FLOATING ENCLOSURES FOR EUTROPHICATION CONTROL

This application claims the benefit of priority of Singapore Patent Application No. 10202204079T, filed 19 April 2022, the content of it being hereby incorporated by reference in its entirety for all purposes.

Technical Field

The present invention relates to the containment of pollutants present in the aquatic environment, and for implementing bioremediation for alleviating eutrophication in tropical and temperate coastal zone seawater and inland water bodies.

Background

Eutrophication refers to water bodies becoming enriched with nutrients, and experiencing an increase in the amount of plant and algae growth. Harmful algal blooms, dead zones, and fish deaths often occur due to eutrophication. Approximately sixty-five percent of the estuaries and coastal waters in the contiguous states of the United States of America (USA) have been found to be moderately to severely containing excessive nutrient levels. These excessive nutrients lead to algal blooms and waters with low oxygen levels (or, hypoxic water) that can kill fish, seagrass and also reduce essential fish habitats. However, on the other hand, many of these water bodies also support bivalve mollusk populations (e.g., oysters, clams, scallops) which naturally reduce nutrients through filter-feeding activities.

Eutrophication sets off a chain reaction in the ecosystem, starting with an overabundance of algae and plants. The excess algae and plant matter eventually decompose,

producing large amounts of carbon dioxide. This lowers the pH level of the relevant water body (ocean), a process known as ocean acidification. Such acidification slows the growth of fish and shellfish and can prevent shell formation in bivalve mollusks. This can lead to a reduced catch for commercial and recreational fisheries, thereby resulting in smaller harvests and more expensive seafood.

The major nutrients that cause over-enrichment are nitrogen and phosphorus. The sources of nutrients vary among regions and are very often remote from the body of affected water. Agriculture, human sewage, urban run-off, industrial effluent, and fossil fuel combustions are the most common sources. The intensive use of commercial fertilizers over the last 50 years has increased crop yield but much of the nutrients not absorbed by crops find their way into local rivers and lakes, and are carried downstream to the estuaries, and eventually to the sea. Human activities have resulted in the near doubling of nitrogen and tripling of phosphorus flows to the environment when compared to natural causes. Trends in agricultural practices, energy use, and population growth indicate that eutrophication, especially in coastal areas, will be an ever-growing problem.

Nutrient over-enrichment often leads to algal bloom resulting in changes in biodiversity and loss of habitats (e.g., decline of coral reef), and appearance of "dead zones" where most animals are driven away or die of hypoxia. Changes in abundance and diversity of living organisms can have adverse impacts on the environmental quality of lakes and coastal waters.

Eutrophication is characterized by excessive plant and algal growth due to the increased availability of one or more limiting growth factors needed for photosynthesis such as sunlight, carbon dioxide, and nutrient fertilizers. Eutrophication occurs naturally over centuries as lakes age and are filled in with sediments. However, human activities which include aquaculture operations have accelerated the rate and extent of eutrophication through both point-source discharges and non-point loadings of limiting nutrients, such as nitrogen and phosphorus, into aquatic ecosystems (i.e., cultural eutrophication), with dramatic consequences for drinking water sources, fisheries, and recreational water bodies. For example, aquaculture scientists and pond managers often intentionally cause eutrophication in water bodies by adding fertilizers to enhance primary productivity and increase the density and biomass of recreationally and economically important fishes via bottom-up effects on higher trophic levels. However, during the 1960s and 1970s, scientists linked algal blooms to nutrient enrichment resulting from anthropogenic activities such as agriculture, industry, and sewage disposal. The known consequences of cultural eutrophication include blooms of blue-green algae (cyanobacteria), tainted drinking water supplies, degradation of recreational opportunities, and hypoxia. The estimated cost of damage mediated by eutrophication in the USA alone is approximately USD2.2 billion annually.

A common symptom of nutrient over-enrichment is the excessive production of algal biomass. Where excess algal proliferation is dominated by certain species of toxic algae, mass mortalities of wild fish and shellfish, birds and even mammals occur; fish farms that use these waters are also impacted. Paralysis, diarrhea, and amnesia are

associated with human consumption of contaminated seafood. In 1997, hundreds of dead fish were found in Chesapeake Bay and Pokomoke River near Maryland in the United States, resulting in waterway closures and public rejection of aquatic produce from the area. The economic loss suffered by the local seafood industry was estimated to be USD43 million. In 1998, an algal bloom wiped out 90 percent of the entire stock of Hong Kong's fish farms and resulted in an estimated economic loss of USD40 million.

Corals and associated organisms in a reef ecosystem in general are adapted to oligotrophic (having a deficiency of plant nutrients that is usually accompanied by an abundance of dissolved oxygen) conditions. Thus, nutrient enrichment resulted from various anthropogenic activities on the coast and land can dramatically influence the functioning of the reef ecosystem. Terrestrial runoff from rivers and streams carrying excess nutrients (inorganic nutrients such as ammonium, nitrate, and phosphate) due to the agricultural, industrial, and various urban activities are the major source of nutrient pollution in the coral environment. Direct discharge of untreated sewage in coastal waters increases the concentration of inorganic nutrients such as ammonium, nitrate, nitrite, and phosphate. A study showed a sharp increase in the nitrate (75-fold), phosphate (31-fold), and ammonium concentrations (3-fold) in the reef environment as a result of terrestrial runoff after heavy rainfall. Another major source of nutrient pollution is coastal fish farming, which increases the organic content of the reef ecosystem near the aquaculture farm through waste like feed pellets, feces, and other organic matter. Similarly, agriculture activities near coastal areas contribute to the pesticide and dissolved inorganic nutrients in the reef ecosystem.

The huge growth in tourism adds up to direct or indirect nutrient pollution in the reef ecosystem. Submarine groundwater discharge was reported to increase nutrient pollution in the Santiago reef flat in Northwestern Philippines.

