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(54) **Title:** METHODS FOR TREATMENT OF WASTEWATER USING MICROALGAL-BACTERIAL CONSORTIA, AND THEIR APPLICATIONS FOR WATER REUSE

(57) **Abstract:** The field of the disclosure lies in the treatment of wastewater. The present disclosure relates to a method for the treatment of wastewater. The present disclosure also relates to a device for the treatment of wastewater. The present disclosure further relates to a closed system comprising a lower part and an upper part, wherein wastewater and microalgae and/or a microalgae-bacteria consortium are located in the lower part of the closed system, and a root area of a crop is located in the upper part of the closed system. Moreover, the present disclosure relates to uses of a device as defined herein, or a closed system as defined herein.

METHODS FOR TREATMENT OF WASTEWATER USING MICROALGAL-BACTERIAL CONSORTIA, AND THEIR APPLICATIONS FOR WATER REUSE

BACKGROUND

[0001] The field of the DISCLOSURE lies in the treatment of wastewater.

[0002] The present disclosure relates to a method for the treatment of wastewater.

[0003] The present disclosure also relates to a device for the treatment of wastewater.

[0004] The present disclosure further relates to a closed system comprising a lower part and an upper part, wherein wastewater and microalgae and/or a microalgae-bacteria consortium are located in the lower part of the closed system, and a root area of a crop is located in the upper part of the closed system.

[0005] Moreover, the present disclosure relates to uses of a device, or a closed system as defined herein.

DESCRIPTION OF THE RELATED ART

[0006] The "background" description provided herein is for the purpose of generally presenting the context of the disclosure. Work of the presently named inventors, to the extent it is described in this background section, as well as aspects of the description which may not otherwise qualify as prior art at the time of filing, are neither expressly nor impliedly admitted as prior art against the present disclosure.

[0007] The decline of freshwater resources has been recognized as one of the main environmental problems on global level, and the reuse of wastewater is increasingly viewed as a pragmatic tool for water conservation.

[0008] There are different types of wastewaters, such as domestic wastewater generated in households, and wastewater from manufacturing and/or industrial operations. One type of domestic wastewater is greywater, which includes water from baths, washing machines, dishwashers, and kitchen sinks. Since greywater is a dilute wastewater stream, it is an attractive stream for the extraction of non-potable water. Greywater usually has a high load of heavy metals, xenobiotic compounds, nitrates, phosphates, and quaternary ammonium compounds originating from body lotions, hair dyes and make-up are also common contaminants. Therefore, greywater needs to be treated before it can be safely reused, for example for crops irrigation, to ensure that maximum crop yield will be realized, and disastrous plant toxicity issues will not develop. To date, greywater treatment technologies are broadly classified into biological, physical, chemical, and combined systems.

[0009] Wastewater from manufacturing and/or industrial operations, such as food processing or metal refining, is referred to as industrial waste or trade waste, which includes liquid waste from any process (e.g., water used to cool machinery or clean plant and equipment). Importantly, industrial waste/trade waste must be treated, and contaminants removed before it can be recycled or discharged to the sewerage system. Since discharge into waterways is regulated by local governments, granted upon licenses use, and at the expenses of the manufacturing/process units – there is always continuous interest on how a specific industry can avoid generating unnecessary industrial waste/trade waste, minimize the amount of water used, minimize the strength of contaminants, and treat, recycle and re-use wastewater.

[0010] Most of the technologies for wastewater treatment include several steps, from pre-treatment (to reduce the number of particles and oil and grease) to post-treatment disinfection (to meet microbiological safety requirements). So far, the most applied method for treating wastewater such as greywater is sand infiltration. However, many issues including clogging, scarcity of well graded sand in some regions as well

as high transportation costs due to the high bulk density are obstacles in its use.

SUMMARY

[0011] In the following, the elements of the invention will be described. These elements are listed with specific embodiments, however, it should be understood that they may be combined in any manner and in any number to create additional embodiments. The variously described examples and preferred embodiments should not be construed to limit the present invention to only the explicitly described embodiments. This description should be understood to support and encompass embodiments which combine two or more of the explicitly described embodiments or which combine the one or more of the explicitly described embodiments with any number of the disclosed and/or preferred elements. Furthermore, any permutations and combinations of all described elements in this application should be considered disclosed by the description of the present application unless the context indicates otherwise.

[0012] The present disclosure provides a method for the treatment of wastewater, the method comprising the following steps:

a. contacting the wastewater with microalgae, wherein the wastewater comprises bacteria, optionally wherein the microalgae is a suspension of microalgae in an aqueous solution, a paste, a gel, or capsules, or wherein the microalgae are microalgae growing on or in a supportive medium, such as a mesh, a membrane, or a foam;
b. culturing the microalgae in the wastewater;
c. removing a part of the culture from step b., and
d. separating the part of the culture of step c. into treated water and a microalgae biomass,

wherein the conditions during the contacting in step a. and/or the culturing in step b. allow a microalgae-bacteria consortium to be established, and wherein the contacting of the wastewater with the microalgae in step a. and/or the removing of the part of the culture in step c. is carried out in form of a batch modus, a fed-batch modus, a repeated fed-batch and harvesting modus, an adaptive modus, such as a fast track adaptive evolution modus, and/or in a titration modus, preferably wherein the contacting of the wastewater with the microalgae in step a. and/or the removing of the part of the culture in step c. is carried out in form of a repeated fed-batch and harvesting modus, a fast track adaptive evolution modus, or in a titration modus.

[0013] The present disclosure provides a device for the treatment of wastewater, wherein the device is in particular for use in a method as defined herein comprising:

(i) at least one wastewater collection tank;
(ii) optionally, at least one pH preconditioning unit;
(iii) at least one wastewater dosing unit;
(iv) at least one algae starter tank;
(v) at least one wastewater processing tank for combining microalgae with wastewater comprising bacteria, and/or for culturing the microalgae in the wastewater comprising the bacteria under conditions to allow a microalgae-bacteria consortium to be established within the at least one wastewater processing tank, and
(vi) optionally, at least one separation unit for separating the content of the processing tank into treated water and a microalgae biomass,

wherein the wastewater from the at least one wastewater dosing unit is supplied to the at least one wastewater processing tank in a batch modus, a fed-batch modus, a repeated fed-batch and harvesting

modus, an adaptive modus, such as a fast track adaptive evolution modus, and/or in a titration modus, preferably wherein the wastewater from the at least one wastewater dosing unit is supplied to the at least one wastewater processing tank in a repeated fed-batch and harvesting modus, a fast track adaptive evolution modus, or in a titration modus.

[0014] The present disclosure provides a closed system comprising a lower part and an upper part, wherein wastewater and microalgae and/or a microalgae-bacteria consortium is located in the lower part of the closed system, and a root area of a crop is located in the upper part of the closed system, wherein the microalgae and/or the microalgae-bacteria consortium and the root area of the crop are in direct contact or wherein the microalgae and/or the microalgae-bacteria consortium and the root area of the crop are separated by an interlayer (such as a mesh, a membrane, a semi-permeable membrane, etc.), optionally wherein microalgae and/or the microalgae-bacteria consortium is embedded in the interlayer, or wherein inactivated microalgae cells are used as the interlayer to enhance the binding capacity of the microalgae-bacteria consortium, and/or lower the nutrient requirement of the crop(s), further optionally wherein the bacteria, the microalgae, and/or the microalgae-bacteria consortium is embedded in a protective matrix, such as a biofilm, a membrane matrix, and/or an encapsulation matrix.

