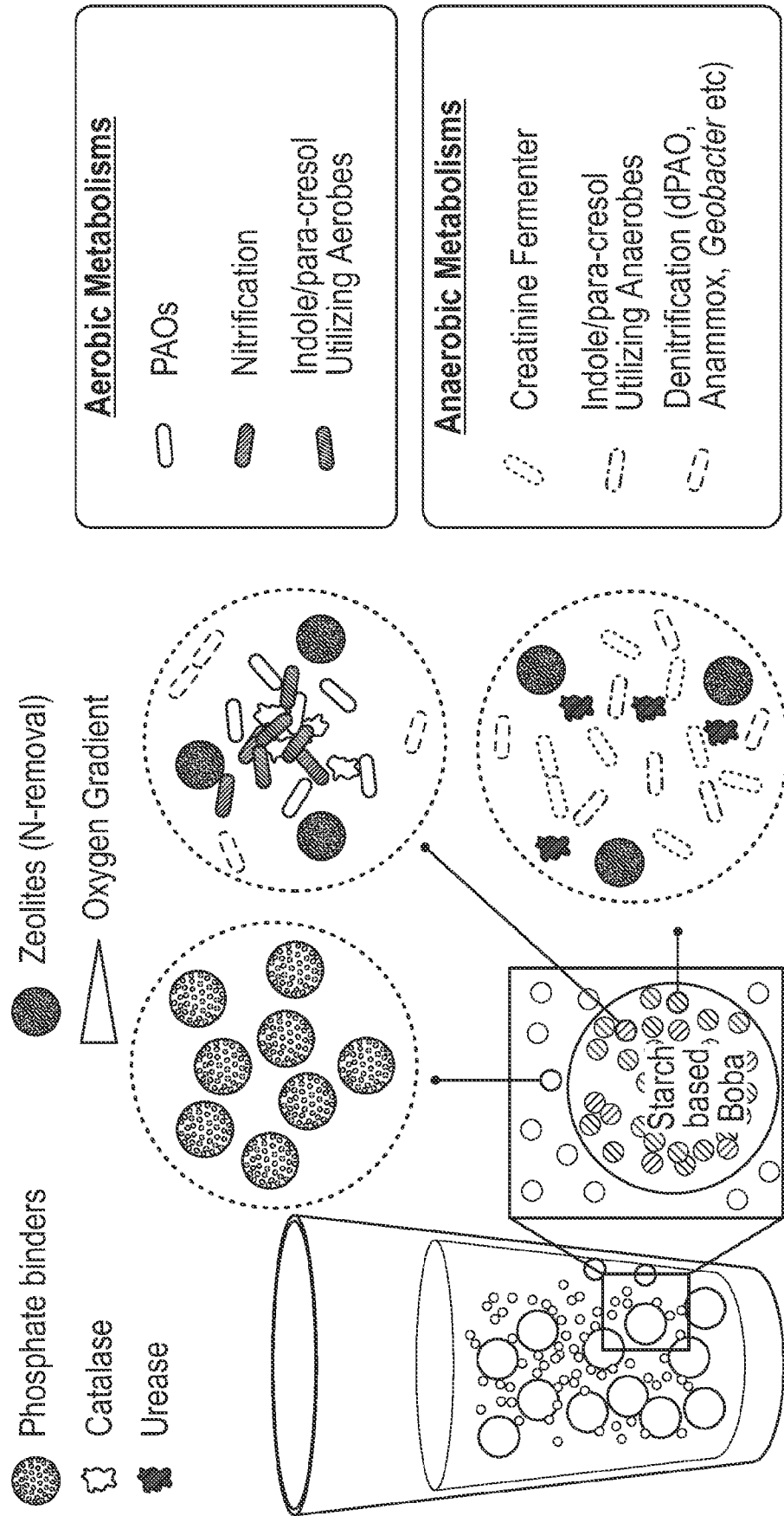


FIG. 4

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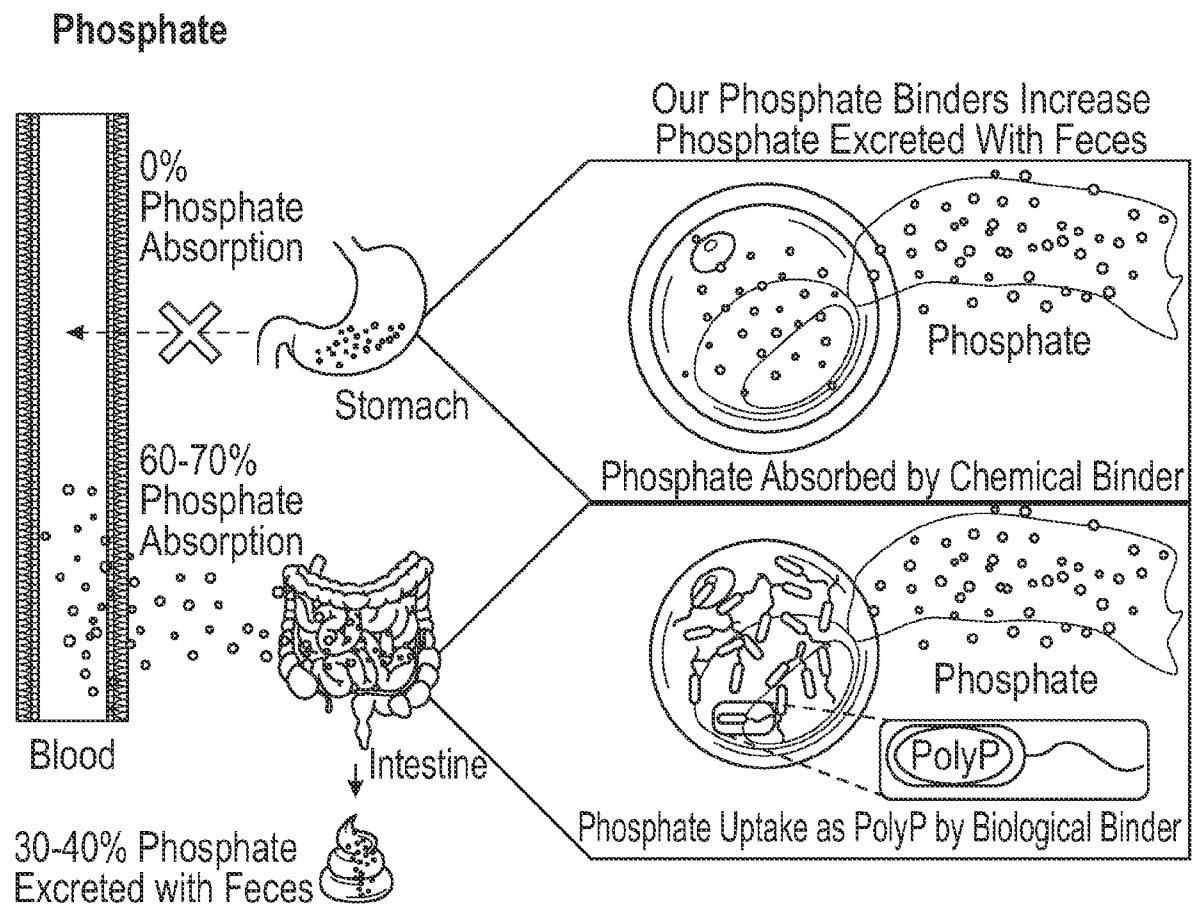
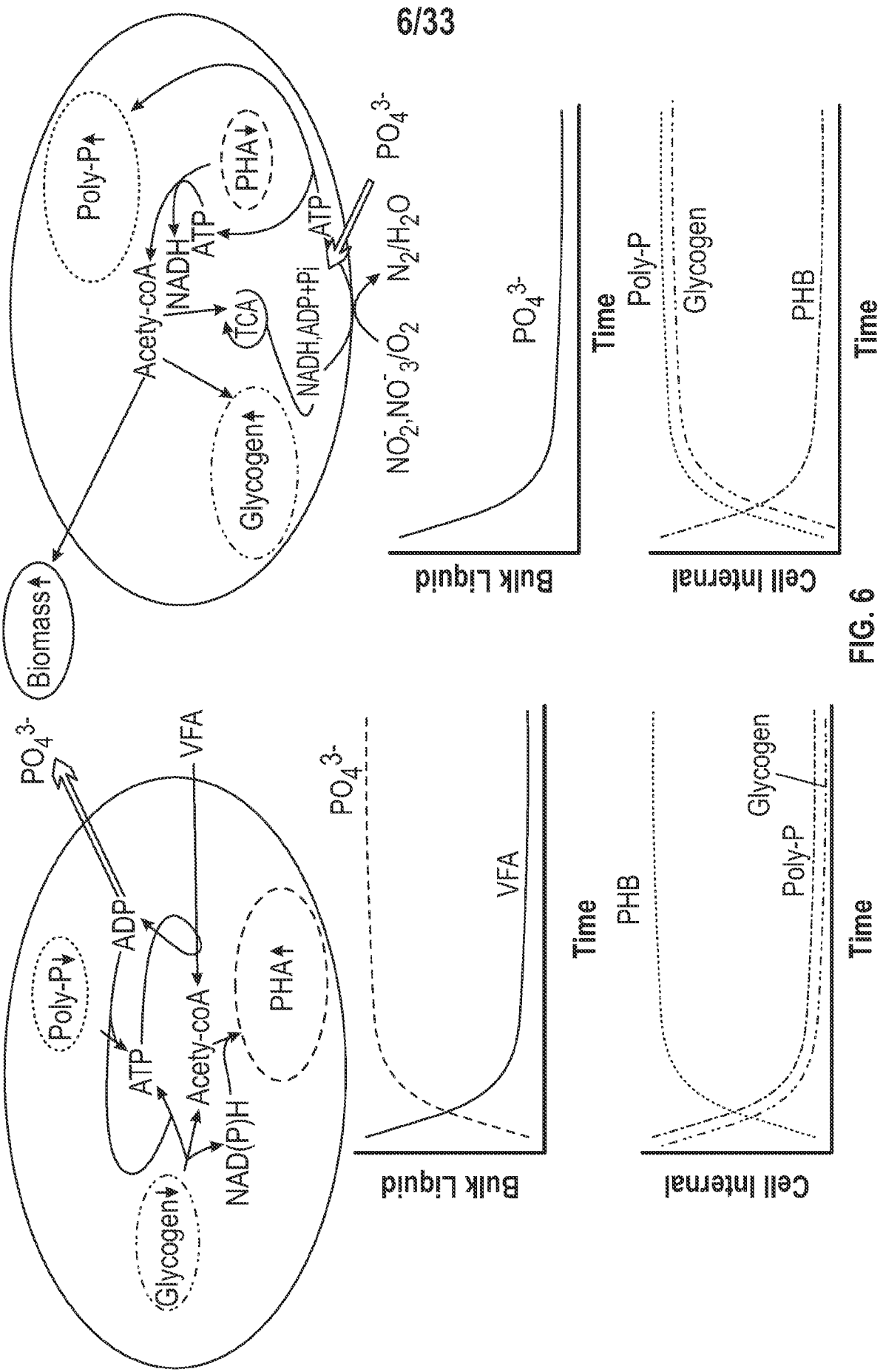


FIG. 5



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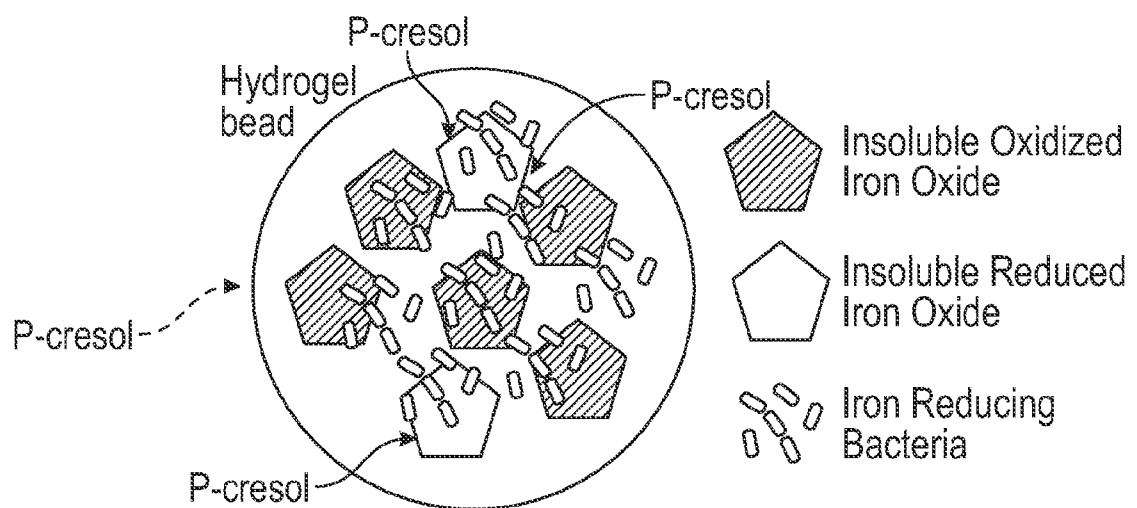