Nutrient enrichment in the coral environment influences the shift of the benthic community from corals towards more algae and sponges. A study showed that turf algae and opportunistic macroalgae will primarily benefit from chronic nutrient exposure, which will ultimately outcompete the reef-building corals. The prevalence of algae in a coral ecosystem has severe ecological implications, which include coral bleaching, reduced coral growth, and coral mortality. For instance, an increase in calcification rate was observed at elevated phosphorous levels, which in long term stresses the coral animals and damage their skeletal integrity (causing coral skeletal diseases, CSDs). Another study reported the decrease in coral resilience and new coral recruit settlement in the presence of macroalgae, algal turfs and sediment. Increased nutrients promote zooxanthellae growth, which in turn increases oxidative stress in the symbiont and induces bleaching and susceptibility of corals to disease. Nutrient-rich water promotes the growth of plankton-feeding larvae of Crown-of-Thorn Starfish (COTS), the coral predator. Nutrient pollution changes the microbial balance around the coral animals and promotes the growth of opportunistic microbes that eventually make the coral animal susceptible to coral disease such as yellow blotch and black band disease. The scenario worsens when combined with other stressors like sea surface temperature rise (SST) and ocean acidification (OA). Overall, the nutrient pollution in the reef environment introduces an imbalance in the reef ecosystem.

The floating enclosure and its embodiments as put forth, together with the methods, system and use of the system associated with them provide a solution to the difficulties encountered in the preventing, controlling or ameliorating of eutrophication in water bodies.

Summary

In the first aspect, there is provided a floating enclosure suitable for remediating pollutants of interest in eutrophication, said floating enclosure comprising the following components:

(i) a centrally positioned grid frame;
(ii) a plurality of planktivores and/or macroalgae and/or bacteria positioned within the grid frame of (i), and
(iii) a curtain structure positioned at the periphery of the floating enclosure.

Advantageously, such a floating enclosure of the present invention allows the flexibility of its deployment in almost any location where there is a body of eutrophic water and at any time of the year.

The plurality of planktivores of the first aspect may be selected from one or more of the following: oysters, mussels, scallops, clams and abalone. Advantageously, these planktivores as positioned within the grid frame of the floating enclosure can be moved horizontally along a river or a coastal region as well as vertically at various depths in a water body.

The plurality of microalgae of the first aspect may be selected from one or more of the following: raphidophytes, dinoflagellates and diatoms. Advantageously, this eliminates the need for biomanipulative, biostimulative or bioaugmentative methods to curb or control eutrophication.

The plurality of bacteria in the first aspect may be selected from one or more of the following: Bacillus licheniformis (TSB27), Lactobacillus thuringiensis, Bacillus Plantarum, Bacillus subtilis (B46), Shewanella putrefaciens Pdp11, Bacillus pumilus H2, Bacillus subtilis WB60, Lac. pentosus BD6, Lac. fermentum LW2, Bacillus subtilis E20, Saccharomyces cerevisiae P13, Pseudoalteromonas sp., Pseudoalteromonas sp., Lactobacillus plantarum, Bacillus subtilis, Lactobacillus rhamnosus, Lactobacillus casei, Bacillus sp. MVF1, Bacillus subtilis, Bacillus licheniformis, Kocuria SM1, Rhodococcus SM2, Vibrio lentus, Lactobacillus plantarum, Bacillus megaterium PTB 1.4, Lactobacillus rhamnosus, Lactobacillus acidophilus, Bacillus subtilis, Lactobacillus bulgaricus, Saccharomyces cerevisiae, Bacillus megaterium, Bacillus polymyxa Lactobacillus delbrueckii, Enterococcus casseliflavus, Pseudoalteromonas sp., Pseudoalteromonas sp. Cepa MLms gA3, Bacillus sp., Pediococcus sp., Lactobacillus plantarum (LP20), Lactobacillus mesenteroides SMM69, Weissella cibaria P71, Bacillus subtilis, Bacillus licheniformis, Bacillus sp., Pediococcus sp., Enterococcus faecalis, Proteobacteria (including Alphaproteobacteria, betaproteobcteria and gammaproteobacteria), *Nitrobacter*, *Nitrospira*, *Nitrospi*, *Prochlorococcus*, *Synechococcus*, *Trichodesmium*, Heterocystus, *Crocosphaera*, Spirulina (Arthrospira platensis, Arthrospira fusiformis and/or Arthrospira maxima), Pseudomonadota or photosynthetic Bacteria (purple non-sulphur bacteria

including Rhodovulum sulfidophilum and/or Rhodobacter sphaeroides).

The curtain structure (3) of the first aspect may consist of a series of connected wave breaking devices (5), each as shown exemplarily in Figure 1, Figure 2(a), Figure 2(b) and Figure 2(c), and/or a biofilter perimeter fence. A wave breaking device dampens incoming wave energy from tides, winds and vessel wakes, advantageously preventing any damage caused to the components of the floating enclosure and at the same time also, advantageously allowing a reduction of ripple effects thereby allowing sunlight penetration beyond the water depth since the scattering of light at the water surface is reduced. The biofilter perimeter fence of the curtain structure filters out phytoplankton and traps silt particles through the use of filter feeders like shellfish (e.g. mussels), advantageously increasing water clarity, leading to improved sunlight penetration which is beneficial for the cultivation of macroalgae. The curtain structure may be kept afloat using floatation devices, e.g. barrel floats (7).

Component (i) and component (ii), and/or component (iii) of the first aspect may be anchored and/or moored. Advantageously, this allows for a circular shape of the series of connected wave breaking devices and/or a biofilter perimeter fence to be maintained by anchoring to the seabed, if required. Further advantageously, the floating enclosure may be suitably moored at a location where local tides and currents provide natural water exchange.

The plurality of plaktivores and/or macroalgae and/or bacteria as positioned within the centrally positioned grid frame (i) of the first aspect may provide shade to the water body underneath the grid frame. Advantageously, this can reduce the average temperature of the entire water body and also the acidification of it.

The curtain structure as positioned at the periphery of the floating enclosure in the first aspect may be discontinuous as shown in Figure 1, by way of an entrance (1) for access to the centrally positioned grid frame (2). Advantageously, this allows for access to the central components of the floating enclosure for preventive maintenance, repairs or other purposes requiring such access.