[0015] The present disclosure provides the use of a device as defined herein, or a closed system as defined herein, in a method as defined herein, or for the treatment of wastewater, for the irrigation of a plant, such as a crop, for manufacturing processes, such as for supplying water for semiconductor fabrication plants, for example cooling water for manufacturing processes and/or water for the air conditioning of semiconductor fabrication plants, and/or for supplying water for industrial processes, such as for the cleaning of machinery in industrial processes.

[0016] As used herein, the term “comprising” is to be construed as encompassing both “including” and “consisting of”, both meanings being specifically intended, and hence individually disclosed embodiments in accordance with the present disclosure. Where used herein, “and/or” is to be taken as specific disclosure of each of the two specified features or components with or without the other. For example, “A and/or B” is to be taken as specific disclosure of each of (i) A, (ii) B and (iii) A and B, just as if each is set out individually herein. In the context of the present disclosure, the terms “about” and “approximately” denote an interval of accuracy that the person skilled in the art will understand to still ensure the technical effect of the feature in question. The term typically indicates deviation from the indicated numerical value by ±20%, ±15%, ±10%, and for example ±5%. As will be appreciated by the person of ordinary skill, such specific deviation for a numerical value for a given technical effect will depend on the nature of the technical effect. For example, a natural or biological technical effect may generally have a larger deviation than one for a man-made or engineering technical effect. As will be appreciated by the person of ordinary skill, the specific deviation for a numerical value for a given technical effect will depend on the nature of the technical effect. For example, a natural or biological technical effect may generally have a larger deviation than one for a man-made or engineering technical effect. Where an indefinite or definite article is used when referring to a singular noun, e.g., "a", "an" or "the", this includes a plural of that noun unless something else is specifically stated.

[0017] The foregoing paragraphs have been provided by way of general introduction and are not intended to limit the scope of the following claims. The described embodiments, together with further advantages, will be best understood by reference to the following detailed description taken in conjunction with the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

[0018] A more complete appreciation of the disclosure and many of the attendant advantages thereof will be readily obtained as the same becomes better understood by reference to the following detailed description when considered in connection with the accompanying drawings, wherein:

[0019] Figure 1 shows a schematic representation of the general method for the treatment of wastewater according to the present disclosure.

[0020] Figure 2 shows a schematic representation of the Repeated Fed-Batch and Harvesting Modus.

[0021] Figure 3 shows a schematic representation of the Fast Track Adaptive Evolution Modus of the Microalgal-Bacterial Consortium.

[0022] Figure 4 shows a schematic representation of the Titration Modus for Titration-like addition of wastewater.

[0023] Figure 5 shows a schematic representation of hydroponic cultures and integrated hydroponic systems according to the present disclosure for simultaneous carbon fixation, wastewater treatment and crops growth bio-stimulation.

[0024] Figure 6 shows the experimental results of greywater filtration by culturing different percentages of grey water with microalgae.

[0025] Figure 7 shows the experimental results of greywater filtration by culturing different percentages of grey water with microalgae with the pH being adjusted to pH 7 in some of the cultures.

[0026] Figure 8 shows the comparison of algae biomass growth results for *C. vulgaris* grown in 25% greywater mixtures without pH pre-adjustment (pH ~ 9) and with pH pre-adjustment to pH 7.

[0027] Figure 9 shows the results for different concentrations of greywater mixtures without pH pre-adjustment (pH ~ 9) and with pH pre-adjustment to pH 7, on the Total Suspended Solids (TSS), the Biochemical Oxygen Demand (BOD), Nitrates consumption, and Phosphates consumption.

[0028] Figure 10 shows the results for different concentrations of greywater mixtures on the Zinc consumption, the Molybdenum consumption, and the total Iron consumption, as well as the pH trend in different concentrations of greywater mixtures on day 0 and day 7 after pH pre-adjustment to pH 7.

DETAILED DESCRIPTION OF THE EMBODIMENTS

[0029] As discussed above, the present disclosure provides a method for the treatment of wastewater, the method comprising the following steps:

a. contacting the wastewater with microalgae, wherein the wastewater comprises bacteria, optionally wherein the microalgae is a suspension of microalgae in an aqueous solution, a paste, a gel, or capsules, or wherein the microalgae are microalgae growing on or in a supportive medium, such as a mesh, a membrane, or a foam;
b. culturing the microalgae in the wastewater;
c. removing a part of the culture from step b., and
d. separating the part of the culture of step c. into treated water and a microalgae biomass,

wherein the conditions during the contacting in step a. and/or the culturing in step b. allow a microalgae-bacteria consortium to be established, and wherein the contacting of the wastewater with the microalgae in step a. and/or the removing of the part of the culture in step c. is carried out in form of a batch modus, a fed-batch modus, a repeated fed-batch and harvesting modus, an adaptive modus, such as a fast track adaptive evolution modus, and/or in a titration modus, preferably wherein the contacting of the wastewater

with the microalgae in step a. and/or the removing of the part of the culture in step c. is carried out in form of a repeated fed-batch and harvesting modus, a fast track adaptive evolution modus, or in a titration modus.

[0030] The present disclosure discloses a novel biological method for the treatment of wastewater.

[0031] Having put focus on how clean water, food and feed can be sustainably produced, the inventors have developed a simple yet elegant, sustainable and environmentally friendly solution with methods offering simultaneous treatment of wastewater streams and their reuse in agriculture applications, i.e. irrigation.

[0032] Biological methods for the treatment of wastewater represent a promising option, because technically valuable microorganisms can be used to mitigate the effects of pollutants thanks to the variety of enzymes and secondary metabolites they produce. Microalgae can play a significant role in biological methods for the treatment of wastewater by, *e.g.*, maintaining a near neutral pH, immobilizing bacteria, and facilitating the settlement of suspended biomass. However, microalgae require specific conditions for growth. Moreover, the presence of complex mixtures of pollutants in wastewater can greatly influence algal productivity and therefore the efficiency of wastewater treatment to acceptable standards for its safe re-use. Thus, biological wastewater treatment and reuse of wastewater faces many complex challenges.

[0033] According to the methods of the present disclosure, wastewater is supplied in a way ensuring that the microalgae and bacteria can efficiently adapt to the presence of pollutants and other compounds in the wastewater. Importantly, enabling microalgae and bacteria to self-adapt to different types of wastewater provides methods for the treatment of wastewater that are very versatile in their use (i.e., are useful for the treatment of different types of greywater, industrial waste and trade waste). Thereby, a stable, specialized, and/or adapted microalgae-bacteria consortium can be established that can effectively and timely remove and/or degrade organic and inorganic pollutants, as well as other compounds, from wastewater.

[0034] In one embodiment, the presence of microalgae and bacteria in the consortium promotes each other´s growth.

[0035] Of note, after a "scale-up phase" (also referred to as "germination" or "warming-up" phase) of culturing the microalgae in the wastewater (step b.), the density of microalgae in the culture is increasing exponentially. Thus, the methods of the present disclosure are also advantageous, because due to the simultaneous microalgae biomass production, less external nutrient substrates need to be added to supply the bacteria and/or the microalgae-bacteria consortium with sufficient nutrients. This further reduces the costs of the methods according to the present disclosure compared to state-of-the-art methods.