FIG. 7

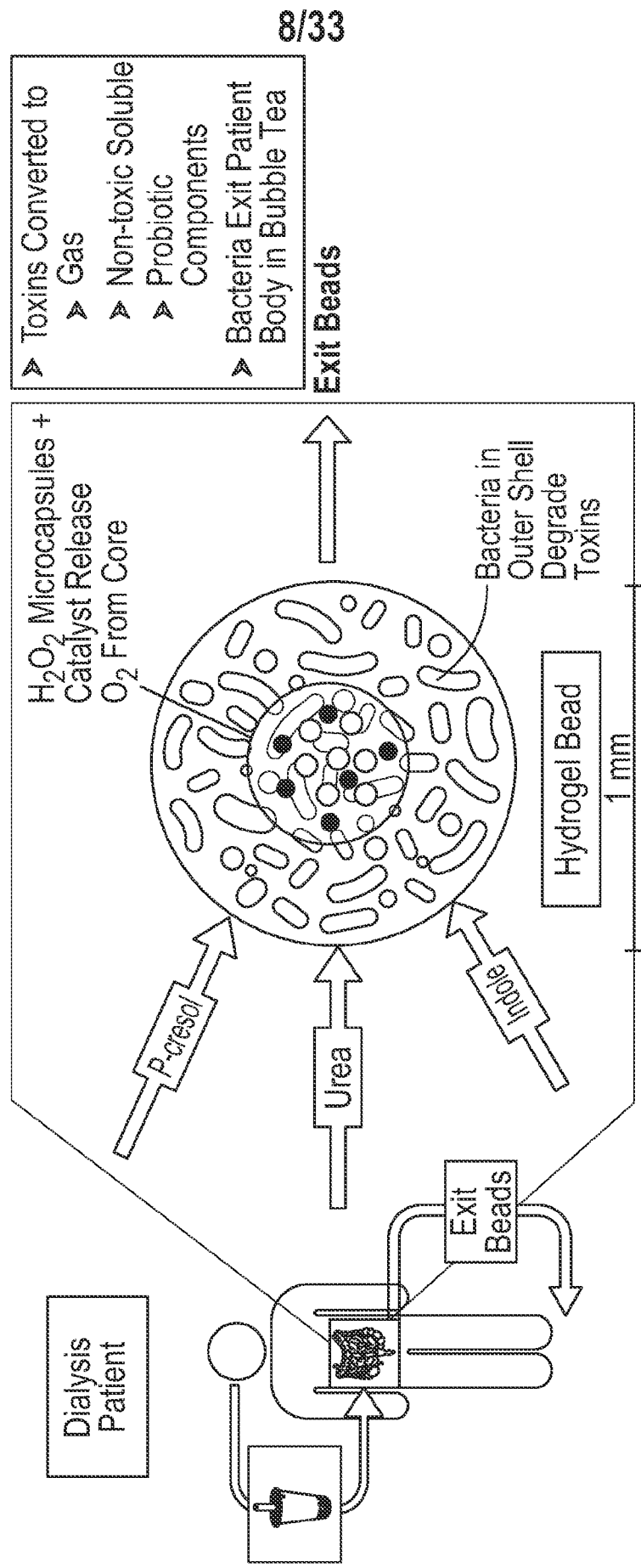


FIG. 8

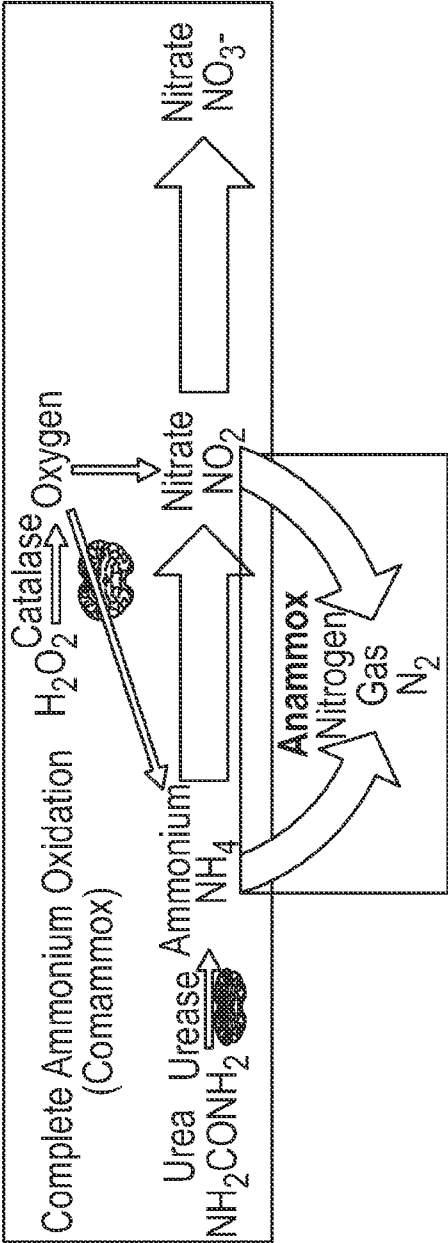


FIG. 9

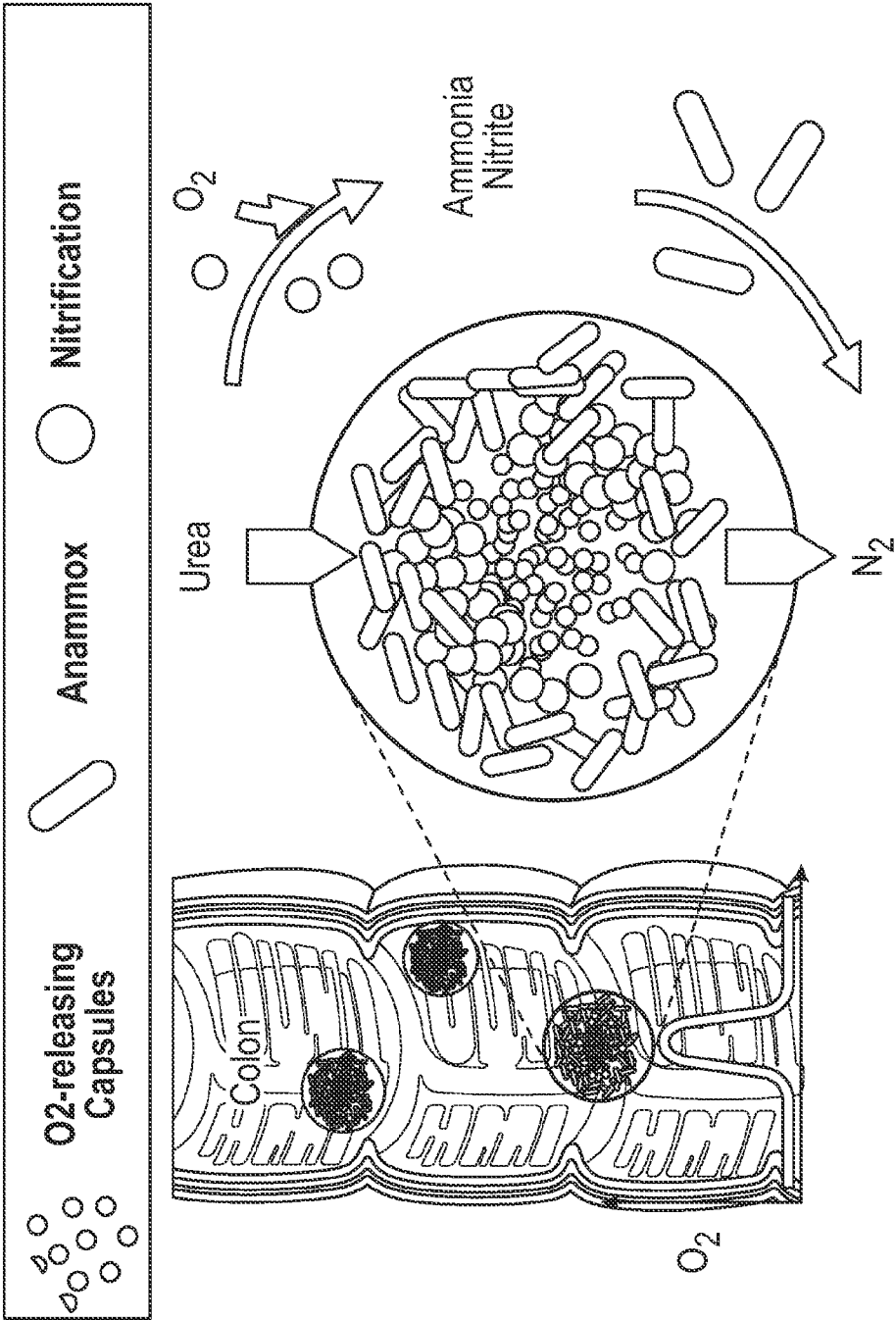


FIG. 10

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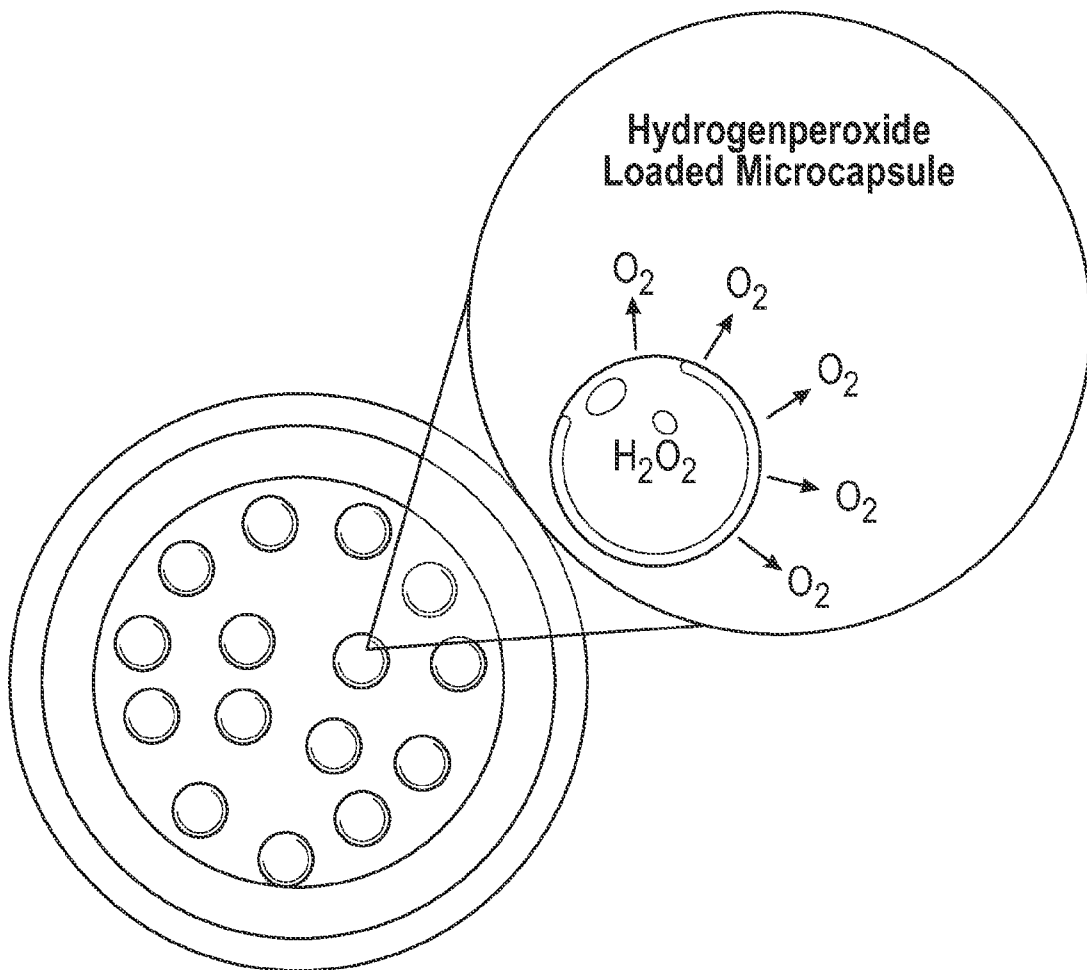


FIG. 11

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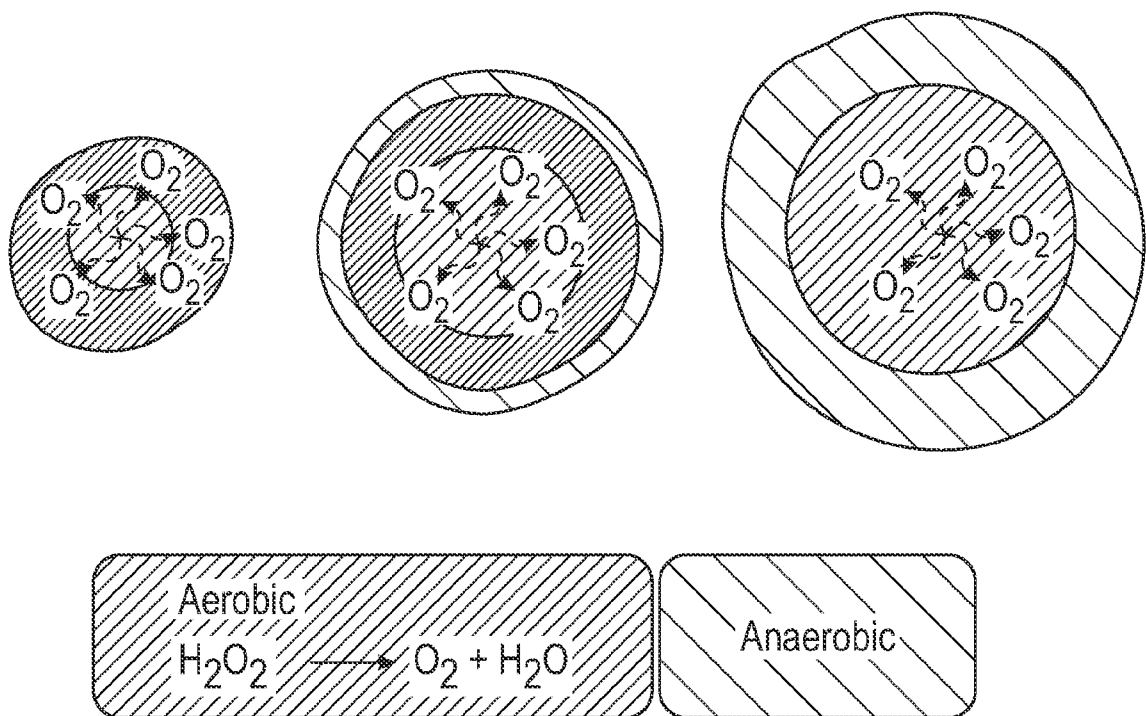