In a second aspect, there is provided a method of remediating eutrophication in a water body, comprising the following steps:

(i) Positioning a floating enclosure according to the first aspect and its variations into the water body;
(ii) Allowing the plurality of planktivores and/or macroalgae and/or bacteria as positioned within the grid frame of the floating enclosure to feed on nutrients present in the water body, thereby limiting the availability of such nutrients to prevent, reduce or ameliorate an algae bloom.

Advantageously, the method as put forth in the second aspect allows for an effective framework to remediate eutrophication in a water body.
The algae bloom as prevented, reduced or ameliorated in the method of the second aspect may be determined by way of remote sensing and/or water sampling. Advantageously, this

allows for the extent of the algae bloom event to be quantifiable and statistically recorded.

The dissolved oxygen level of the water body in step (ii) of the second aspect during the prevention, reduction or amelioration of the algae bloom may be on the average more than 3mg/L within the floating enclosure and its vicinity. Advantageously, this avoids hypoxia or anoxia which can kill marine life, disrupt their migration and habitats, and changes (disrupts) a benthic community structure.

The following step (iii) may be further present in regard of the method in the second aspect:

(iii) Introducing one or more fish species into the floating enclosure.

Advantageously, the one or more fish species as introduced into the floating enclosure will feed on the nutrients in the water body experiencing eutrophication.

Yet further, the following step (iv) may be present in the variation of the second aspect mentioned above where one or more fish species are introduced into the floating enclosure:

(iv) Harvesting from the plurality of planktivores and/or macroalgae and/or bacteria and/or fish species.

Advantageously, step (iv) allows for the processing of the harvest to provide valuable products, for instance: biofuel, specialty chemicals, nutraceuticals, food, and/or alternative protein as fish meal replacement.

The nutrients in the second aspect may contain one or more of the following elements: nitrogen, phosphorous, iron and silicon. Advantageously, these elements as identified to be limited make up one or more of the nutrients which lead up to an algae bloom.

The species found in the algae bloom of the second aspect may be one or more of the following species: Anabaena, Phenomenon, Microcystis, Noctiluca, Alexandrium, Prorocentrum, Gymnodinium, Ceratium, Pfiesteria, Dinophysis, Gyrodinium, Heterosigma, Chattonella, Skeletonema, Synedropsis, Pseudonitzschia, Leptocylindrus, Chaetoceros; Phaeocystis, Nannochloris, Stichococcus, Aureococcus, Miraltia, Guinardia, Spermatozopsis, Urosolenia, Nitschia, Cyclotella, Cryptomonas, and Pedinophora.

Advantageously, knowledge of one or more of the species found in the algae bloom provides an understanding of the potential interaction between the species and existing marine life and anthropogenic activities.

In the third aspect, there is provided a system of at least two floating enclosures, wherein each of the at least two floating enclosures is selectable from the floating enclosure as provided in the first aspect and its variations.

Advantageously, such a system allows for an integrated and widespread implementation of the floating enclosure provided in the first aspect, allowing for the prevention, reduction or amelioration of an algae bloom on a scaled up basis involving more than one floating enclosure.

In the fourth aspect, there is provided the use of the system mentioned in the third aspect to prevent, reduce or ameliorate eutrophication and/or the harvesting of the plurality of planktivores and/or macroalgae and/or bacteria and additionally and/or fish species. Advantageously, the use of the system allows not only for prevention, reduction or amelioration of eutrophication, but also allows garnering value added products as part of an overall eutrophication control strategy.

Definitions

The following words and terms used herein shall have the meaning indicated:

The term "algae" refers to any organisms with chlorophyll and a thallus not differentiated into roots, stems and leaves, and encompasses prokaryotic and eukaryotic organisms that are photoautotrophic or photo auxotrophic. The term "algae" includes macroalgae (commonly known as seaweed) and microalgae. The terms "microalgae" and "phytoplankton," used interchangeably herein, refer to any microscopic algae, photoautotrophic or photoautotrophic eukaryotes (such as, protozoa), photoautotrophic or photoautotrophic prokaryotes. The use of the term "algal" also relates to microalgae and thus encompasses the meaning of "microalgal."

The term "algal bloom" as used herein refers to the presence of algae in a body of water comprising at least about 1×106 algal cells per ml or greater, such as but not limited to about 5×106 algal cells per ml, about 107 algal cells per ml, about 5×107 algal cells per ml, and about 108 algal cells per ml.

The term "vicinity" is used to refer to a locus within a radius of up to 30m of a location.

The words "essentially" and "substantially" do not exclude "completely" e.g. a composition which is "substantially free" from Y may be completely free from Y. Where necessary, the word "substantially" may be omitted from the definition of the invention.

Unless specified otherwise, the terms "comprising" and "comprise", and grammatical variants thereof, are intended to represent "open" or "inclusive" language such that they include recited elements but also permit inclusion of additional, unrecited elements.

As used herein, the term "about", typically means +/- 10% of the stated value, more typically +/- 9% of the stated value, more typically +/- 8% of the stated value, more typically +/- 7% of the stated value, more typically +/- 6% of the stated value, more typically +/- 5% of the stated value, more typically +/- 4% of the stated value, more typically +/- 3% of the stated value, more typically +/- 2% of the stated value, more typically +/- 1% of the stated value, and even more typically +/- 0.5% of the stated value.

The term "slightly", in the context of any value described in the specification means typically +/- 10% of the stated value, more typically +/- 9% of the stated value, more typically +/- 8% of the stated value, more typically +/- 7% of the stated value, more typically +/- 6% of the stated value, more typically +/- 5% of the stated value, more typically +/- 4% of the stated value, more typically +/- 3% of the stated value, more typically, +/- 2% of the stated value, more typically +/- 1% of the stated value, and even more typically +/- 0.5% of the stated value.

The word “similar” is to be inferred as “comparable with” with the intention to neglect any non-major and/or minor features present.

Throughout this disclosure, certain embodiments may be disclosed in a range format. It should be understood that the description in range format is merely for convenience

and brevity and should not be construed as an inflexible limitation on the scope of the disclosed ranges.

Accordingly, the description of a range should be considered to have specifically disclosed all the possible sub-ranges as well as individual numerical values within that range. For example, the description of a range such as from 1 to 6 should be considered to have specifically disclosed sub-ranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 3, 4, 5, and 6. This applies regardless of the breadth of the range.