[0036] In one embodiment, a part of the culture from step b. of the method is removed, wherein the part is at least 0,01% of the culture, at least 0,1% of the culture, at least 0,5% of the culture, at least 1% of the culture, at least 10% of the culture, at least 20% of the culture, at least 30% of the culture, at least 40% of the culture, at least 50% of the culture, at least 60% of the culture, at least 70% of the culture, at least 80% of the culture, at least 90% of the culture, or around 100% of the culture. Thus, the term "a part" of the culture can also refer to the entire culture.

[0037] The part of the culture that is removed can then be added to a subsequent batch or to a separation unit, which separates the part of the culture that is removed into clean water and microalgae biomass.

[0038] In one embodiment, the culturing in step b. of the method is carried out in at least two separate batches, optionally wherein the contacting in step a. of the method and/or the removing of the part of the culture in step c. is carried out periodically and/or stepwise increasingly from one batch to a subsequent

batch, such as from a first batch to a second batch, from the second batch to a third batch, from the third batch to a fourth batch, and so on.

[0039] In one embodiment, in the contacting in step a. of the method, the ratio of wastewater to microalgae is 1% to 75% wastewater to 25% to 99% microalgae, preferably 10% to 40% wastewater to 60% to 90% microalgae, more preferably 20% to 30% wastewater to 70% to 80% microalgae, even more preferably 22.5% to 27.5% wastewater to 72.5% to 77.5% microalgae, and most preferably around 25% wastewater to around 75% microalgae.

[0040] In one embodiment, the contacting of the wastewater with the microalgae in step a. of the method and/or the removing of the part of the culture in step c. of the method is carried out in form of the repeated fed-batch and harvesting modus.

[0041] In one embodiment, when the contacting of the wastewater with the microalgae in step a. of the method and/or the removing of the part of the culture in step c. of the method is carried out in form of the repeated fed-batch and harvesting modus, an amount of a liquid is added to the culture comprising wastewater and microalgae in the contacting of step a. of the method of a subsequent batch, such as the second batch, the third batch, or the fourth batch, that equals the amount of the part of the culture that is removed in step c. of the method of the previous batch, such as the first batch, the second batch, or the third batch, optionally wherein the liquid is wastewater, further optionally wherein the amount of the liquid that is added to the culture comprising wastewater and microalgae in the contacting in step a. of the subsequent batch, such as the second batch, the third batch, or the fourth batch, that equals the part of the culture that is removed in step c. of the method of the previous batch is increasing from batch to batch, such as is doubled from one batch to the subsequent batch. In this embodiment, the amount of the liquid that is added to the culture comprising wastewater and microalgae in the contacting in step a. of the subsequent batch is added “stepwise increasingly”.

[0042] In one embodiment, the contacting of the wastewater with the microalgae in step a. of the method and/or the removing of the part of the culture in step c. of the method is carried out in form of the fast- track adaptive evolution modus.

[0043] In one embodiment, when the contacting of the wastewater with the microalgae in step a. of the method and/or the removing of the part of the culture in step c. of the method is carried out in form of the fast-track adaptive evolution modus, the part of the culture that is removed in step c. of the method of a previous batch, such as the first batch, the second batch, or the third batch, is added in the contacting of step a. to the wastewater of the subsequent batch, such as the second batch, the third batch, or the fourth batch, wherein the ratio of wastewater to microalgae in the culture that is added in the contacting of step a. to the wastewater of the subsequent batch is increasing compared to the ratio of wastewater to microalgae of the previous batch, such as the first batch, the second batch, or the third batch.

[0044] Microalgae–bacteria co-cultures can show a wide range of relationships other than symbiosis, including commensalism, parasitism, competition, amensalism or neutralism, which might alter their efficacy. Also, these associations are dynamic and can evolve over time. By increasing the initial inoculation ratio slowly from batch to batch, a robust and stable microalgae-bacteria consortium can be assembled, because microalgae and bacteria can slowly acclimatize to the novel conditions and an accelerated evolution-like mechanism is enabled. These robust and stable microalgae-bacteria consortia can treat wastewater efficiently and effectively.

[0045] In one embodiment, the concentration of microalgae in the culture of a subsequent batch is lower than in the previous batch.

[0046] In one embodiment, the concentration of wastewater in the culture of a subsequent batch is higher than in the previous batch.

[0047] In one embodiment, when the contacting of the wastewater with the microalgae in step a. of the method and/or the removing of the part of the culture in step c. of the method is carried out in form of the fast-track adaptive evolution modus, the part of the culture that is removed in step c. of the method of a previous batch that is added in the contacting of step a. to the wastewater of the subsequent batch is not increasing from batch to batch, i.e. an equal or an almost equal amount of the culture is removed in step c. of the method of a previous batch compared to the amount that is added in the contacting of step a. to the wastewater of the subsequent batch.

[0048] In one embodiment, the contacting of the wastewater with the microalgae in step a. of the method and/or the removing of the part of the culture in step c. of the method is carried out in form of the titration modus.

[0049] In one embodiment, when the contacting of the wastewater with the microalgae in step a. of the method and/or the removing of the part of the culture in step c. of the method is carried out in form of the titration modus, the wastewater is titrated to a culture comprising the microalgae in step a. of the method. In this embodiment, the wastewater is supplied in a titration-like modus, in order to give the microalgae-bacteria consortium sufficient time to slowly adapt and react. Thereby, microalgae have sufficient time to acclimate to the titrated wastewater, including pollutants and bacteria. This modus can prevent and/or lower a fast consortium response against the addition of the wastewater, which then allows and/or enhances a fast treatment of the wastewater. In this embodiment, the microbial consortium is established based on both spatial and temporal "segregation", which allows algae and bacteria to slowly adapt to the new conditions.

[0050] In one embodiment, when the contacting of the wastewater with the microalgae in step a. of the method and/or the removing of the part of the culture in step c. of the method is carried out in form of the titration modus, the algae culture is preferably supplied at an optimal starting density, such as a density within a range of between 1 and 3 g/L. In general, an optimal algae starting density according to the present disclosure is a high density, because a high algae starting density allows the microalgal-bacterial consortium to withstand the new environmental conditions of the added wastewater, which includes pollutants and other compounds that might hinder the microalgal-bacterial consortium to be stable established.

[0051] In one embodiment, the microalgae-bacteria consortium established during the contacting in step a. and/or the culturing in step b. of the method is a liquid culture.

[0052] In one embodiment, the microalgae-bacteria consortium established during the contacting in step a. and/or the culturing in step b. is not a biofilm microbial consortium.

[0053] In one embodiment, establishing the microalgae-bacteria consortium during the contacting in step a. and/or the culturing in step b. of the method does not require an organic fish emulsion fertilizer.