FIG. 12

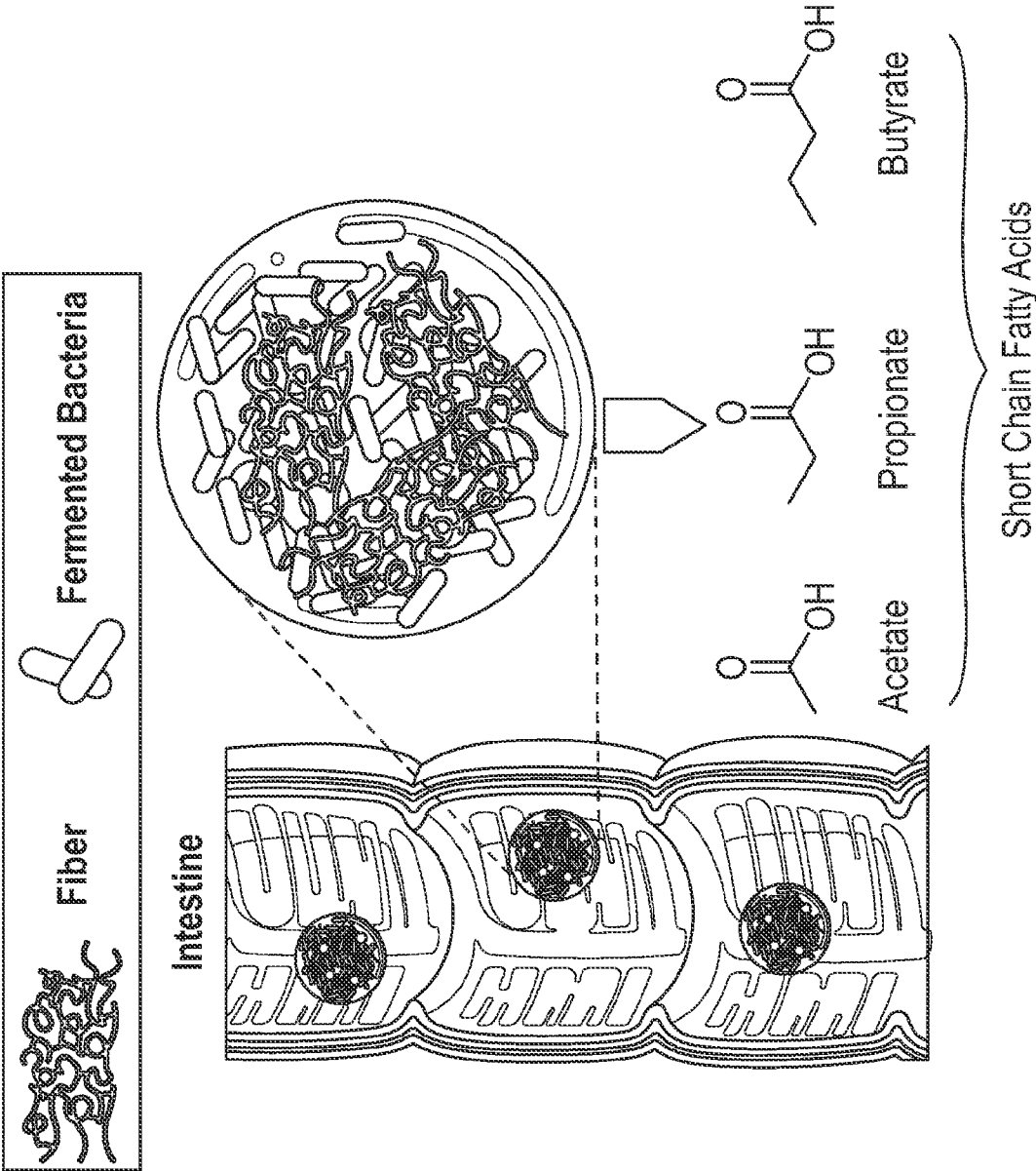


FIG. 13A

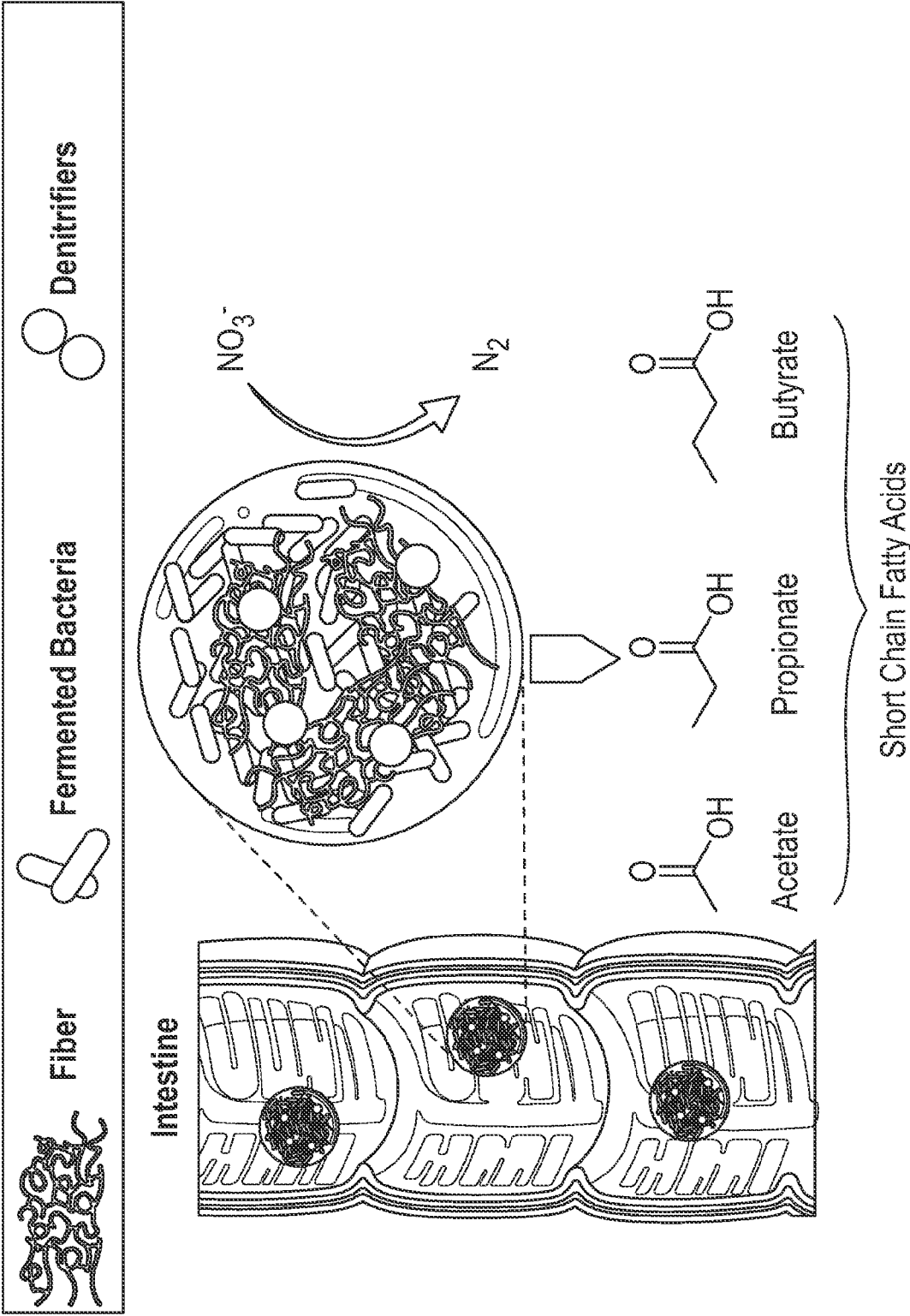
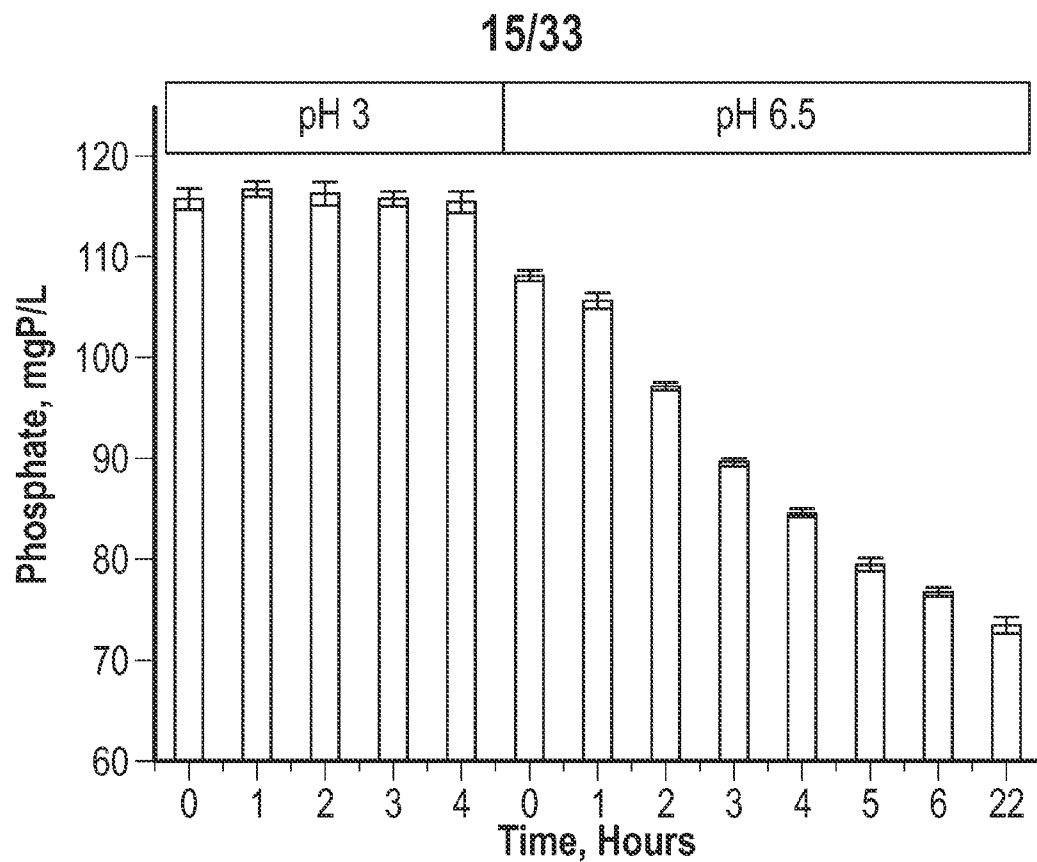
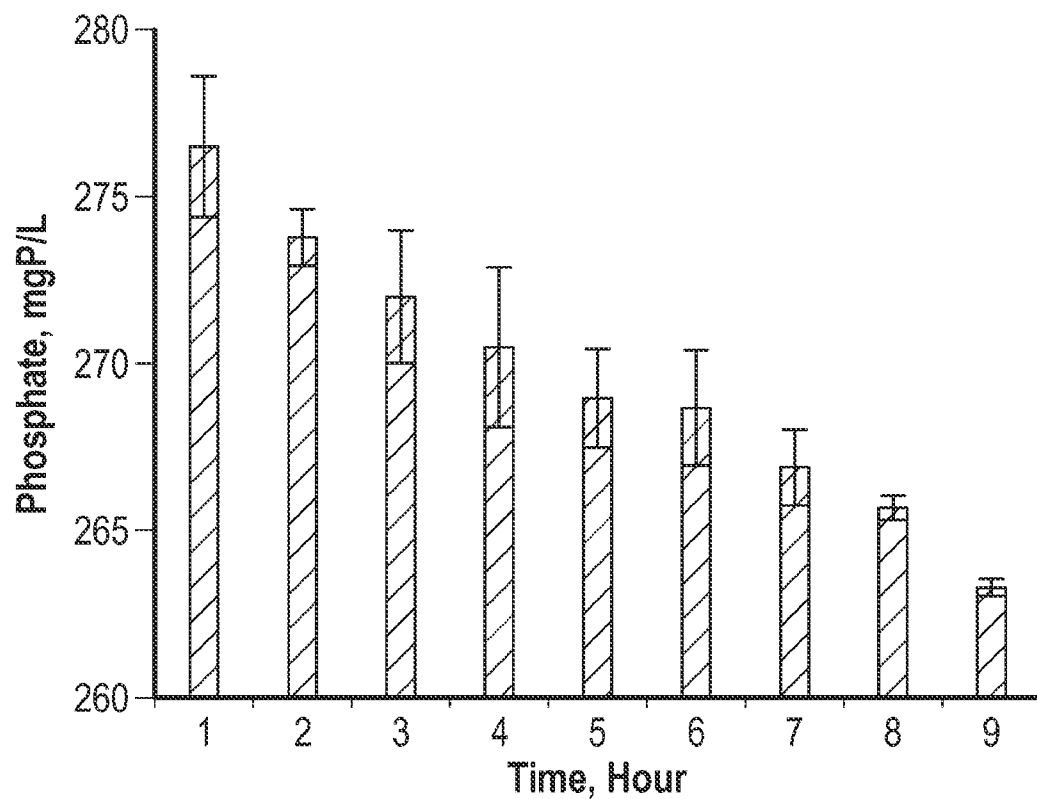