Disclosure of Optional Embodiments

Exemplary, non-limiting embodiments according to the first, second, third and fourth aspects will now be disclosed.

In an embodiment of the first aspect, the grid frame, is made of units, each unit (as shown in Figure 4a, Figure 4b and Figure 4c) made of any suitable material (including wood) and is of any of the dimensions as follows: 1m x 1m, 2m x 2m, 3m x 3m, 5m x 10m or any other suitable dimension or shape, with the presence of securing long lines (of which may be in arrayed form). Vertical ropes (4a) may be attached to these long, essentially horizontal spanning lines (4b), as shown in Figure 4(b)and Figure 4(c).

In another embodiment, the plurality of the planktivores are filter feeders of the pelagic or benthic filter feeding types. In yet another embodiment, one or more species of seaweed (macroalgae) and or in combination with planktivores may be positioned within the grid frame. In an embodiment, the population of dominant species of seaweed (macroalgae) may constitute greater than about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 75%, about 80%, about 90%, about 95%, about 97%, about 98% of the seaweed present in the population. In certain embodiments, several major seaweed (macroalgae) species may each constitute greater than about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, or about 80% of the seaweed present in the population.

In another embodiment, seaweed indigenous to the region at which the floating enclosure of the current application are installed, are used. Preferably, seaweed species from the

same climatic region, same salinity environment, or same ecosystem are used. Most preferably, the seaweed (macroalgae)and planktivores are derived from a naturally occurring trophic system.

In another embodiment, the planktivores are shellfish of the freshwater, brackish water or marine (saltwater) types. The shellfish of the present application include but are not limited to oysters, mussels, scallops, clams, and more particularly, Crassostrea species such as C. gigas, C. virginica, C. ariakensis, C. rivularis, C. angulata, C. eradelie, C. commercialis, Saccostrea species such as S. glomerata, S. cucculata and S. commercialis; Mercenaria species such as M. mercenaria and M. campechensis; Ostrea species such as O. edulis, O. chilensis, and O. lurida, Area transversa, Panope generosa, Saxodomus nuttili; Mytilus species such as M. edulis (blue mussel), M, coruscus, M. chilensis, M. trossulus, and M. galloprovincialis (Mediterranean mussel), Aulacomya ater, Choromytilus chorus, Tapes semidecussatus; Perna species such as P. viridis, P. canaliculus; Venerupis species such as V. decussata, V. semidecussata, Sinonovacula constricta (Razor clam), Mya arenaria (soft shell clams), Spisula solidissima (surf clams), Amusium balloti, Argopecten irradians (bay scallops); Pectan species such as P. alba, P. yessonsis, P. maximus; and Chlamys species such as C. farreri, C. opercularis, C. purpuratus and C. varia.

In another embodiment, the plurality of planktivores (shellfish) and macroalgae are both cultured within the same body of eutrophic water, in proximity to each other in the same floating enclosure. In yet another embodiment, the plurality of bacteria are one or more of the following bacteria:

Bacillus licheniformis (TSB27), Lactobacillus thuringiensis, Bacillus Plantarum, Bacillus subtilis (B46), Shewanella putrefaciens Pdp11, Bacillus pumilus H2, Bacillus subtilis WB60, Lac. pentosus BD6, Lac. fermentum LW2, Bacillus subtilis E20, Saccharomyces cerevisiae P13, Pseudoalteromonas sp., Pseudoalteromonas sp., Lactobacillus plantarum, Bacillus subtilis, Lactobacillus rhamnosus, Lactobacillus casei, Bacillus sp. MVF1, Bacillus subtilis, Bacillus licheniformis, Kocuria SM1, Rhodococcus SM2, Vibrio lentus, Lactobacillus plantarum, Bacillus megaterium PTB 1.4, Lactobacillus rhamnosus, Lactobacillus acidophilus, Bacillus subtilis, Lactobacillus bulgaricus, Saccharomyces cerevisiae, Bacillus megaterium, Bacillus polymyxa Lactobacillus delbrueckii, Enterococcus casseliflavus, Pseudoalteromonas sp., Pseudoalteromonas sp. Cepa MLms gA3, Bacillus sp., Pediococcus sp., Lactobacillus plantarum (LP20), Lactobacillus mesenteroides SMM69, Weissella cibaria P71, Bacillus subtilis, Bacillus licheniformis, Bacillus sp., Pediococcus sp., Enterococcus faecalis, Proteobacteria (including Alphaproteobacteria, betaproteobcteria and gammaproteobacteria), *Nitrobacter*, *Nitrospira*, *Nitrospi*, *Prochlorococcus*, *Synechococcus*, *Trichodesmium*, Heterocystus, *Crocosphaera*, Spirulina (Arthrospira platensis, Arthrospira fusiformis and/or Arthrospira maxima), Pseudomonadota or photosynthetic Bacteria (purple non-sulphur bacteria including Rhodovulum sulfidophilum and/or Rhodobacter sphaeroides).

In an embodiment, the macroalgae can be macroscopic, multicellular, marine algae species which includes some types of Rhodophyta (red), Phaeophyta (brown) and Chlorophyta (green) macroalgae and ephemeral macroalgae like Ulva, Caldophora and Chaetomorpha.

In an embodiment, umbrella culture or rack culture is used in cultivating shellfish as the plurality of planktivores.

In an embodiment, the curtain structure positioned at the periphery of the floating enclosure is kept afloat by flotation devices like barrel type floats (7) as shown in Figure 2(a), Figure 2(b) and Figure 2(c). In another embodiment, a biofilter perimeter fence of the curtain structure is made up of filter feeders (e.g. mussels or other shellfish) attached on vertical ropes which in turn are attached to essentially horizontal spanning ropes, a singularity of which is exemplarily depicted in Figure 2(c), item (8) in a unit of the curtain structure.

In another embodiment, the floating enclosure is free-floating and moves with local currents.

In yet another embodiment, the floating enclosure is unrestrained and can be moved using mechanical means, e.g. using a propulsion or propeller boat engine.