[0054] In one embodiment, the bacteria are a bacterial monoculture selected from *Aeromonadaceae*; *Alcaligenaceae*; *Bacillaceae*; *Comamonadaceae*; *Enterobacteriaceae*; *Nitrosomonadaceae*; *Nocardiaceae*; *Microbacteriaceae*; *Pseudomonadaceae*; *Shewanellaceae*; *Sphingomonadaceae*; *and Streptococcaceae*; or a multi- and/or mixed culture selected from a culture comprising *Aeromonadaceae*, *Rhodobacteraceae*, and *Chromatiaceae*; *Alcaligenaceae*, and *Burkholderiaceae*; *Brucellaceae* (various strains); *Enterobacteriaceae* (genetically modified *Enterobacteriaceae* types or not genetically modified

Enterobacteriaceae types); *Methylococcaceae* (various strains); *Mycobacteriaceae* (various strains); *Peronosporaceae*, and *Bacillaceae*; *Pseudomonadaceae* (various strains); *Pseudomonadaceae*, and *Methylococcaceae*; *Pseudomonadaceae*, and *Nocardiaceae*; *Pseudomonadaceae*, and *Sphingomonadaceae*; *Rhizobiaceae*, *Sphingomonadaceae*, *Xanthomonadaceae*, and *Actinomycetaceae*; *Rhodocyclaceae*, *Burkholderiaceae*, *Methylophilaceae*, *Sphingomonadaceae*, and *Pseudomonadaceae*; *Sphingobacteriaceae*, *Flavobacteriaceae*, and *Alcaligenaceae*; *Sphingomonadaceae*, *Pseudomonadaceae*, and *Comamonadaceae*; and *Xanthomonadaceae*, and *Sphingomonadaceae*; optionally wherein the bacterial monoculture, or the multi- and/or mixed culture comprises bacteria selected from *Brevibacillus borstelensis*; *Streptomyces albogriseolus*; *Bacillus subtilis*; *Bacillus amyloliquefaciens*; *Bacillus pumilus SE34*; and an isolated consortium augmented with *Aspergillus versicolor*.

[0055] In one embodiment, the bacteria are not oil-degrading bacteria.

[0056] In one embodiment, the bacteria are not *B. cepacian* bacteria.

[0057] In one embodiment, the bacteria are not separately grown and/or precultured (pre-cultivated).

[0058] In one embodiment, the microalgae are a microalgal monoculture selected from *Chlamydomonadaceae*; *Chlorellaceae*; *Chlorococcaceae*; *Chroococcaceae*; *Eustigmataceae*; *Goniaceae*; *Naviculaceae*; *Scenedesmaceae*; *Selenastraceae*, *and Stephanodiscaceae*; or a microalgal and microbial mixed culture selected from a culture comprising *Chlorellaceae (various strains)*; *Chlorellaceae, Chaetophoraceae*, and *Diatoms*; *Chaetophoraceae, Chlorellaceae, Diatoms*, and *Selenastraceae*; *and Chlorophyceae*, and *Cyanobacteria*; optionally wherein the microalgal monoculture, or the microalgal and microbial mixed culture comprises a microalgae selected from *Chlorella vulgaris*; *Chlorella sorokiniana*; *Scenedesmus obliquus*; *Scenedesmus quadricauda*; *Desmodesmus spp.*; *Raphidocelis subcapitata (Selenastrum capricornutum)*; *Chlamydomonas reinhardtii*; *Phaeodactylum tricornutum*; *Thalassiosira spp.*; *Skeletonema spp.*; *Thalassiosira spp.*; *Synechococcus spp.*; and *Spirulina (Arthrospira platensis)*.

[0059] In one embodiment, the microalgae are not oil-acclimated algae.

[0060] In one embodiment, the bacteria and/or microalgae are not genetically modified.

[0061] In one embodiment referring to the contacting in step a. of the method, the microalgae are present in an algal cultivation medium, such as 3N-BBM + V medium, BBM + V medium, Spirul medium, or F/2 medium.

[0062] In one embodiment, the microalgae are supplied at an optimal starting density, such as a starting density within a range of between 1 and 3 g/L.

[0063] In one embodiment, the wastewater comprises greywater and/or wastewater from manufacturing operations or production factories and/or wastewater from industrial operations, such as food processing, metal refining, semiconductor wafer production, water used to cool machinery, or clean plant and equipment, optionally wherein the greywater includes water from baths, washing machines, dishwashers, and kitchen sinks. In this embodiment, the wastewater comprises industrial waste (IW) or trade waste (TW).

[0064] In one embodiment, the wastewater is and/or comprises silicon containing wastewater.

[0065] In one embodiment, the wastewater comprises at least one of a heavy metal, such as Zinc (Zn), Iron (Fe^{2+} or Fe^{3+}), Copper (Cu), or Manganese (Mn), a xenobiotic compound, a pathogenic contaminant, an organic contaminant, an inorganic contaminant, an ion, a nitrate, a phosphate, Molybdenum (Mo), Boron (B), Chloride (Cl), Sodium (Na), Silicon, or an Ammonium compound, such as a quaternary ammonium compound originating from a body lotion, a hair dye or a make-up product, optionally wherein the wastewater is greywater. Of note, any of these compounds or atoms can be toxic for plants, such as crops.

In one embodiment, the wastewater comprises a contaminant that is toxic for plants, such as crops. Thus, any contaminant shall be included in the present disclosure, without being limited to contaminants selected from Zinc (Zn), Iron (Fe^{2+} or Fe^{3+}), Copper (Cu), Manganese (Mn), a xenobiotic compound, a pathogenic contaminant, an organic contaminant, an inorganic contaminant, an ion, a nitrate, a phosphate, Molybdenum (Mo), Boron (B), Chloride (Cl), Sodium (Na), Silicon, and an Ammonium compound.

[0066] While greywater usually has a rather low pathogenic and/or organic contaminant load, the load of compounds like ammonium compounds, such as quaternary ammonium compounds originating from a body lotion, a hair dye or a make-up product, is usually high.

[0067] In one embodiment, the treatment reduces at least one of Zinc (Zn), Molybdenum (Mo), Iron (Fe^{2+} or Fe^{3+}), Copper (Cu), Manganese (Mn), Boron (B), Chloride (Cl), Sodium (Na), nitrate/nitrogen (NO_3/N), phosphate/phosphorus (PO_4/P), the turbidity, the biochemical oxygen demand (BOD), the total suspended solids (TSS), and/or the Sodium Adsorption Ratio (SAR), in the wastewater.

[0068] Some of the requirements for water to be reused as source for irrigation according to EU standards (such as according to Articles 4 and 5 of the Directive on the Regulation on minimum requirements for water reuse for agricultural irrigation) require the water to have a Biochemical oxygen demand (BOD5 at 20°C) (without nitrification) lower than 25 mg/L oxygen, a chemical oxygen demand (COD) lower than 125 mg/L oxygen, total dissolved solids (TDS)/total suspended solids (TSS) lower than 960 mg/L, a total alkalinity ($CaCO_3$) between 30 mg/L and 100 mg/L, a hardness (Calcium and Magnesium) between 50 mg/L and 150 mg/L, a Calcium level between 40 mg/L and 100 mg/L, a Magnesium level above 25 mg/L, and an electrical conductivity (EC) lower than 1.5 mmhos/cm.

[0069] In one embodiment, the total iron reduction is at least 80%, preferably at least 90%, more preferably at least 93%, even more preferably at least 96%, even more preferably at least 99%, and most preferably around 100%.

[0070] In one embodiment, the zinc reduction is at least 30%, preferably at least 33%, more preferably at least 40%, even more preferably at least 42%, even more preferably at least 68%, even more preferably at least 90%, and most preferably around 100%.

[0071] In one embodiment, the molybdenum reduction is at least 0.27%, preferably at least 10%, more preferably at least 20%, even more preferably at least 26%, even more preferably at least 40%, even more preferably at least 50%, and most preferably at least 60%.

[0072] In one embodiment, the nitrate reduction is at least 6%, preferably at least 8%, more preferably at least 10%, even more preferably at least 15%, even more preferably at least 30%, and most preferably at least 45%.

[0073] In one embodiment, the phosphate reduction is at least 7%, preferably at least 12%, more preferably at least 20%, even more preferably at least 25%, even more preferably at least 30%, and most preferably at least 50%.