FIG. 13B

**FIG. 14****FIG. 15**

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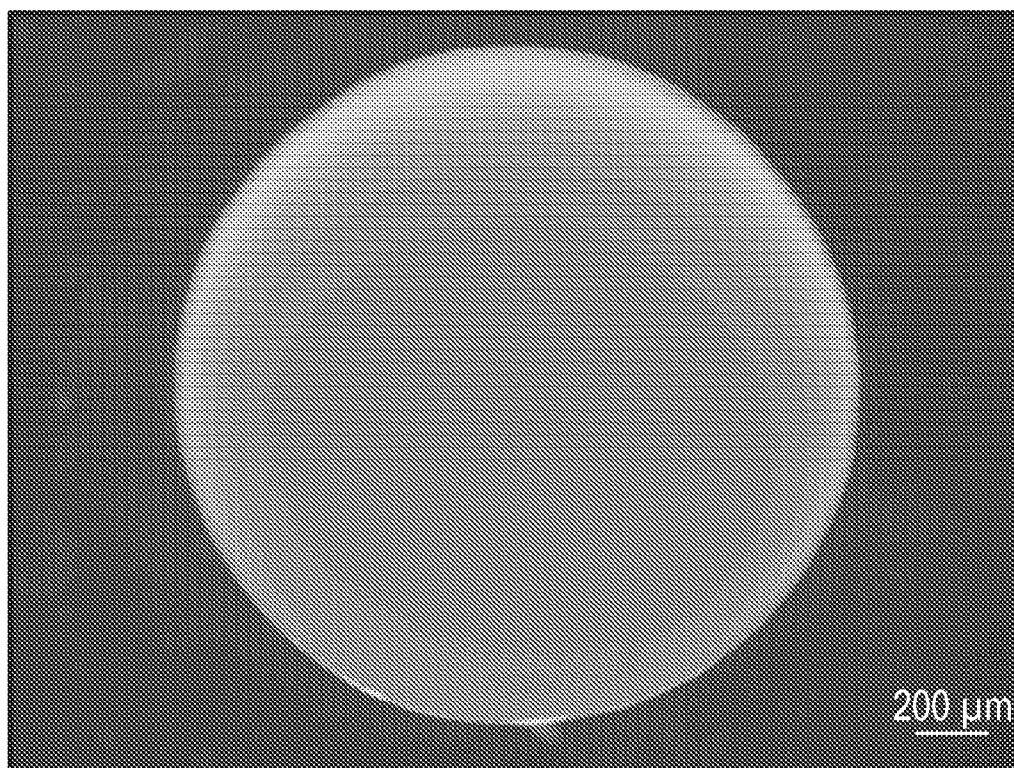