In an embodiment, the planktivores are shellfish, including but not limited to abalone, coneshell, oysters, mussels, scallops, clams, and more particularly, Crassostrea species such as C. gigas, C. virginica, C. ariakensis, C. rivularis, C. angulata, C. eradelie, C. commercialis, Saccostrea species such as S. glomerata, S. cucculata and S. commercialis; Mercenaria species such as M. mercenaria and M. campechensis; Ostrea species such as O. edulis, O. chilensis, and O. lurida, Area transversa, Panope generosa, Saxodomus nuttili; Mytilus species such as M. edulis (blue mussel), M, coruscus, M. chilensis, M. trossulus, and M. galloprovincialis (Mediterranean mussel), Aulacomya ater, Choromytilus chorus, Tapes semidecussatus; Perna species

such as P. viridis, P. canaliculus; Venerupis species such as V. decussata, V. semidecussata, Sinonovacula constricta (Razor clam), Mya arenaria (soft shell clams), Spisula solidissima (surf clams), Amusium balloti, Argopecten irradians (bay scallops); Pectan species such as P. alba, P. yessonsis, P. maximus; and Chlamys species such as C. farreri, C. opercularis, C. purpuratus and C. varia.

In another embodiment, the series of connected wave breaking devices of the curtain structure are floating drum-like structures made of high density polyethylene (HDPE) material mixed with seaweed bioplastics, arrayed in a circular form with regular intervals of long nylon ropes anchored to the sea floor to maintain a circular shape.

In an embodiment, the series of connected wave breaking devices, not only dampens incoming wave energy from tides, winds and vessel wakes but also acts as a mechanical filter barrier to reduce suspended particles from entering the macroalgae cultivation zone within the grid frame. In certain embodiments, each of the wave breaking devices has curved surfaces (6), diagrammatically depicted in Figure 3.

In some embodiments, the components (i) and (iii) of the floating enclosure are moored physically to a fixed point on a seabed or to a fixed structure which is erected in water. In another embodiment, the floating enclosure is moored at a fixed location where local tides and currents provide natural water exchange. In yet another embodiment, the floating enclosure can be anchored and/or moored in a reversible fashion such as to allow mobility of the floating enclosure when necessary.

In an embodiment of the second aspect, the water body may contain freshwater, brackish water or seawater, or may be coastal waters, inland waters, oceans, lakes, rivers and waterways.

In another embodiment, the grid frame found within the floating enclosure, of any suitable material (including wood) is each unit (as depicted in Figure 4a, Figure 4b and Figure 4c) of dimensions as follows: 1m x 1m, 2m x 2m, 3m x 3m, 5m x 10m or any other suitable dimension or shape, with the presence of securing long lines (item 4b as depicted in Figures 4(a)-4(c), of which may be in arrayed form). Vertical ropes (4a) may be attached to these essentially horizontal long lines (4b).

In another embodiment, the plurality of the planktivores are filter feeders of the pelagic or benthic filter feeding types. In yet another embodiment, one or more species of seaweed (macroalgae) and or in combination with planktivores may be positioned within the grid frame within the floating enclosure. In an embodiment, the population of dominant species of seaweed (macroalgae) may constitute greater than about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 75%, about 80%, about 90%, about 95%, about 97%, about 98% of the seaweed present in the population. In certain embodiments, several major seaweed (macroalgae) species may each constitute greater than about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, or about 80% of the seaweed present in the population.

In another embodiment, seaweed indigenous to the water body at which the method of the second aspect of the current application is practised, are used. Preferably, seaweed

species from the same climatic region, same salinity environment, or same ecosystem are used. Most preferably, the seaweed (macroalgae)and planktivores are derived from a naturally occurring trophic system.

In another embodiment, the planktivores are shellfish of the freshwater, brackish water or marine (saltwater) types. The shellfish include but are not limited to oysters, mussels, scallops, clams, and more particularly, Crassostrea species such as C. gigas, C. virginica, C. ariakensis, C. rivularis, C. angulata, C. eradelie, C. commercialis, Saccostrea species such as S. glomerata, S. cucculata and S. commercialis; Mercenaria species such as M. mercenaria and M. campechensis; Ostrea species such as O. edulis, O. chilensis, and O. lurida, Area transversa, Panope generosa, Saxodomus nuttili; Mytilus species such as M. edulis (blue mussel), M, coruscus, M. chilensis, M. trossulus, and M. galloprovincialis (Mediterranean mussel), Aulacomya ater, Choromytilus chorus, Tapes semidecussatus; Perna species such as P. viridis, P. canaliculus; Venerupis species such as V. decussata, V. semidecussata, Sinonovacula constricta (Razor clam), Mya arenaria (soft shell clams), Spisula solidissima (surf clams), Amusium balloti, Argopecten irradians (bay scallops); Pectan species such as P. alba, P. yessonsis, P. maximus; and Chlamys species such as C. farreri, C. opercularis, C. purpuratus and C. varia.

In another embodiment, the plurality of planktivores (shellfish) and macroalgae are both cultured within the same body of eutrophic water, in proximity to each other in the same floating enclosure as positioned in the method of the second aspect. In yet another embodiment, the plurality of bacteria are one or more of the following

bacteria: Bacillus licheniformis (TSB27), Lactobacillus thuringiensis, Bacillus Plantarum, Bacillus subtilis (B46), Shewanella putrefaciens Pdp11, Bacillus pumilus H2, Bacillus subtilis WB60, Lac. pentosus BD6, Lac. fermentum LW2, Bacillus subtilis E20, Saccharomyces cerevisiae P13, Pseudoalteromonas sp., Pseudoalteromonas sp., Lactobacillus plantarum, Bacillus subtilis, Lactobacillus rhamnosus, Lactobacillus casei, Bacillus sp. MVF1, Bacillus subtilis, Bacillus licheniformis, Kocuria SM1, Rhodococcus SM2, Vibrio lentus, Lactobacillus plantarum, Bacillus megaterium PTB 1.4, Lactobacillus rhamnosus, Lactobacillus acidophilus, Bacillus subtilis, Lactobacillus bulgaricus, Saccharomyces cerevisiae, Bacillus megaterium, Bacillus polymyxa Lactobacillus delbrueckii, Enterococcus casseliflavus, Pseudoalteromonas sp., Pseudoalteromonas sp. Cepa MLms gA3, Bacillus sp., Pediococcus sp., Lactobacillus plantarum (LP20), Lactobacillus mesenteroides SMM69, Weissella cibaria P71, Bacillus subtilis, Bacillus licheniformis, Bacillus sp., Pediococcus sp., Enterococcus faecalis, Proteobacteria (including Alphaproteobacteria, betaproteobcteria and gammaproteobacteria), *Nitrobacter*, *Nitrospira*, *Nitrospi*, *Prochlorococcus*, *Synechococcus*, *Trichodesmium*, Heterocystus, *Crocosphaera*, Spirulina (Arthrospira platensis, Arthrospira fusiformis and/or Arthrospira maxima), Pseudomonadota or photosynthetic Bacteria (purple non-sulphur bacteria including Rhodovulum sulfidophilum and/or Rhodobacter sphaeroides).