[0074] In one embodiment, the turbidity reduction is at least 75%, preferably at least 80%, more preferably at least 85%, even more preferably at least 90%, and most preferably at least 97%.

[0075] The person of skill is well aware of methods for determining the biochemical oxygen demand (BOD), such as the BOD5 at 20 °C, total suspended solids (TSS)/total dissolved solids (TDS), and the Sodium Adsorption Ratio (SAR).

[0076] In one embodiment, the BOD reduction is at least 10%, preferably at least 20%, more preferably at least 30%, even more preferably at least 38%, and most preferably at least 39%. The BOD can be

determined by different methods, such as in a homogenized, unfiltered and undecanted sample, wherein the dissolved oxygen is determined before and after a five-day incubation at 20°C ± 1°C in complete darkness. A further method that can be used to determine the BOD is to determine the TOC (total organic carbon), since BOD and TOC can be correlated with the following exponential equation:

BOD = 1.813(TOC)0.4244

Accordingly, the BOD can be indirectly determined by measuring the TOC.

[0077] In one embodiment, the TSS/TDS reduction is at least 50%, preferably at least 60%, more preferably at least 70%, even more preferably at least 79%, and most preferably at least 86%. The TSS/TDS can be determined by different methods, such as by filtering of a representative sample through a 0,45 µm filter membrane, drying at 105°C and weighing the remaining solids. Alternatively, the TSS/TDS can be determined by centrifuging of a representative sample for at least five minutes with a mean acceleration of 2800 to 3200 g, drying at 105°C and weighing the remaining solids.

[0078] In one embodiment, a contaminant other than Zinc (Zn), Iron (Fe^{2+} or Fe^{3+}), Copper (Cu), Manganese (Mn), a xenobiotic compound, a pathogenic contaminant, an organic contaminant, an inorganic contaminant, an ion, a nitrate, a phosphate, Molybdenum (Mo), Boron (B), Chloride (Cl), Sodium (Na), Silicon, or an Ammonium compound, is reduced by the method for the treatment of wastewater of the present disclosure.

[0079] In one embodiment, a pretreatment of the wastewater is performed, optionally wherein the pretreatment is an adjustment of the pH, and wherein the pH is adjusted to a pH of 6.0 to 8.0, preferably to a pH of 6.5 to 7.5, more preferably to a pH of 6.8 to 7.2, even more preferably to a pH of 6.9 to 7.1, and most preferably to a pH of around 7.0. The person of skill is well aware of methods for preadjusting the pH, such as by addition of an acid like HCl or by CO_2 injection.

[0080] In one embodiment, the method does not comprise a preliminary sterilization, filtration, chlorine treatment, mechanical treatment, and/or coagulation-flocculation of the wastewater. Thus, in the method of the present disclosure, no primary and/or secondary conventional treatments are required, such as a preliminary sterilization, filtration, chlorine treatment, mechanical treatment, and/or coagulation-flocculation of the wastewater. Accordingly, the method of the present disclosure is a single-step method, wherein wastewater is directly treated in a continuous process from entry as wastewater to exit as “clean water”/ “treated water”.

[0081] In one embodiment, the only pretreatment of the wastewater that is performed is an adjustment of the pH, optionally wherein the pH is adjusted to a pH of 6.0 to 8.0, preferably to a pH of 6.5 to 7.5, more preferably to a pH of 6.8 to 7.2, even more preferably to a pH of 6.9 to 7.1, and most preferably to a pH of around 7.0.

[0082] In one embodiment, the treated water of step d. of the method is used for the irrigation of plants, such as crops, for manufacturing processes, such as in semiconductor fabrication plants, for example as cooling water and/or for the air conditioning of semiconductor fabrication plants, and/or for industrial processes, such as for the cleaning of machinery in industrial processes, optionally wherein the microalgae biomass of step d. of the method is used as a crop and/or as a fertilizer. In this embodiment, the treated water of step d. of the method is reused, for example in agricultural applications, such as in irrigation, or in production factories, in semiconductor fabs or in labs. The regeneration of wastewater according to the present disclosure maximizes the sustainable use of resources.

[0083] The sustainable use of resources of the present disclosure is further enhanced by the simultaneous production of microalgal and/or crops biomass.

[0084] In one embodiment, the microalgae biomass separated from the part of the culture of step c. of the method has a biomass density [cells/ml] of at least $2 \cdot 10^6$ cells/ml, preferably at least $3 \cdot 10^6$ cells/ml, more preferably at least $4 \cdot 10^6$ cells/ml, even more preferably at least $5 \cdot 10^6$ cells/ml, even more preferably at least $6 \cdot 10^6$ cells/ml, even more preferably at least $7 \cdot 10^6$ cells/ml, and most preferably at least $7{,}5 \cdot 10^6$ cells/ml. The biomass density [cells/ml] in the microalgae biomass separated from the part of the culture of step c. of the method can depend on the duration of cultivation. In one embodiment, the cultivation duration is 1 week.

[0085] The inventors found that when cultures were maintained at pH 7, the cultures experienced less toxicity effects and also grew to reach significantly higher biomass densities (~$4.4 \cdot 10^6$ cells/ml when cultures were cultivated for 1 week at a pH of around 9 compared to $7.5 \cdot 10^6$ cells/ml when the pH of the cultures was adjusted to pH 7 and maintained during the cultivation for 1 week at pH 7).

[0086] In one embodiment, in the removing a part of the culture in step c. of the method, older cells and/or accumulated nutrients are removed from the culture, optionally wherein the removal of older cells increases the light utilization in the culture, and/or enhances the nutrient and pollutant uptake from the wastewater. Importantly, the removal of older cells increases the light utilization in the culture by reducing the likelihood of self-shading effects of older cells in the culture. The removal of older cells and/or accumulated nutrients from the culture maintains a vigorous growth of the microalgal-bacterial consortium in the culture.

[0087] In one embodiment, the method does not generate secondary waste.

[0088] In one embodiment, the method does not comprise the use of chemicals, or only comprises the use of a chemical for the adjustment of the pH but no additional chemical.

[0089] In one embodiment, the method further comprises one or more steps selected from:

e. monitoring the quality of the process and/or the content of pollutants in the water,
f. supplying air and/or carbon dioxide to the culture,
g. drying the microalgae biomass separated in step d. of the method, and
h. extracting metals from the microalgae biomass of step d. of the method.

[0090] In one embodiment, the method further comprises the growing of a crop, and wherein the microalgae-bacteria consortium and the crop are in direct contact or wherein the microalgae-bacteria consortium and the crop are separated by an interlayer (such as a mesh, a membrane, a semi-permeable membrane, etc.), optionally wherein the microalgae and/or the microalgae-bacteria consortium is embedded in the interlayer, or wherein inactivated microalgae cells are used as the interlayer to enhance the binding capacity of the microalgae-bacteria consortium, and/or lower the nutrient requirement of the crop.

[0091] According to the present disclosure, any kind of interlayer can be used that can separate the microalgae and/or the microalgae-bacteria consortium and the root area of the crop. An example of an interlayer that could be used for the present disclosure, without being limited thereto, is a mesh or a membrane, such as a semi-permeable membrane and/or a dialysis membrane.

[0092] In one embodiment, microalgae and crop roots are in direct contact, wherein symbiotic associations can be established and maintained. This allows simultaneous water bioremediation and crop growth stimulation in a *hydroponics* design, such as a *Syneco-hydroponics* design. The term *Syneco*, as used herein, refers to an ecosystem that is self-sustained.