FIG. 16

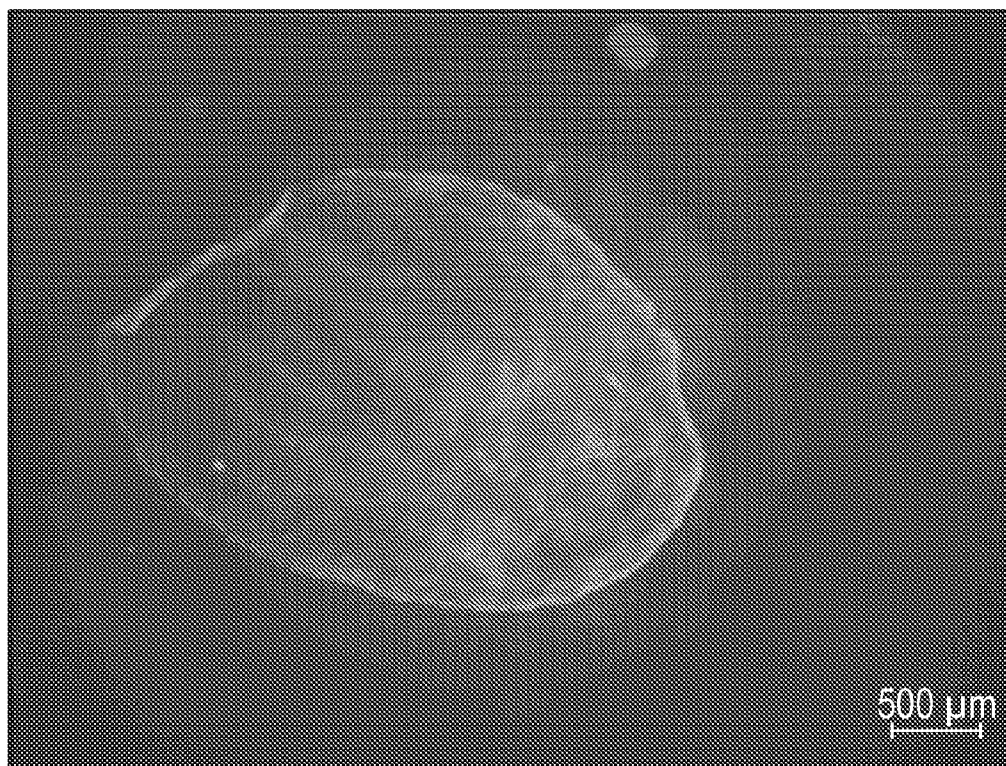


FIG. 17

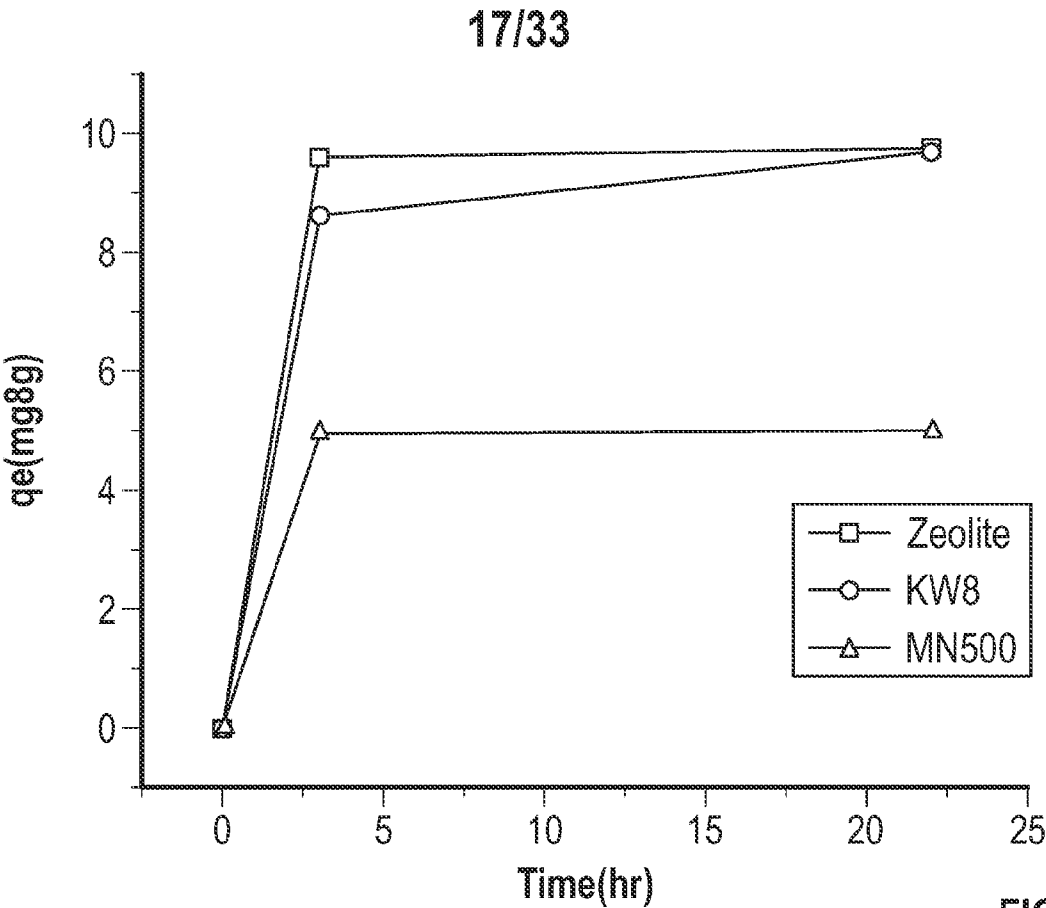


FIG. 18

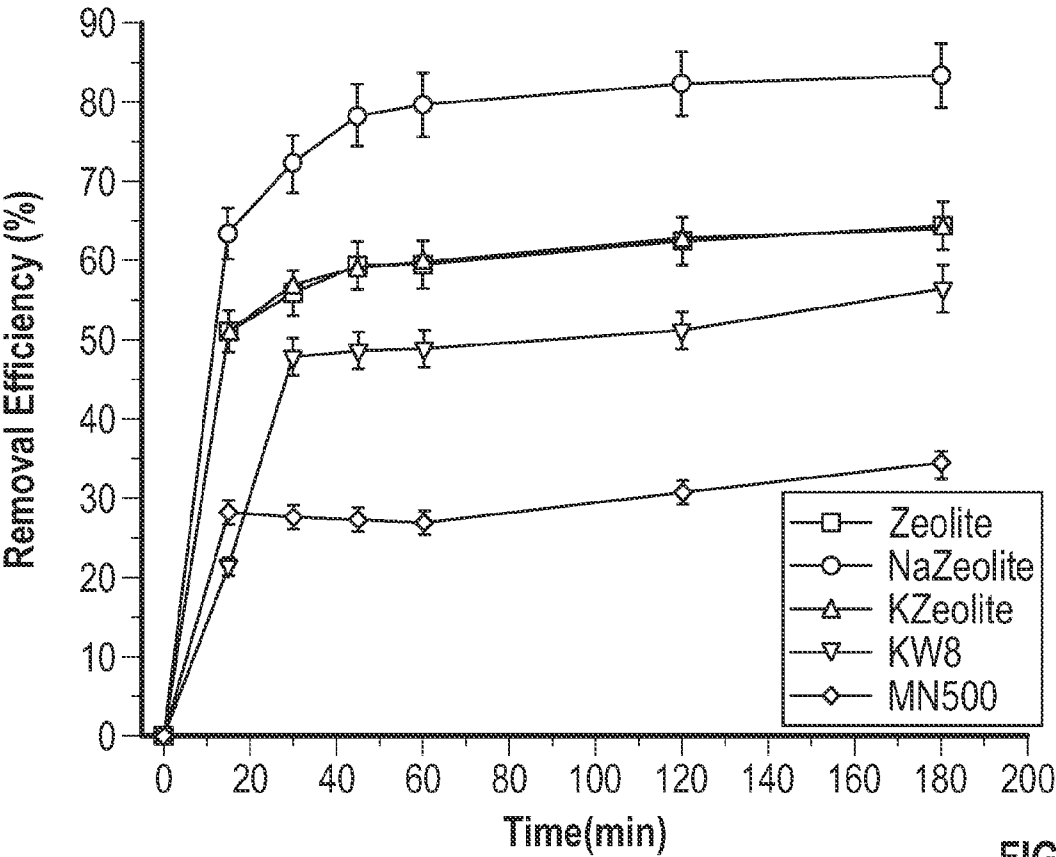


FIG. 19

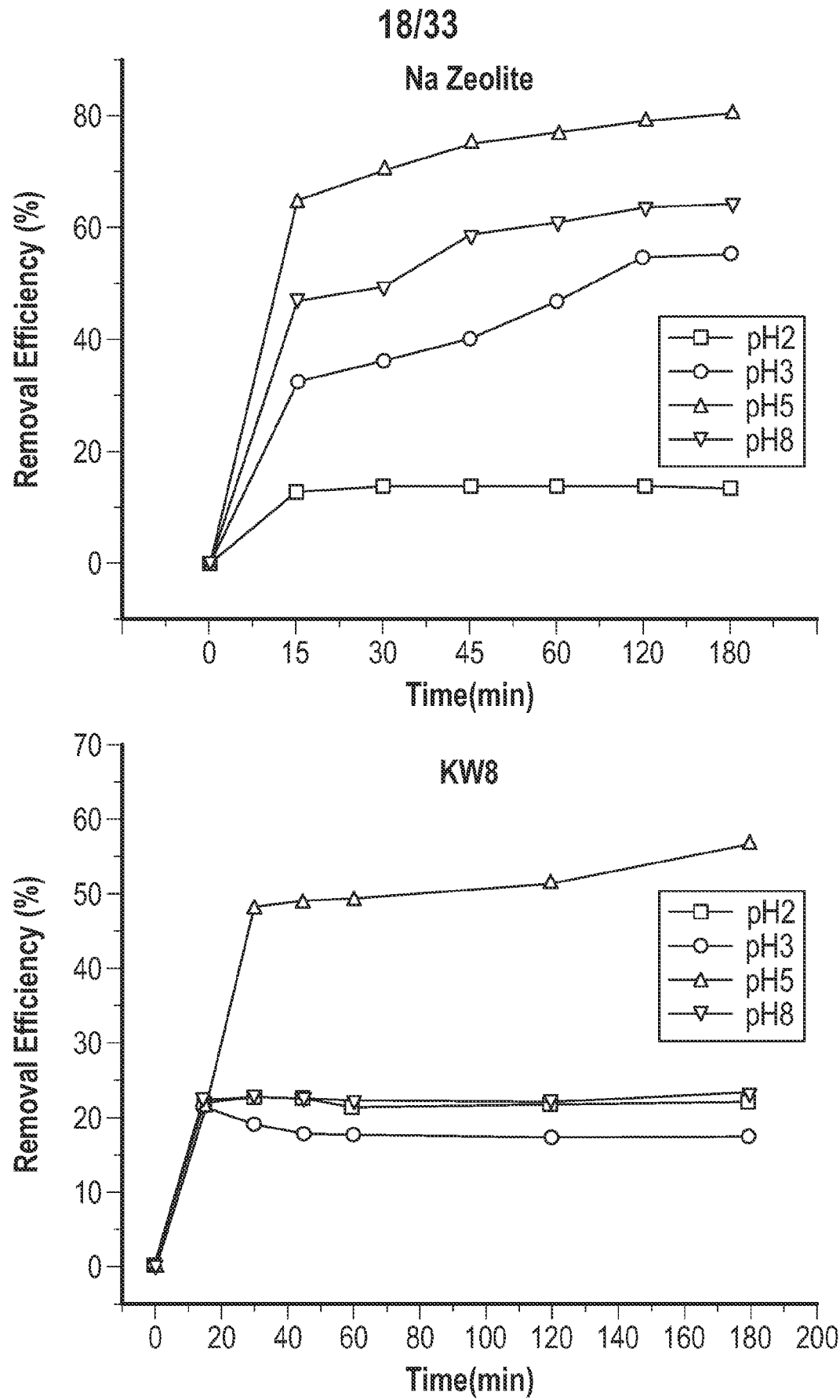


FIG. 20

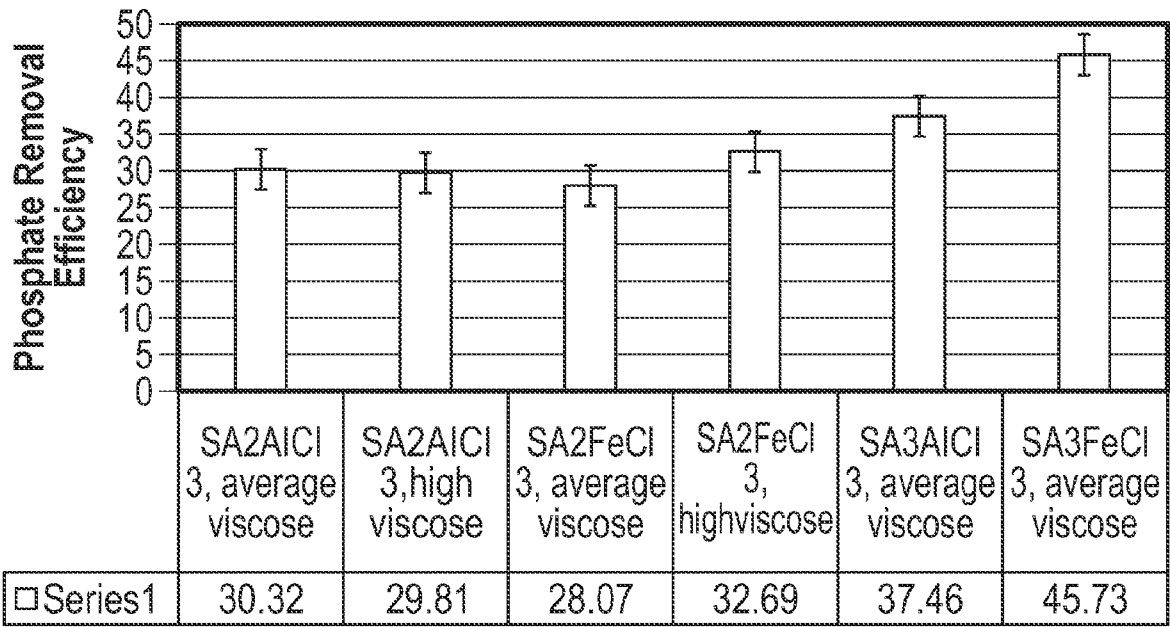


FIG. 21

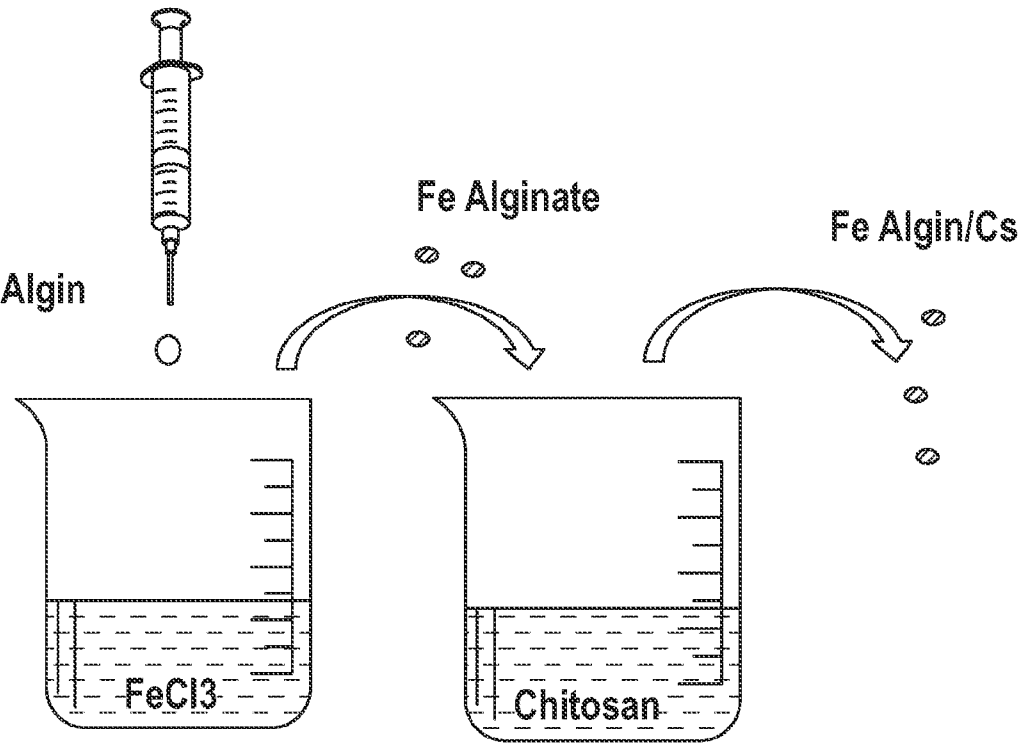


FIG. 22

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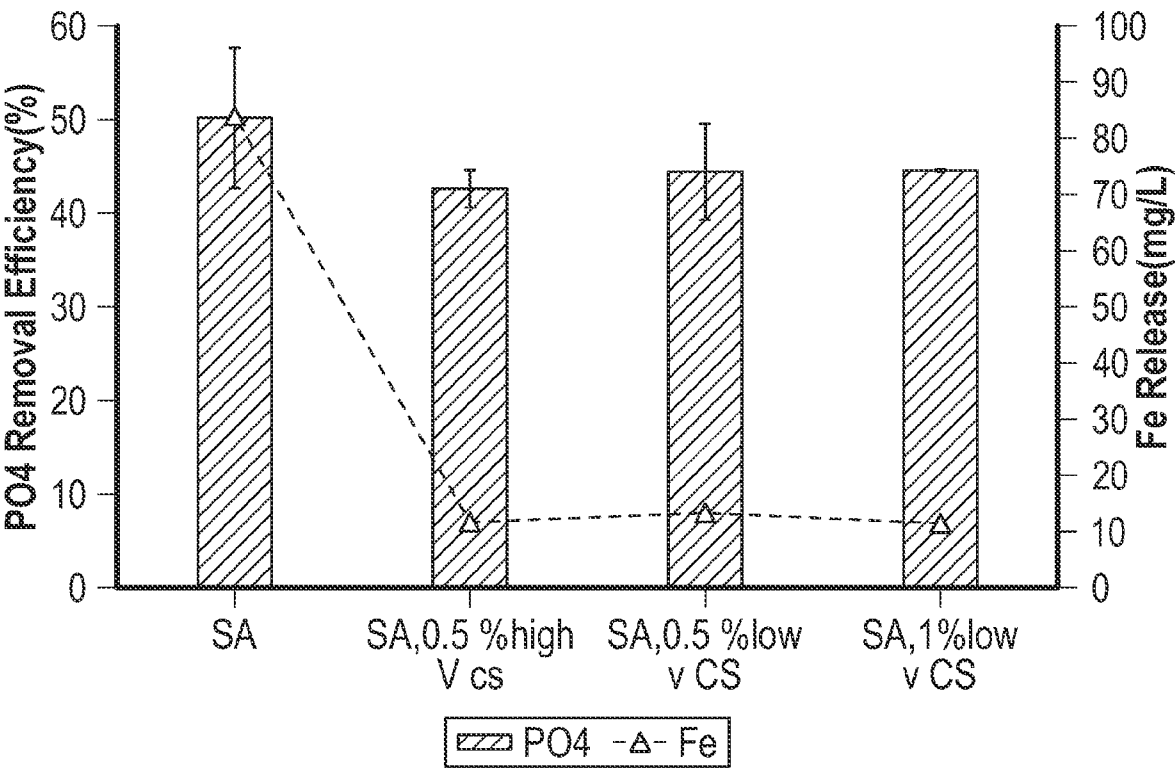


FIG. 23

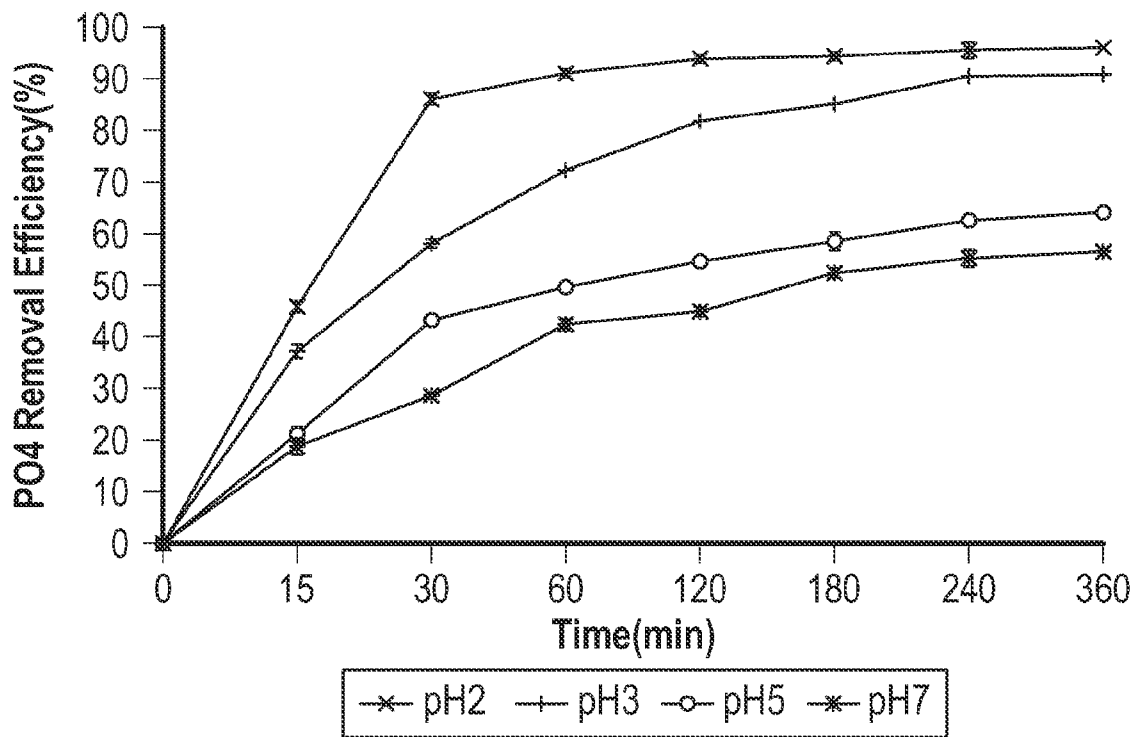


FIG. 24

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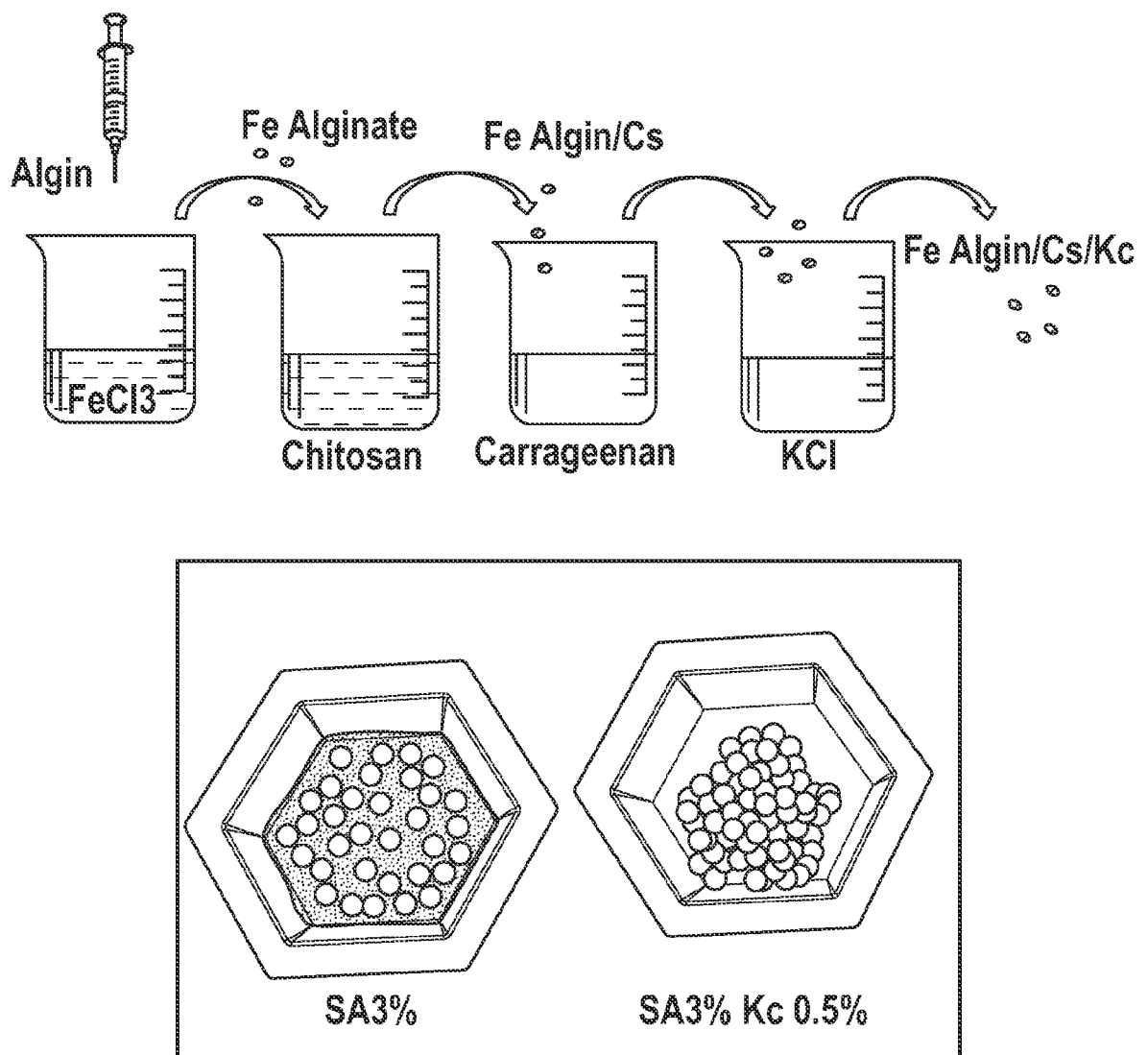