In an embodiment, the macroalgae can be macroscopic, multicellular, marine algae species which includes some types of Rhodophyta (red), Phaeophyta (brown) and Chlorophyta (green) macroalgae and ephemeral macroalgae like Ulva, Caldophora and Chaetomorpha.

In an embodiment, umbrella culture or rack culture is used in cultivating shellfish to form the plurality of planktivores.

In an embodiment, the curtain structure at the periphery of the floating enclosure which is positioned in the method of the second aspect is kept afloat by flotation devices e.g. barrel type floats (7).

In another embodiment, the floating enclosure after being positioned, is free-floating and allowed to move with local currents.

In another embodiment, the floating enclosure as positioned is unrestrained and can be moved using mechanical means, e.g. using a propulsion or propeller type boat engine.

In an embodiment of the second aspect, the plurality of planktivores are shellfish, including but not limited to abalone, coneshell, oysters, mussels, scallops, clams, and more particularly, Crassostrea species such as C. gigas, C. virginica, C. ariakensis, C. rivularis, C. angulata, C. eradelie, C. commercialis, Saccostrea species such as S. glomerata, S. cucculata and S. commercialis; Mercenaria species such as M. mercenaria and M. campechensis; Ostrea species such as O. edulis, O. chilensis, and O. lurida, Area transversa, Panope generosa, Saxodomus nuttili; Mytilus species such as M. edulis (blue mussel), M, coruscus, M. chilensis, M. trossulus, and M. galloprovincialis (Mediterranean mussel), Aulacomya ater, Choromytilus chorus, Tapes semidecussatus; Perna species such as P. viridis, P. canaliculus; Venerupis species such as V. decussata, V. semidecussata, Sinonovacula constricta (Razor clam), Mya arenaria (soft shell clams), Spisula

solidissima (surf clams), Amusium balloti, Argopecten irradians (bay scallops); Pectan species such as P. alba, P. yessonsis, P. maximus; and Chlamys species such as C. farreri, C. opercularis, C. purpuratus and C. varia.

In another embodiment, the series of connected wave breaking devices of the curtain structure found in the floating enclosure are positioned in a floating drum-like structure made of high density polyethylene (HDPE) material mixed with seaweed bioplastics, arrayed in a circular form with regular intervals of long nylon ropes anchored to the sea floor to maintain an essentially circular shape (as depicted in Figure 1). In a further embodiment, the series of connected wave breaking devices not only dampens incoming wave energy from tides, winds and vessel wakes but also acts as a mechanical filter barrier to reduce suspended particles from entering the macroalgae cultivation zone within the grid frame. In certain further embodiments, each of the wave breaking devices has curved surfaces (6) as depicted in Figure 3.

In some embodiments, components (i) and (iii) of the floating enclosure to be positioned are moored physically to a fixed point on a seabed or to a fixed structure which is erected in water. In another embodiment, the floating enclosure to be positioned is moored at a fixed location where local tides and currents provide natural water exchange. In yet another embodiment, the floating enclosure when being positioned can at the same time be anchored and/or moored in a reversible fashion such as to allow mobility of the floating enclosure when necessary.

In an embodiment, the remote sensing techniques which may be used to determine the algae bloom include the use of

data systems that comprise remote sensing (e.g. AVHRR, SEAWIFS, LANDSAT and MODIS based on satellite technology and also hyperspectral sensors) and water sampling subsystems, data integration, modeling subsystems and other advanced techniques (e.g. fourth derivative analysis). In another embodiment, the water sampling techniques used to determine the algae bloom include the use of measurement devices adapted to record the parameters of the water body in which the algae bloom is present; a data processing device having programming instructions for applying an algorithm that converts data into nutrient level data, optionally linked to geographic data; a transmitter for transmitting data from the measuring device or processor to a site remote from the site where the measurement takes place, an early warning system for eutrophication and excessive temperature built-up; and a report generator for producing a report of the amounts of nutrient and algal biomass in the water over an area.

In an embodiment, the dissolved oxygen level in step (ii) during the prevention, reduction or amelioration of the algae bloom is, on the average more than 3mg/L in the water body within the floating enclosure and its vicinity of within a radius of 30m from the centre of the floating enclosure.

In an embodiment, the method of the second aspect is practiced with the additional step (iii), i.e., introducing one or more fish species in the likes of finfish (e.g. salmon, bass, porgy, tuna, flounder, haddock, halibut, catfish, red snapper, whiting, cod, and ocean perch) into the floating enclosure. In yet a further embodiment, the following step (iv) is present: harvesting from the plurality of planktivores and/or macroalgae and/or bacteria

and/or fish species, in which the fish species are finfish including salmon, bass, porgy, tuna, flounder, haddock, halibut, catfish, red snapper, whiting, cod, and ocean perch.

In an embodiment, the nutrients present in the water body include both phosphorous and nitrogen.

In an embodiment, the system of the third aspect consists of two or more floating enclosures positioned at about equidistance apart.

In an embodiment of the fourth aspect, the use of the system to prevent, reduce or ameliorate eutrophication and/or the harvesting of the plurality of planktivores and/or macroalgae and/or bacteria and additionally and/or fish species in which, the fish species one or more of the following: salmon, bass, porgy, tuna, flounder, haddock, halibut, catfish, red snapper, whiting, cod, and ocean perch.