[0093] In an alternative embodiment, microalgae and crop roots are separated by an interlayer, such as a mesh, a membrane, a semi-permeable membrane, and so on.

[0094] In one embodiment, the microalgae-bacteria consortium and the crops are separated by an interlayer, wherein the interlayer prevents the movement of at least one ion and/or at least one pollutant, such as an organic and/or an inorganic pollutant, from the wastewater to the crop, and wherein the interlayer allows the diffusion of at least one crop-growth promoting compound and/or treated water to the crop, optionally wherein the at least one crop-growth promoting compound is released by the microalgae-bacteria consortium, and/or the at least one pollutant is metabolized and/or degraded by the microalgae-bacteria consortium and/or accumulated within their cells.

[0095] In one embodiment, the interlayer is an internal interlayer.

[0096] In one embodiment, the interlayer is a mesh and/or a membrane, preferably an internal mesh and/or an internal membrane.

[0097] In one embodiment, the mesh and/or the interlayer and/or the membrane is a stacked repetitive configuration of sequential meshes and/or sequential interlayers and/or sequential membranes.

[0098] When coculture systems, such as cocultures of microalgae and bacteria, are difficult to operate due to different conditions required for growth of strains, product inhibition, substrate competition, and so on, a spatially segregated design, such as the one shown on the left side of Figure 5 of the present disclosure, with a semi-permeable submerged interlayer, can overcome the issues of the incompatibility of the members of the consortium, while also helping the exchange of beneficial metabolites only. Importantly, the interlayer allows only for the mixture of clean water and growth promoting compounds to reach the upper part of the closed system, where the crop roots are located, i.e. allows a selective diffusion of clean water and beneficial compounds.

[0099] The microalgae-bacteria consortium metabolizes and/or degrades at least one pollutant of the wastewater, thereby preventing pollutants to reach the roots of the crop.

[00100] In one embodiment, the interlayer allows the at least one crop to grow under ideal conditions by reducing product inhibition, reducing substrate competition, and/or improving the required growth conditions of different strains.

[00101] In one embodiment, any of the steps a. to h. of the method is repeated by a certain amount, such as once, twice, three times, four times, five times, six times, seven times, and so on.

[00102] As discussed above, the present disclosure provides a device for the treatment of wastewater, wherein the device is in particular for use in a method according to the present disclosure, comprising:

(i) at least one wastewater collection tank;

(ii) optionally, at least one pH preconditioning unit;

(iii) at least one wastewater dosing unit;

(iv) at least one algae starter tank;

(v) at least one wastewater processing tank for combining microalgae with wastewater comprising bacteria, and/or for culturing the microalgae in the wastewater comprising the bacteria under conditions to allow a microalgae-bacteria consortium to be established within the at least one wastewater processing tank, and

(vi) optionally, at least one separation unit for separating the content of the processing tank into treated water and a microalgae biomass,

wherein the wastewater from the at least one wastewater dosing unit is supplied to the at least one wastewater processing tank in a batch modus, a fed-batch modus, a repeated fed-batch and harvesting modus, an adaptive modus, such as a fast track adaptive evolution modus, and/or in a titration modus, preferably wherein the wastewater from the at least one wastewater dosing unit is supplied to the at least one wastewater processing tank in a repeated fed-batch and harvesting modus, a fast track adaptive evolution modus, or in a titration modus.

[00103] In one embodiment, the device does not require the presence of a separation unit if microalgae and crops are in direct contact. In other embodiments, a separation unit for separating the content of the processing tank into treated water and a microalgae biomass is beneficial.

[00104] In one embodiment, the device comprises

(i) at least one wastewater collection tank;
(ii) at least one pH preconditioning unit;
(iii) at least one wastewater dosing unit;
(iv) at least one algae starter tank;
(v) at least one wastewater processing tank for combining microalgae with wastewater comprising bacteria, and/or for culturing the microalgae in the wastewater comprising the bacteria under conditions to allow a microalgae-bacteria consortium to be established within the at least one wastewater processing tank, and
(vi) at least one separation unit for separating the content of the processing tank into treated water and a microalgae biomass.

[00105] In one embodiment, the pH is adjusted in the at least one pH preconditioning unit to a pH of 6 to 8, preferably a pH of 6.5 to 7.5, more preferably a pH of 6.8 to 7.2, even more preferably to a pH of 6.9 to 7.1, and most preferably to a pH of around 7.0.

[00106] In one embodiment, the at least one wastewater dosing unit is an automatic wastewater dosing, such as a timed wastewater pump.

[00107] In one embodiment, the bacteria are a bacterial monoculture selected from *Aeromonadaceae*; *Alcaligenaceae*; *Bacillaceae*; *Comamonadaceae*; *Enterobacteriaceae*; *Nitrosomonadaceae*; *Nocardiaceae*; *Microbacteriaceae*; *Pseudomonadaceae*; *Shewanellaceae*; *Sphingomonadaceae*; *and Streptococcaceae*; or a multi- and/or mixed culture selected from a culture comprising *Aeromonadaceae*, *Rhodobacteraceae*, and *Chromatiaceae*; *Alcaligenaceae*, and *Burkholderiaceae*; *Brucellaceae* (various strains); *Enterobacteriaceae* (genetically modified *Enterobacteriaceae* types or not genetically modified *Enterobacteriaceae* types); *Methylococcaceae* (various strains); *Mycobacteriaceae* (various strains); *Peronosporaceae*, and *Bacillaceae*; *Pseudomonadaceae* (various strains); *Pseudomonadaceae*, and *Methylococcaceae*; *Pseudomonadaceae*, and *Nocardiaceae*; *Pseudomonadaceae*, and *Sphingomonadaceae*; *Rhizobiaceae*, *Sphingomonadaceae*, *Xanthomonadaceae*, and *Actinomycetaceae*; *Rhodocyclaceae*, *Burkholderiaceae*, *Methylophilaceae*, *Sphingomonadaceae*, and *Pseudomonadaceae*; *Sphingobacteriaceae*, *Flavobacteriaceae*, and *Alcaligenaceae*; *Sphingomonadaceae*, *Pseudomonadaceae*, and *Comamonadaceae*; and *Xanthomonadaceae*, and *Sphingomonadaceae*; optionally wherein the bacterial monoculture, or the multi- and/or mixed culture comprises bacteria selected from *Brevibacillus borstelensis*; *Streptomyces albogriseolus*; *Bacillus subtilis*; *Bacillus amyloliquefaciens*; *Bacillus pumilus SE34*; and an isolated consortium augmented with *Aspergillus versicolor*.

[00108] In one embodiment, the bacteria are not oil-degrading bacteria.

[00109] In one embodiment, the bacteria are not *B. cepacian* bacteria.