FIG. 25

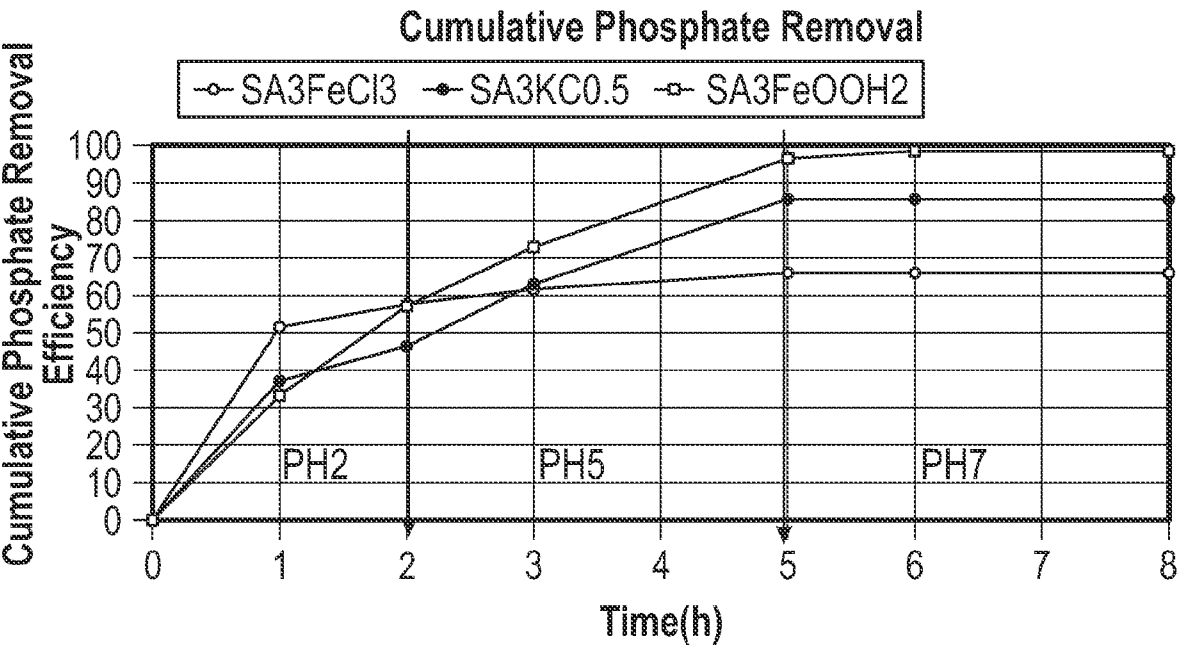


FIG. 26

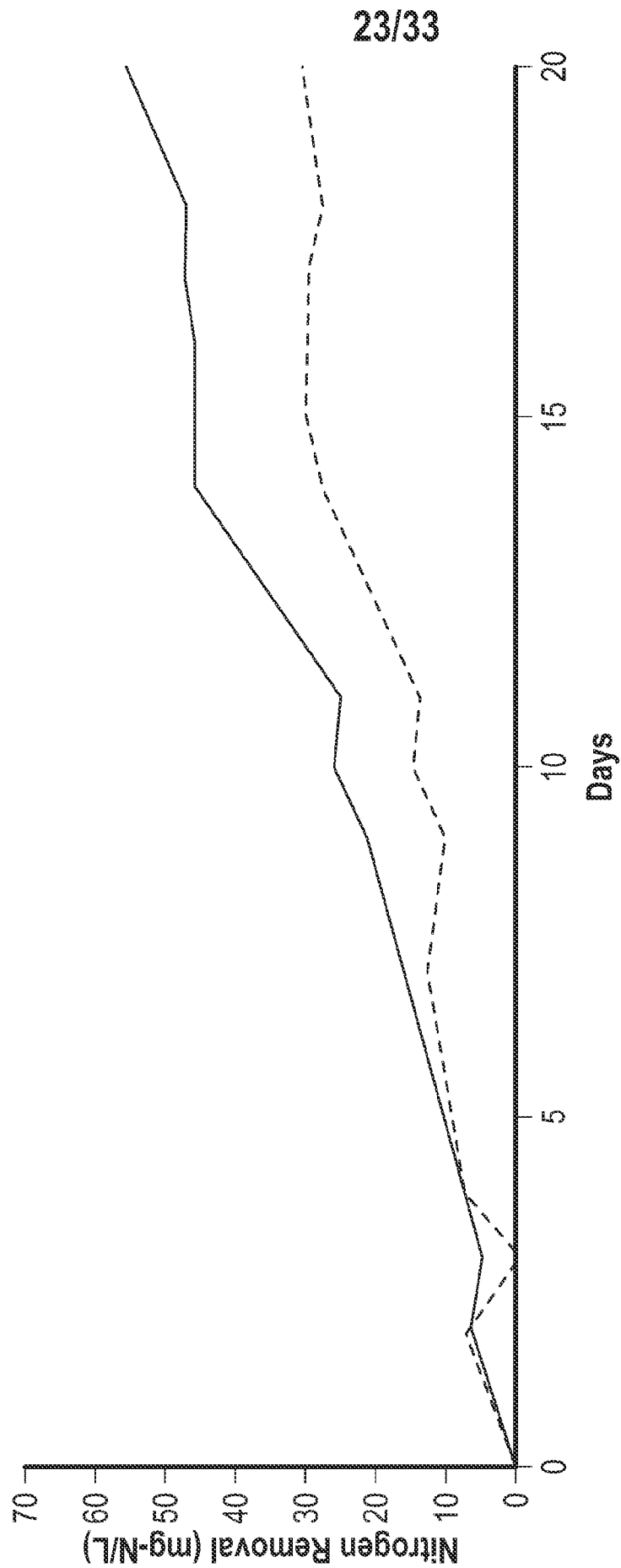


FIG. 27

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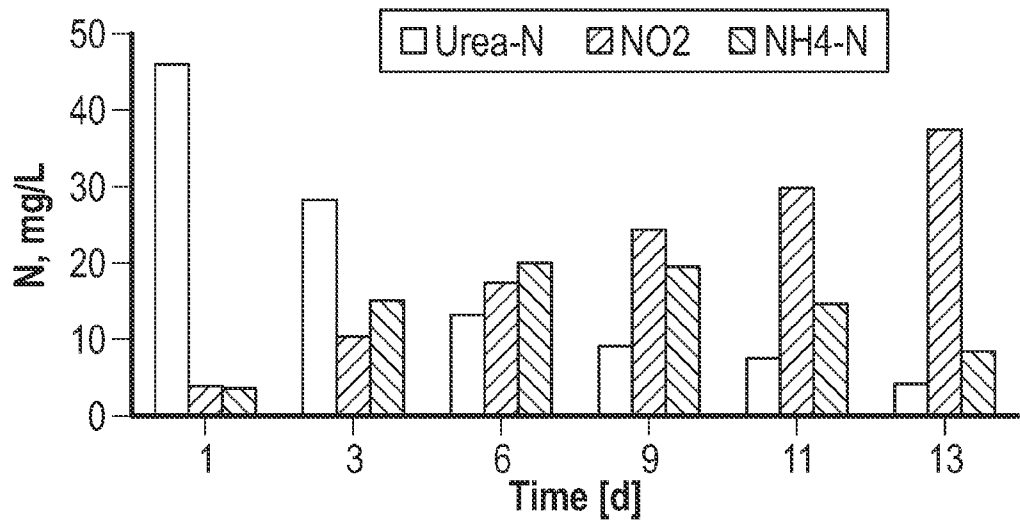


FIG. 28

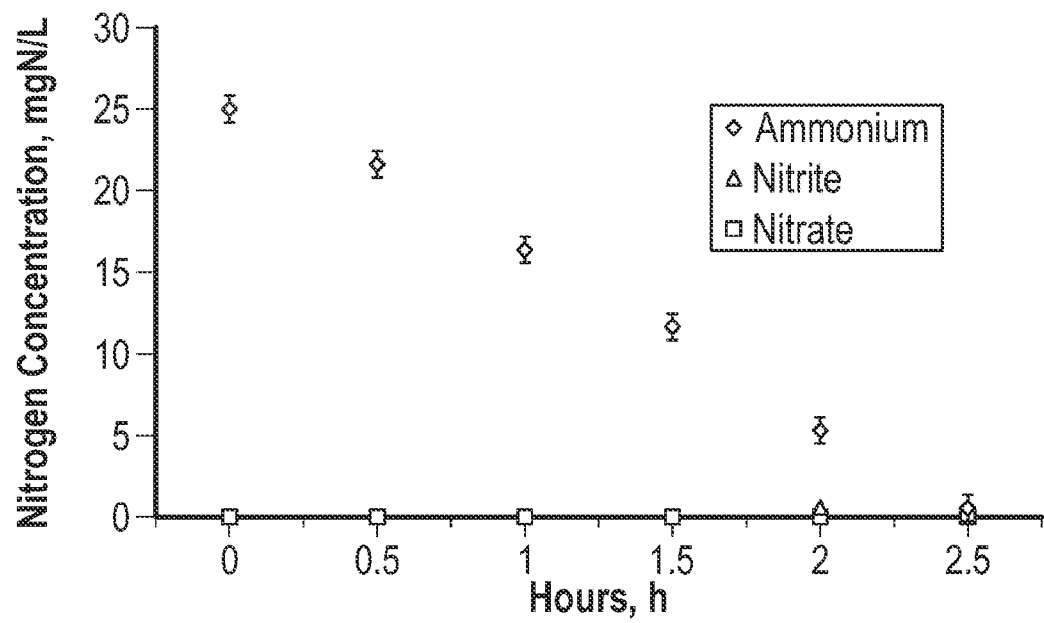


FIG. 29

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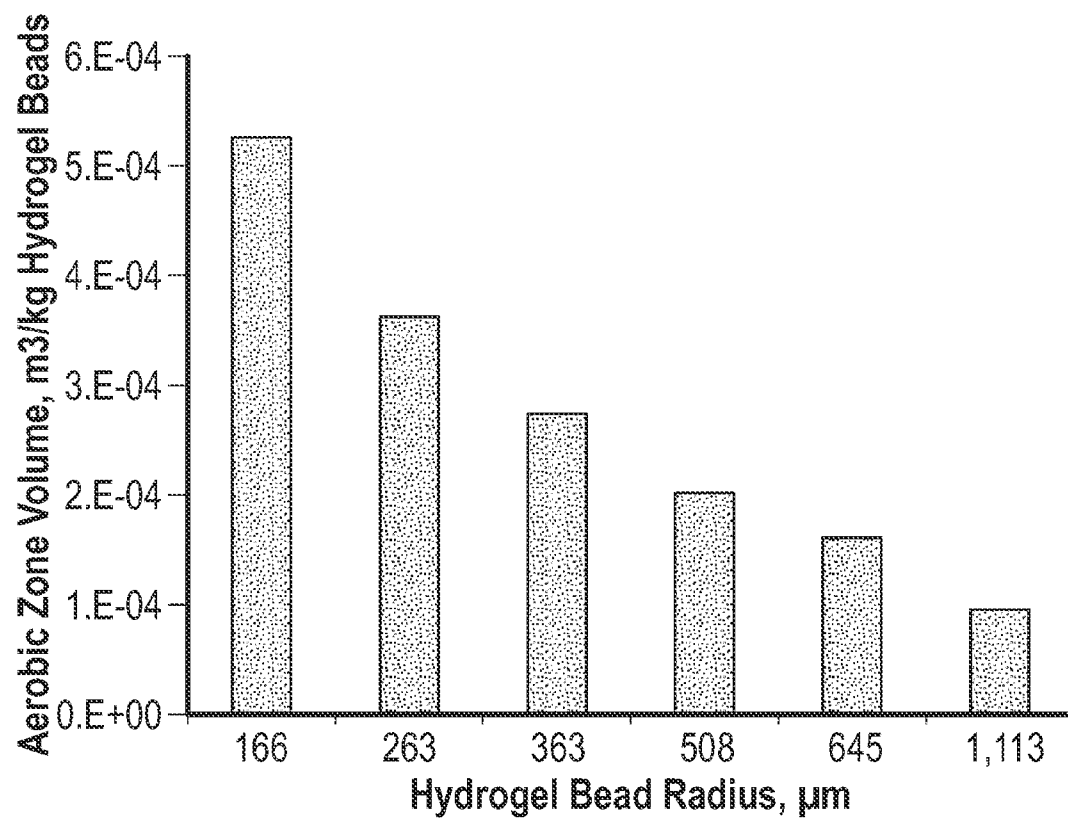