Brief Description Of Drawings/Table

The accompanying drawings and tables illustrate disclosed embodiments and serve to explain the principles of the disclosed embodiments. It is to be understood, however, that the drawings and table are designed for purposes of illustration only, and not as a definition of the limits of the invention.

Fig. 1 shows the plan view of an exemplary floating enclosure.

Fig. 2(a) shows the plan view of an exemplary curtain structure of the floating enclosure.

Fig. 2(b) shows the side view of an exemplary curtain structure of the floating enclosure.

Fig. 2(c) shows an isometric view of an exemplary curtain structure of the floating enclosure.

Fig. 3 shows various views of an exemplary wave breaking device suitable to be used in the floating enclosure as a series of connected wave breaking devices.

Fig. 4(a) shows the plan view of an exemplary centrally positioned grid frame unit together with a plurality of organisms (planktivores and/or macroalgae and/or bacteria) positioned within the grid frame.

Fig. 4(b) shows the side view of an exemplary centrally positioned grid frame unit together with a plurality of organisms (planktivores and/or macroalgae and/or bacteria) positioned within the grid frame.

Fig. 4(c) shows the isometric view of an exemplary centrally positioned grid frame unit together with a plurality of organisms (planktivores and/or macroalgae and/or bacteria) positioned within the grid frame.

Table 1 provides a list of bacteria which can act as probiotics to fish in the floating enclosures and their related methods or systems.

Examples

Non-limiting examples of the invention will be further described in greater detail by reference to specific Examples. These examples should not be construed as in any way limiting the scope of the invention.

Example 1: Dimensions of a unit of the grid frame in the floating enclosure

In this example, the dimensions of the unit of the grid frame in the floating enclosure are shown in centimetres in Figure 4(a) in which the overall length and width of the unit is approximately 1000cm and 500cm.

Example 2: Dimensions of a unit of a single wave breaking device in the curtain structure

In this example, the dimensions of a unit of a single wave breaking device in the curtain structure are shown in centimetres in Figure 3, in which the characteristic length is approximately 50cm, the shortest linear distance between the two edges of each curved face is 25cm, and the diameter ø of the circular cross-section of the unit is approximately 50cm.

Example 3: Floating enclosures in multiplicity

Floating enclosures of the present application can be interconnected to form a system with at least two floating enclosures. An island like installation will be visually

apparent when the number of interconnected floating enclosures increases.

Example 4: Detecting, quantifying and analyzing an algae bloom/eutrophic water

Eutrophic water and algal bloom is identified by one or more techniques, such as but not limited to analysis of water samples and remote sensing. Data collected is analyzed in combination with historical data for a region. For example, eutrophic water can be found in a location where an algal bloom has occurred previously or occurs on a regular, diurnal, seasonal, or annual basis.

Example 5: Application of the floating enclosure in water bodies of different water bodies with varying characteristics

The floating enclosure is useful in eutrophic water found inland or in coastal areas, including but not limited to lakes, river estuaries, continental shelves, lagoons, drowned river beds, embayments, gulfs, and coastal plains. Typically, the source of the nutrients in eutrophic water is agricultural runoff, industrial effluent, sewage, upwelling, aquaculture waste, or a mixture thereof. The oversupply of nutrients in a body of eutrophic water support algal growth leading to an increase in algae biomass, an algal bloom, a harmful algal bloom, or hypoxia. The growth rate of the algae in eutrophic water is limited by the concentration of at least one of these elements: nitrogen, phosphorous, iron, or silicon.

Example 6: Mobility of floating enclosures

The enclosures are not permanently installed and can be relocated to another geographic location. As a further

example, an enclosure is a towage cage or net in which seaweed, planktivorous organisms and shellfish are cultured.

Example 7: Integration of floating enclosures with marine structures

In this example, the floating enclosure can be integrated or adapted to a vessel or an oil/gas production platform.

Example 8: Use of various bacteria as probiotics in the floating enclosures and their related methods and/or systems

The bacteria listed in Table 1 act as probiotics to fish species within the floating enclosures and its related methods and/or systems.

Example 9: Bacteria suitable for use in the floating enclosures and their related methods and/or systems

Marine bacteria are single-celled organisms that can be shaped like little spheres, rods, or (less commonly) spirals. They are often very small, with cell diameters of just a few microns (about 1/100th the width of a human hair). They perform all kinds of chemical processes in the open ocean, including most of the steps in nitrogen cycling.

As examples, alphaproteobacteria and gammaproteobacterial (Proteobacteria) are an extremely diverse group of bacteria that are suitable. Some alphaproteobacteria and gammaproteobacteria carry the nifH gene, and may be able to fix nitrogen.

As further examples, ammonium oxidizing bacteria (AOB) are suitable bacteria that are all involved in a specific biochemical process (nitrification), but which may or may not be related to one another. They are involved in the

first step of nitrification — the conversion of ammonium to nitrite (also known as "ammonium oxidation").

Most of the known ammonium oxidizing bacteria are betaproteobacteria and gammaproteobacteria. These bacteria are believed to be involved in this process because they carry a gene that codes for the ammonia monooxygenase enzyme (*amoA*).

Several different genera of marine bacteria are involved in the second step in the nitrification process—converting nitrite to nitrate (also known as "nitrite oxidation"). These include the suitable *Nitrobacter*, *Nitrospira*, and *Nitrospina*. Of these, *Nitrospira* is believed to be the most important in the open ocean.

Prochlorococcus is a genus of suitable cyanobacteria that is very common in open-ocean areas around the world. Although extremely tiny, with cells only 0.5 to 0.8 microns across, *Prochlorococcus* (along with another genus, *Synechococcus*) are so widespread and abundant that may produce a third of the oxygen in the Earth's atmosphere. *Prochlorococcus* are "obligate photoautotrophs", obtaining all of their energy through photosynthesis. However, they require nitrogen as a nutrient, and can use either nitrate or ammonium as a source of nitrogen. Thus, they are a key part of the "assimilation" process in oceanic nitrogen cycles.