[00110] In one embodiment, the microalgae are a microalgal monoculture selected from *Chlamydomonadaceae*; *Chlorellaceae*; *Chlorococcaceae*; *Chroococcaceae*; *Eustigmataceae*; *Goniaceae*; *Naviculaceae*; *Scenedesmaceae*; *Selenastraceae*, *and Stephanodiscaceae*; or a microalgal and microbial mixed culture selected from a culture comprising *Chlorellaceae (various strains)*; *Chlorellaceae, Chaetophoraceae*, and *Diatoms*; *Chaetophoraceae, Chlorellaceae, Diatoms*, and *Selenastraceae*; *and Chlorophyceae*, and *Cyanobacteria*; optionally wherein the microalgal monoculture, or the microalgal and microbial mixed culture comprises a microalgae selected from *Chlorella vulgaris*; *Chlorella sorokiniana*; *Scenedesmus obliquus*; *Scenedesmus quadricauda*; *Desmodesmus spp.*; *Raphidocelis subcapitata* (*Selenastrum capricornutum*); *Chlamydomonas reinhardtii*; *Phaeodactylum tricornutum*; *Thalassiosira spp.*; *Skeletonema spp.*; *Thalassiosira spp.*; *Synechococcus spp.*; and *Spirulina* (*Arthrospira platensis*); preferably wherein the microalgal and microbial mixed culture comprises *Chlorella vulgaris*.

[00111] In one embodiment, the microalgae are not oil-acclimated algae.

[00112] In one embodiment, the bacteria and/or microalgae are not genetically modified.

[00113] In one embodiment relating to the device of the present disclosure, the microalgae are a suspension of microalgae in an aqueous solution, a paste, a gel, or capsules, or the microalgae are microalgae growing on or in a supportive medium, such as a mesh, a membrane, or a foam.

[00114] In one embodiment, the device additionally comprises one or more of:

(vii) a sensor for process monitoring;

(viii) a unit for supplying air and/or carbon dioxide;

(ix) a sensor for monitoring the water quality of the process and/or the content of pollutants in the water;

(x) a drying unit, and

(xi) an extraction unit, optionally wherein the extraction unit is for extracting metals.

[00115] In one embodiment, the treated water is used for the irrigation of plants, such as crops, in manufacturing processes, such as in semiconductor fabrication plants, for example as cooling water and/or for the air conditioning of semiconductor fabrication plants, and/or for industrial processes, such as for the cleaning of machinery in industrial processes, optionally wherein the microalgae biomass is used as crop and/or as fertilizer.

[00116] As discussed above, the present disclosure provides a closed system comprising a lower part and an upper part, wherein wastewater and microalgae and/or a microalgae-bacteria consortium is located in the lower part of the closed system, and a root area of a crop is located in the upper part of the closed system, wherein the microalgae and/or the microalgae-bacteria consortium and the root area of the crop are in direct contact or wherein the microalgae and the root area of the crop are separated by an interlayer (such as a mesh, a membrane, or a semi-permeable membrane), optionally wherein microalgae and/or the microalgae-bacteria consortium is embedded in the interlayer, or wherein inactivated microalgae cells are used as the interlayer to enhance the binding capacity of the microalgae-bacteria consortium, and/or lower the nutrient requirement of the crop, further optionally wherein the bacteria, the microalgae, and/or the microalgae-bacteria consortium is embedded in a protective matrix, such as a biofilm, a membrane matrix, and/or an encapsulation matrix.

[00117] In one embodiment, the microalgae and the root area of the crop are separated by an interlayer, wherein the interlayer prevents the movement of an ion and/or a pollutant, such as an organic and/or an

inorganic pollutant, from the wastewater from the lower part of the closed system to the root area of the crop in the upper part of the closed system, and wherein the interlayer allows for diffusion of a crop-growth promoting compound and/or clean water to the upper part of the closed system, optionally wherein the crop-growth promoting compound is released by the microalgae-bacteria consortium, and/or a pollutant is metabolized and/or degraded by the microalgae-bacteria consortium and/or accumulated within their cells.

[00118] In one embodiment, the interlayer and/or the microalgae-bacteria consortium reduces crop toxicity, evaporation, product inhibition, and/or substrate competition, and/or increases light utilization, water utilization, nutrient utilization, and/or carbon fixation of the crop, thereby enhancing the crop growth and/or yield.

[00119] In one embodiment, the closed system is a reactor.

[00120] In one embodiment, the closed system is a microalgae-bacteria consortium reactor.

[00121] In one embodiment relating to the closed system of the present disclosure, the microalgae are a suspension of microalgae in an aqueous solution, a paste, a gel, or capsules, or the microalgae are microalgae growing on or in a supportive medium, such as a mesh, a membrane, or a foam.

[00122] In one embodiment, carbon dioxide (CO_2), and/or illumination is supplied artificially, optionally wherein the illumination is an LED illumination.

[00123] In one embodiment, the light intensity and/or LED illumination is 20 to 140 µmol/m2xs, 40 to 100 µmol/m2xs, more preferably around 60 µmol/m2xs.

[00124] In one embodiment, a photoperiod is used, such as a 16:8 light to dark cycle.

[00125] In one embodiment, a constant light is used, i.e., the light to dark cycle is 24:0.

[00126] As discussed above, the present disclosure provides the use of a device according to the present disclosure, or a closed system according to the present disclosure, in a method according to the present disclosure, or for the treatment of wastewater, for the irrigation of a plant, such as a crop, for manufacturing processes, such as for supplying water for semiconductor fabrication plants, for example cooling water for manufacturing processes and/or water for the air conditioning of semiconductor fabrication plants, and/or for supplying water for industrial processes, such as for the cleaning of machinery in industrial processes.

[00127] The present disclosure further provides the use of a microalgae in a method as defined herein, wherein the microalgae are a suspension of microalgae in an aqueous solution, a paste, a gel, or capsules, or wherein the microalgae are microalgae growing on or in a supportive medium, such as a mesh, a membrane, or a foam.

[00128] Note that the present technology can also be configured as described below.

(1) A method for the treatment of wastewater, the method comprising the following steps:

a. contacting the wastewater with microalgae, wherein the wastewater comprises bacteria, optionally wherein the microalgae is a suspension of microalgae in an aqueous solution, a paste, a gel, or capsules, or wherein the microalgae are microalgae growing on or in a supportive medium, such as a mesh, a membrane, or a foam;
b. culturing the microalgae in the wastewater;
c. removing a part of the culture from step b., and
d. separating the part of the culture of step c. into treated water and a microalgae biomass,

wherein the conditions during the contacting in step a. and/or the culturing in step b. allow a microalgae-bacteria consortium to be established, and wherein the contacting of the wastewater with the microalgae in step a. and/or the removing of the part of the culture in step c. is carried out in form of a batch modus, a

fed-batch modus, a repeated fed-batch and harvesting modus, an adaptive modus, such as a fast track adaptive evolution modus, and/or in a titration modus, preferably wherein the contacting of the wastewater with the microalgae in step a. and/or the removing of the part of the culture in step c. is carried out in form of a repeated fed-batch and harvesting modus, a fast-track adaptive evolution modus, or in a titration modus.

(2) The method according to (1), wherein the culturing in step b. of the method is carried out in at least two separate batches, optionally wherein the contacting in step a. of the method and/or the removing of the part of the culture in step c. is carried out periodically and/or stepwise increasingly from one batch to a subsequent batch, such as from a first batch to a second batch, from the second batch to a third batch, from the third batch to a fourth batch, and so on.

(3) The method according to (1) or (2), wherein in the contacting in step a. of the method, the ratio of wastewater to microalgae is 1% to 75% wastewater to 25% to 99% microalgae, preferably 10% to 40% wastewater to 60% to 90% microalgae, more preferably 20% to 30% wastewater to 70% to 80% microalgae, even more preferably 22.5% to 27.5% wastewater to 72.5% to 77.5% microalgae, and most preferably around 25% wastewater to around 75% microalgae.