FIG. 30

Indole Degradation

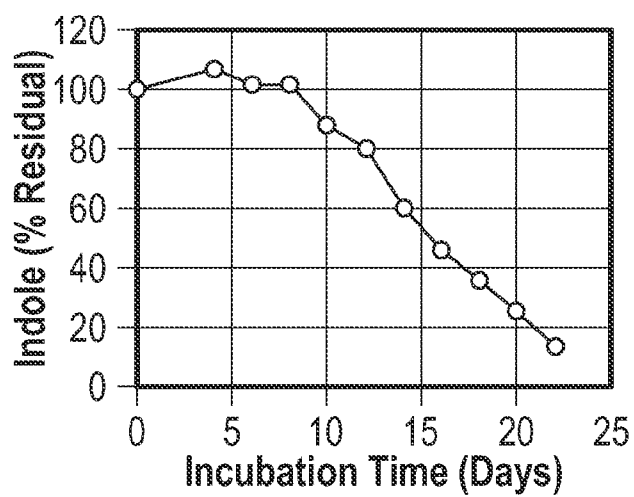


FIG. 31

 SYBR-stained *T. aminoaromatica* in Hydrogels

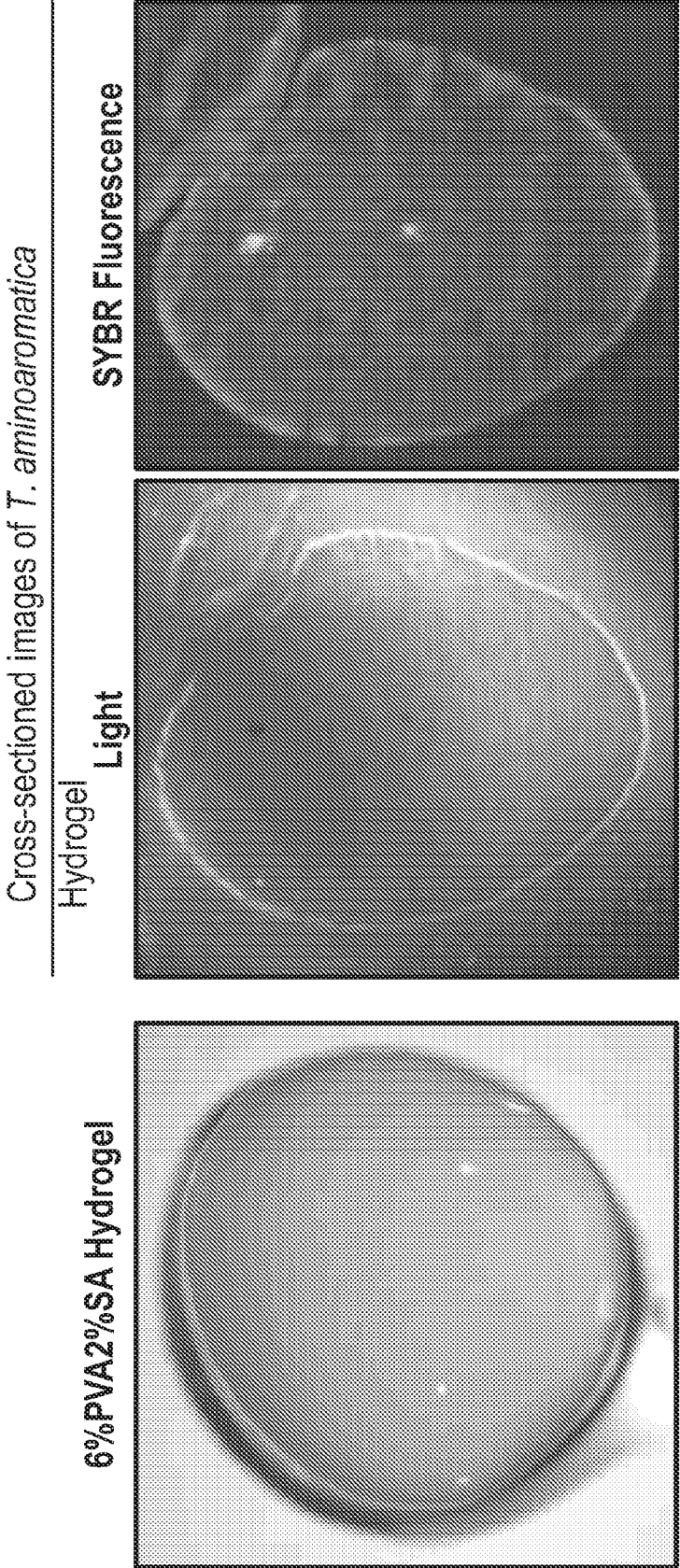


FIG. 32

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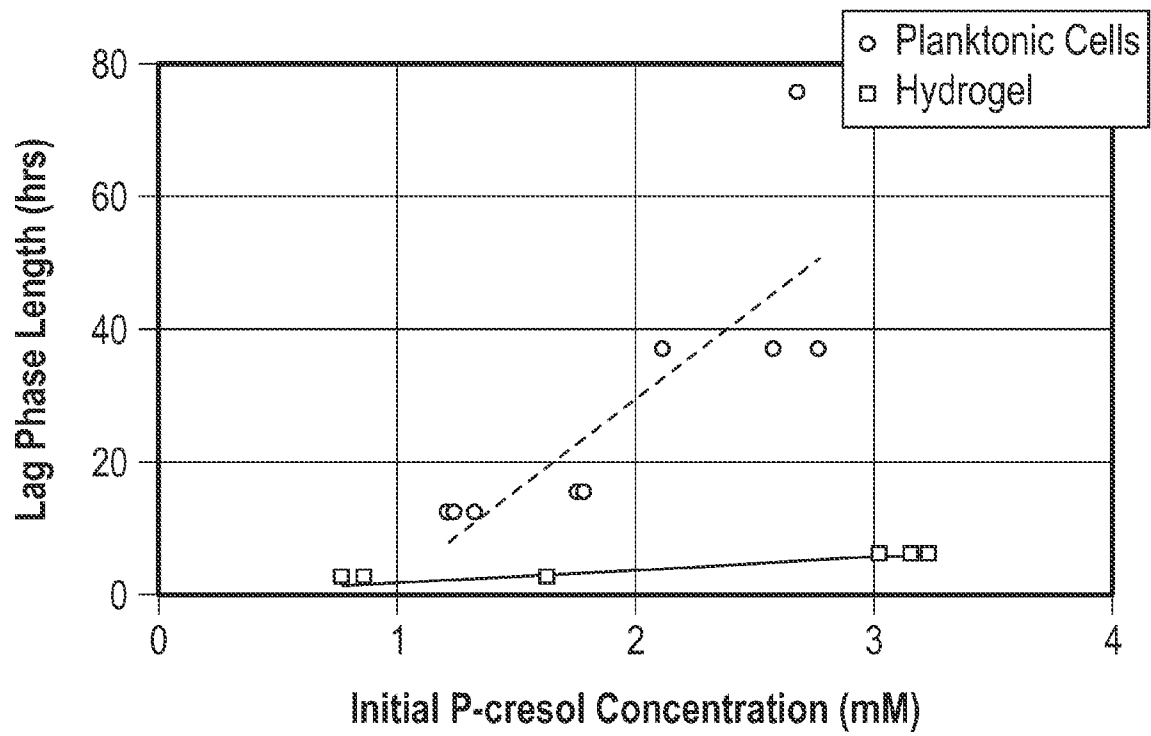


FIG. 33

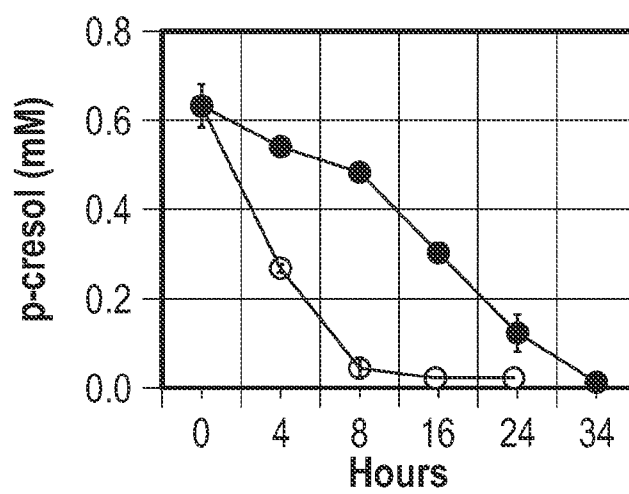


FIG. 34

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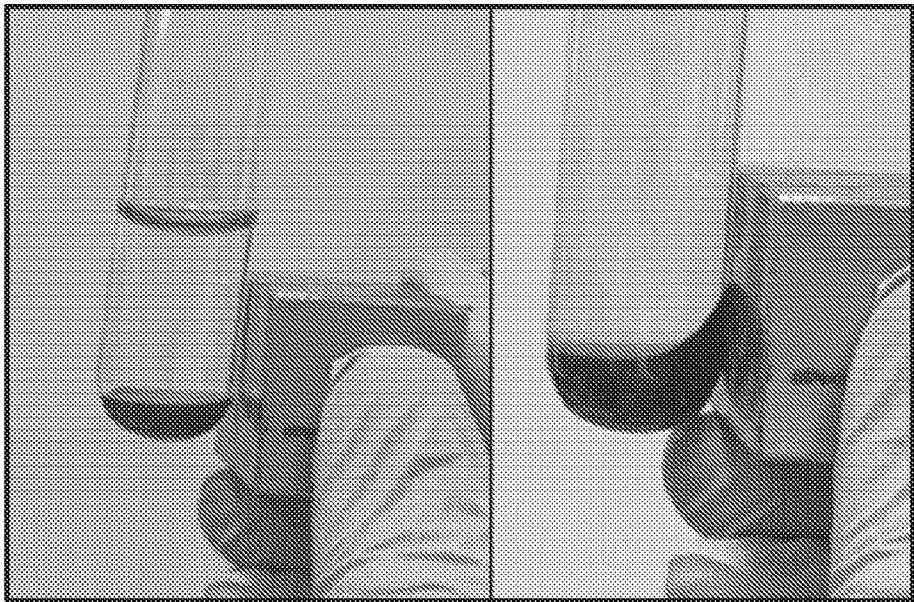
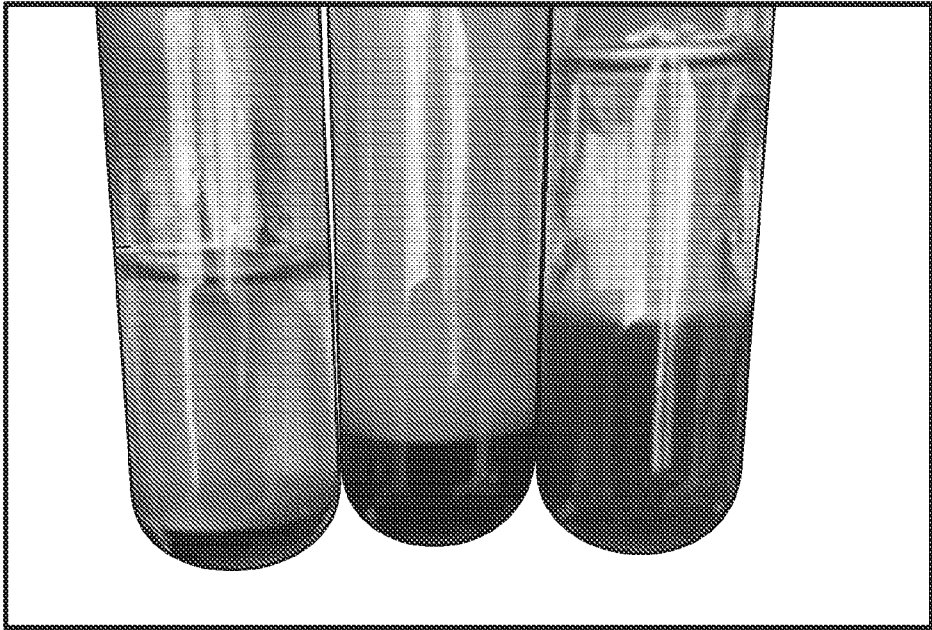


FIG. 35

Ferrozine Assay of FE(II) Spent Growth Media VS Uninoculated Control



Sample	mM Fe(II) Ions
Inoculated 1	1.9 mM
Inoculated 2	0.3 mM
Uninoculated Control	0.1 mM