Synechococcus is another suitable, common type of marine cyanobacteria. It is arguably the second most common group of photosynthetic marine bacteria, after *Prochlorococcus*. It has a similar shape as *Prochlorococcus*, but is typically a little bit larger, at 0.8 to 1.5 microns across.

Like *Prochlorococcus*, *Synechococcus* are "obligate phototrophs", which means that they can only obtain energy through photosynthesis, and require nitrate as a key nutrient. They can use nitrate or ammonium as sources of nitrogen, and are a key part of the "assimilation" process in oceanic nitrogen cycles.

Cyanobacteria are a large group of photosynthetic bacteria, some of which can "fix" nitrogen, converting nitrogen gas into more biologically useful compounds. Cyanobacteria live in all kinds of environments, but are especially important in open-ocean ecosystems. They were formerly known as blue-green algae but are now recognized as a type of bacteria, not a type of algae (algae are eukaryotes). Other suitable forms of cyanobacteria which may be used in the floating enclosures of the present applications are *Trichodesmium*, Heterocystus cyanobacteria, *Crocosphaera and uncultivated cyanobacteria group A (UCYN-A)*. *Trichodesimium* is a genus of colonial cyanobacteria that is one of the most important and well-studied nitrogen-fixing organisms found in open-ocean areas. Heterocystus cyanobacteria are multi-celled organisms that form microscopic filaments and perform nitrogen fixation in the open ocean. The most common genus of Heterocystus cyanobacteria in open-ocean areas is *Richelia*, which is almost always found living inside of diatoms, a type of microscopic marine algae. *Crocosphaera* is a genus of singled-celled cyanobacteria that lives through photosynthesis and can "fix" nitrogen. It grows in many tropical ocean areas where the water is about 24 degrees Celsius. Uncultivated cyanobacteria group A (UCYN-A) is a group of nitrogen-fixing cyanobacteria that were first discovered in the open ocean near Hawaii in 1998. Other suitable non-toxic cyanobacteria include

Spirulina (including Arthrospira platensis, Arthrospira fusiformis and Arthrospira maxima).

The suitable purple non-sulfur bacteria (PNSB) including Rhodovulum sulfidophilum and Rhodobacter sphaeroides are part of a group of Pseudomonadota capable of photosynthesis. PNSB are characterized by their ability to perform anoxygenic photosynthesis and are widely distributed in soil and water. In recent years PNSB are increasingly applied to aquaculture in asia, and their probiotic effect mechanisms on health of aquatic livestocks as well as water quality improvements are gaining more intensive studies and validation. They are proven to increase feed conversion, contain essential dietary components such carotenoids, B vitamins and fatty acids as well as synthesize powerful antibiotic substances. They are demonstrated to remain viable as they pass through an animal's gut. They are highly digestible and present absolutely no threat of pathogenesis. Moreover, they do no harm to, and are indeed known to work synergistically with, other probiotic genera such as Lactobacillus. The ability of PNSB to inhibit pathogenic bacteria such as vibrios and the ability to digest organic matter in water column warrants its application in aquaculture, especially in high density shrimp farming.

Example 10: Other organisms suitable for use in the floating enclosures and their related methods and/or systems

Archaea are single celled organisms that look similar to bacteria, but which are in an entirely separate biological domain. Historically, archaea were thought of as living mostly in extremely hot, acidic, or low oxygen environments. However, in the last decade, using DNA and

RNA analysis, molecular biologists have found them to be very common in both freshwater and saltwater environments. Like ammonium oxidizing bacteria, suitable ammonium oxidizing archaea (AOA) are important in the nitrification process in open-ocean areas. They perform the first step of nitrification - the conversion of ammonium to nitrite, which is also known as "ammonium oxidation." In the process of performing ammonium oxidation process, AOA may produce nitrous oxide, a greenhouse gas that is sometimes released from open-ocean waters into the atmosphere.All of the suitable, known ammonium oxidizing archaea are in the group *Thaumarchaea* (formerly known as *Crenarchaeota*). These archaea are common in soils, in estuaries, and in the deeper parts of the open ocean, where there is little light and oxygen concentrations are relatively low. Similar to ammonium oxidizing bacteria (AOB), AOA are able to produce an enzyme called ammonia monooxygenase.

Applications

The potential applications of the presently disclosed technology are wide-ranging and are described below:

The present technology is useful for the containment of pollutants present in the aquatic environment and for implementing a biological bioremediation method for eutrophication in tropical and temperate coastal zone sea waters and inland water bodies.

The lack of recovery of coral reefs worldwide and the proliferation of Crown-of-Thorn Starfish (COTS) have been largely attributable to eutrophication. In addition, evidence based on research reveals that coral skeletal

diseases (CSDs) and coral bleaching are indeed also promoted by eutrophication. Much of the increased fertility/eutrophication is due to the increased loads of nutrients exported via discharges from coastal developments. The present technology as put forth in the current application can aid authorities to reduce runoff nutrient loads, including both on a smaller scale basis of 1-10 floating enclosures as installed, or to more than 50 such floating enclosures for larger sized water bodies of concern.

The use of the present technology also allows marine science authorities to establish a further and deeper understanding of the relationships of various elements in a eutrophic system to offer the possibility of biological intervention with a view towards abating the deleterious effects of eutrophication, this being possible with the inclusion of remote sensing systems and suitable water sampling techniques integrated within the present technology.

The present technology is centred on the recognition that the presence of nutrients are the major cause of massive eutrophication, and that an algae bloom can be prevented, controlled or ameliorated efficiently by the removal of these nutrients that form the major feed source for algae. In such instances, this could mean a revival of a fishery industry that has been affected by eutrophication and create a positive impact on food security issues.

As the plurality of planktivores and/or macroalgae and/or bacteria as positioned within the grid frame of the present technology can provide shade to the water underneath it, this will also positively impact and potentially stall and

reverse the effects of global warming action on the water bodies.

It will be apparent that various other modifications and adaptations of the invention will be apparent to the person skilled in the art after reading the foregoing disclosure without departing from the spirit and scope of the invention and it is intended that all such modifications and adaptations come within the scope of the appended claims.