FIG. 36

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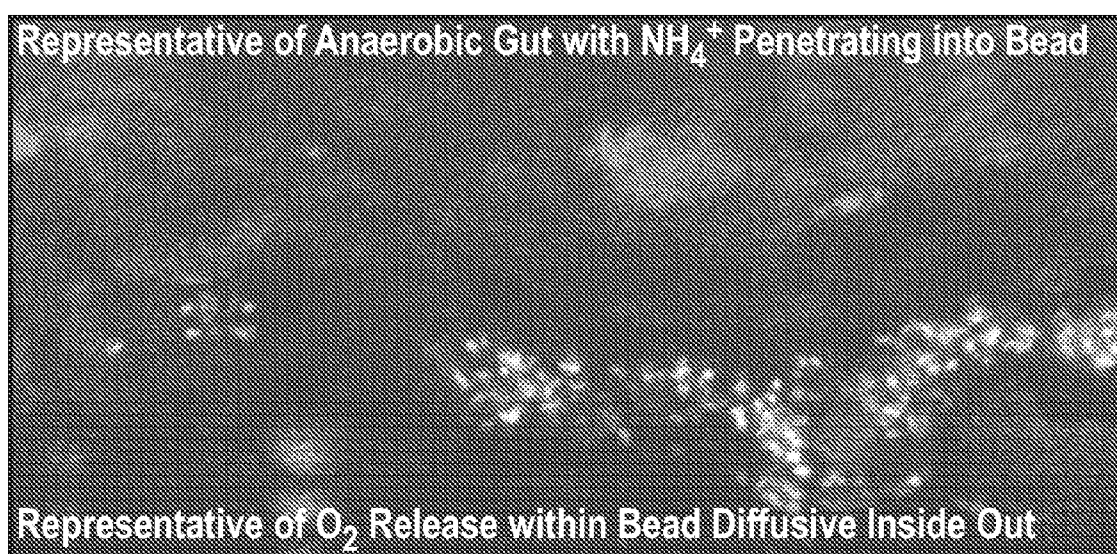


FIG. 37

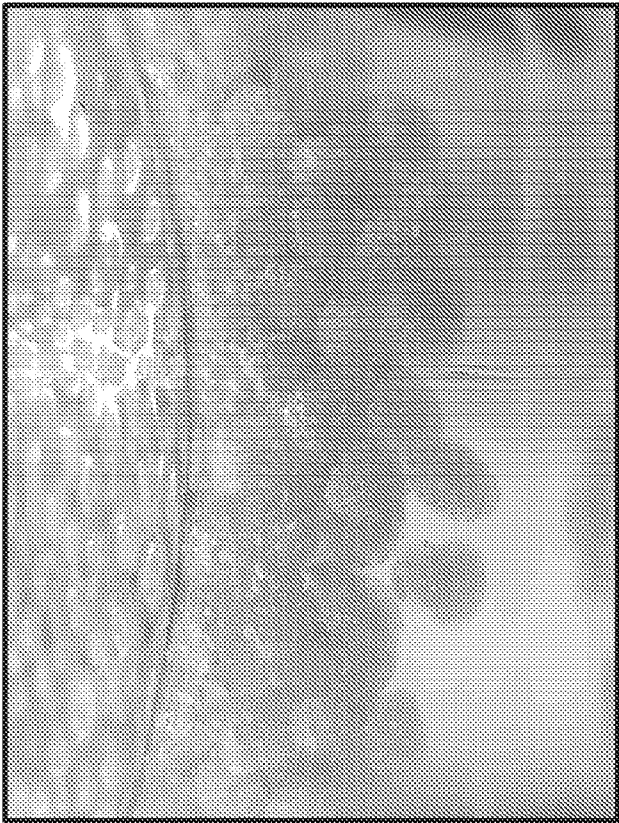


FIG. 38B

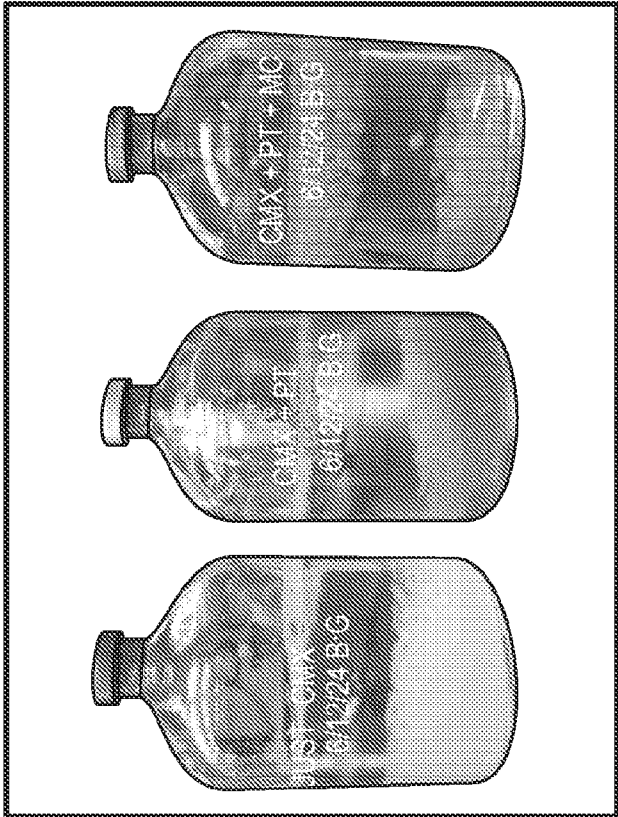


FIG. 38A

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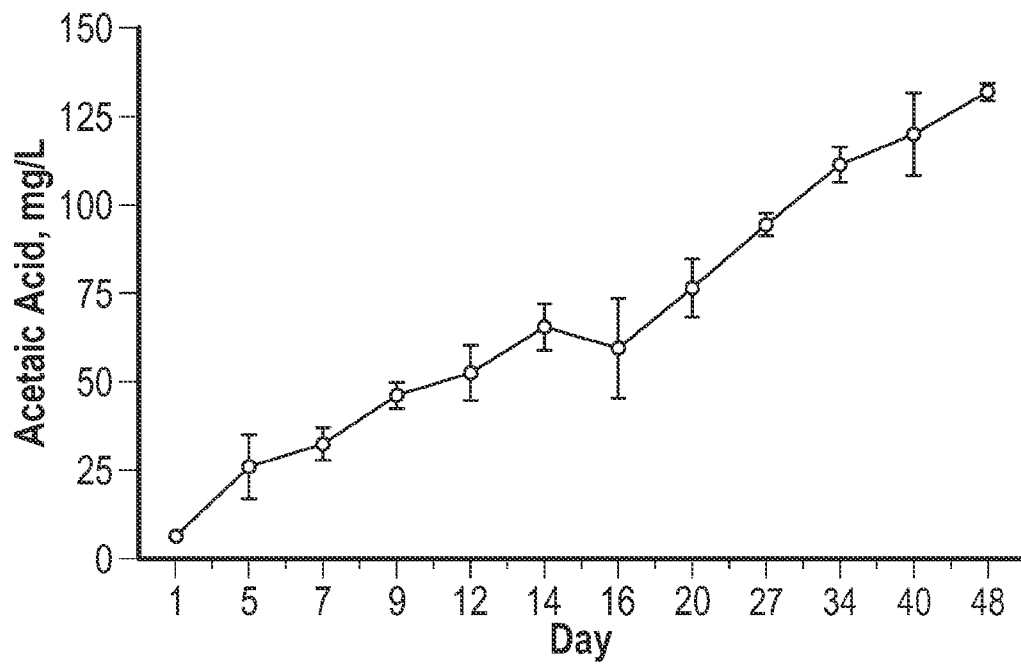


FIG. 39

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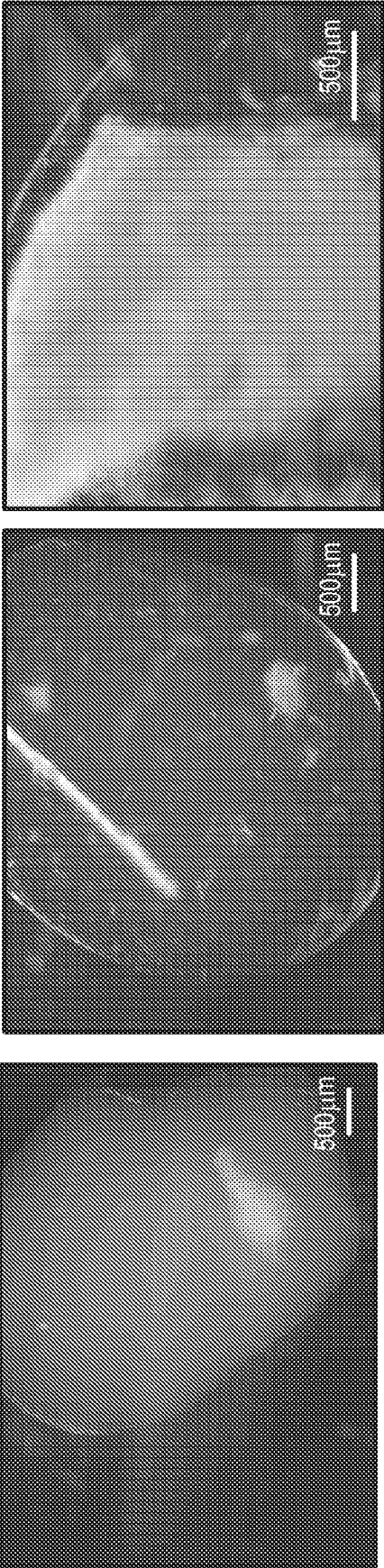


FIG. 40

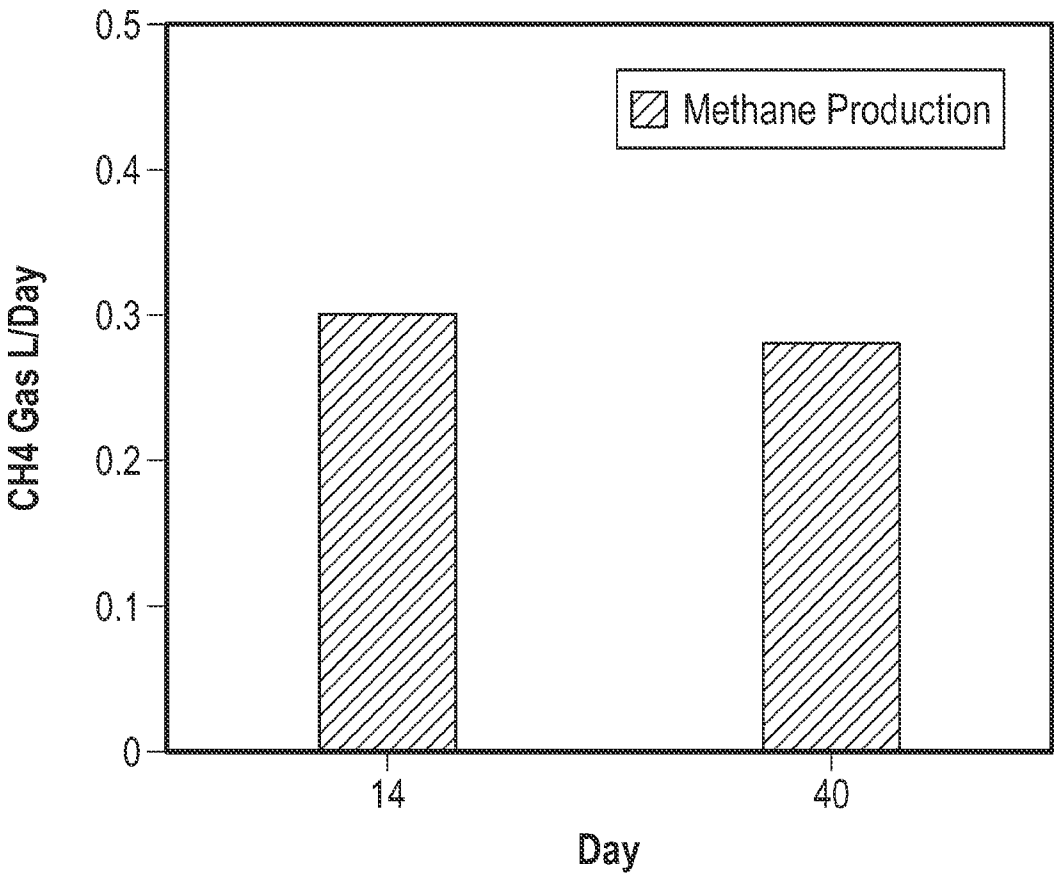


FIG. 41

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2024/034373

A. CLASSIFICATION OF SUBJECT MATTERIPC: *C12N 11/04* (2024.01); *A23L 33/135* (2024.01); *A61K 35/66* (2024.01); *A61K 9/50* (2024.01); *A61P 1/04* (2024.01); *A61P 13/12* (2024.01)CPC: *C12N 11/04*; *A23L 33/135*; *A61K 9/1652*; *A61K 9/1682*; *A61P 1/04*; *A61P 13/12*; *A61K 35/66*; *A61K 9/50*

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History Document

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

See Search History Document

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

See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2022/0000938 A1 (UNIVERSITY OF WASHINGTON) 06 January 2022 (06.01.2022) Entire document	1-6
A	US 2017/0027976 A1 (METHYLATION SCIENCES INTERNATIONAL SRL) 02 February 2017 (02.02.2017) Entire document	1-6
A	US 6,706,287 B2 (RANGANATHAN et al.) 16 March 2004 (16.03.2004) Entire document	1-6
A	WO 2021/076508 A1 (UNIVERSITY OF WASHINGTON) 22 April 2021 (22.04.2021) Entire document	1-6
A	PAPDIMITRIOU et al. Chitosan-g-PEG nanoparticles ionically crosslinked with poly(glutamic acid) and tripolyphosphate as protein delivery systems, Int J Pharm., 10 April 2012, Vol. 430, Nos. 1-2, Pgs. 318-327. Entire document	1-6



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

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

“D” document cited by the applicant in the international application

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

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

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

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

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

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

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

“&” document member of the same patent family

Date of the actual completion of the international search

09 August 2024 (09.08.2024)

Date of mailing of the international search report

20 August 2024 (20.08.2024)

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US
Commissioner for Patents
P.O. Box 1450, Alexandria, VA 22313-1450

Facsimile No. 571-273-8300

Authorized officer

MATOS
TAINA

Telephone No. 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2024/034373

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: **7-48**
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).