(4) The method according to any one of (1) to (3), wherein when the contacting of the wastewater with the microalgae in step a. of the method and/or the removing of the part of the culture in step c. of the method is carried out in form of the repeated fed-batch and harvesting modus, an amount of a liquid is added to the culture comprising wastewater and microalgae in the contacting of step a. of the method of a subsequent batch, such as the second batch, the third batch, or the fourth batch, that equals the amount of the part of the culture that is removed in step c. of the method of the previous batch, such as the first batch, the second batch, or the third batch, optionally wherein the liquid is wastewater, further optionally wherein the amount of the liquid that is added to the culture comprising wastewater and microalgae in the contacting in step a. of the subsequent batch, such as the second batch, the third batch, or the fourth batch, that equals the part of the culture that is removed in step c. of the method of the previous batch is increasing from batch to batch, such as is doubled from one batch to the subsequent batch.

(5) The method according to any one of (1) to (3), wherein when the contacting of the wastewater with the microalgae in step a. of the method and/or the removing of the part of the culture in step c. of the method is carried out in form of the fast track adaptive evolution modus, the part of the culture that is removed in step c. of the method of a previous batch, such as the first batch, the second batch, or the third batch, is added in the contacting of step a. to the wastewater of the subsequent batch, such as the second batch, the third batch, or the fourth batch, wherein the ratio of wastewater to microalgae in the culture that is added in the contacting of step a. to the wastewater of the subsequent batch is increasing compared to the ratio of wastewater to microalgae of the previous batch, such as the first batch, the second batch, or the third batch.

(6) The method according to any one of (1) to (3), wherein when the contacting of the wastewater with the microalgae in step a. of the method and/or the removing of the part of the culture in step c. of the method is carried out in form of the titration modus, the wastewater is titrated to a culture comprising the microalgae in step a. of the method.

(7) The method according to any one of (1) to (6), wherein the bacteria are a bacterial monoculture selected from *Aeromonadaceae*; *Alcaligenaceae*; *Bacillaceae*; *Comamonadaceae*; *Enterobacteriaceae*; *Nitrosomonadaceae*; *Nocardiaceae*; *Microbacteriaceae*; *Pseudomonadaceae*; *Shewanellaceae*; *Sphingomonadaceae*; *and Streptococcaceae*; or a multi- and/or mixed culture selected from a culture comprising *Aeromonadaceae*, *Rhodobacteraceae*, and *Chromatiaceae*; *Alcaligenaceae*, and

Burkholderiaceae; *Brucellaceae* (various strains); *Enterobacteriaceae* (genetically modified *Enterobacteriaceae* types or not genetically modified *Enterobacteriaceae* types); *Methylococcaceae* (various strains); *Mycobacteriaceae* (various strains); *Peronosporaceae*, and *Bacillaceae*; *Pseudomonadaceae* (various strains); *Pseudomonadaceae*, and *Methylococcaceae*; *Pseudomonadaceae*, and *Nocardiaceae*; *Pseudomonadaceae*, and *Sphingomonadaceae*; *Rhizobiaceae*, *Sphingomonadaceae*, *Xanthomonadaceae*, and *Actinomycetaceae*; *Rhodocyclaceae*, *Burkholderiaceae*, *Methylophilaceae*, *Sphingomonadaceae*, and *Pseudomonadaceae*; *Sphingobacteriaceae*, *Flavobacteriaceae*, and *Alcaligenaceae*; *Sphingomonadaceae*, *Pseudomonadaceae*, and *Comamonadaceae*; and *Xanthomonadaceae*, and *Sphingomonadaceae*; optionally wherein the bacterial monoculture, or the multi- and/or mixed culture comprises bacteria selected from *Brevibacillus borstelensis*; *Streptomyces albogriseolus*; *Bacillus subtilis*; *Bacillus amyloliquefaciens*; *Bacillus pumilus SE34*; and an isolated consortium augmented with *Aspergillus versicolor*.

(8) The method according to any one of (1) to (7), wherein the microalgae are a microalgal monoculture selected from *Chlamydomonadaceae*; *Chlorellaceae*; *Chlorococcaceae*; *Chroococcaceae*; *Eustigmataceae*; *Goniaceae*; *Naviculaceae*; *Scenedesmaceae*; *Selenastraceae*, *and Stephanodiscaceae*; or a microalgal and microbial mixed culture selected from a culture comprising *Chlorellaceae (various strains)*; *Chlorellaceae, Chaetophoraceae*, and *Diatoms*; *Chaetophoraceae, Chlorellaceae, Diatoms*, and *Selenastraceae*; *and Chlorophyceae*, and *Cyanobacteria*; optionally wherein the microalgal monoculture, or the microalgal and microbial mixed culture comprises a microalgae selected from *Chlorella vulgaris*; *Chlorella sorokiniana*; *Scenedesmus obliquus*; *Scenedesmus quadricauda*; *Desmodesmus spp.*; *Raphidocelis subcapitata (Selenastrum capricornutum)*; *Chlamydomonas reinhardtii*; *Phaeodactylum tricornutum*; *Thalassiosira spp.*; *Skeletonema spp.*; *Thalassiosira spp.*; *Synechococcus spp.*; and *Spirulina (Arthrospira platensis)*.

(9) The method according to any one of (1) to (8), wherein in the contacting in step a. of the method, the microalgae are present in an algal cultivation medium, such as 3N-BBM + V medium, BBM + V medium, Spirul medium, or F/2 medium, optionally wherein the microalgae are supplied at an optimal starting density, such as a starting density within a range of between 1 and 3 g/L.

(10) The method according to any one of (1) to (9), wherein the wastewater comprises greywater and/or wastewater from manufacturing operations or production factories and/or wastewater from industrial operations, such as food processing, metal refining, semiconductor wafer production, water used to cool machinery, or clean plant and equipment, optionally wherein the greywater includes water from baths, washing machines, dishwashers, and kitchen sinks.

(11) The method according to any one of (1) to (10), wherein the wastewater comprises at least one of a heavy metal, such as Zinc (Zn), Iron (Fe^{2+} or Fe^{3+}), Copper (Cu), or Manganese (Mn), a xenobiotic compound, a pathogenic contaminant, an organic contaminant, an inorganic contaminant, an ion, a nitrate, a phosphate, Molybdenum (Mo), Boron (B), Chloride (Cl), Sodium (Na), and an Ammonium compound, such as a quaternary ammonium compound originating from a body lotion, a hair dye or a make-up product, optionally wherein the wastewater is greywater.

(12) The method according to any one of (1) to (11), wherein the treatment reduces at least one of Zinc (Zn), Molybdenum (Mo), Iron (Fe^{2+} or Fe^{3+}), Copper (Cu), Manganese (Mn), Boron (B), Chloride (Cl^-), Sodium (Na^+), nitrate/nitrogen (NO_3^-/N), phosphate/phosphorus (PO_4^{3-}/P), the Biochemical Oxygen

Demand (BOD), the total suspended solids (TSS), and the Sodium Adsorption Ratio (SAR), in the wastewater.

(13) The method according to any one of (1) to (12), wherein a pretreatment of the wastewater is performed, optionally wherein the pretreatment is an adjustment of the pH, and wherein the pH is adjusted to a pH of 6.0 to 8.0, preferably to a pH of 6.5 to 7.5, more preferably to a pH of 6.8 to 7.2, even more preferably to a pH of 6.9 to 7.1, and most preferably to a pH of around 7.0, further optionally wherein the method does not comprise a preliminary sterilization, filtration, chlorine treatment, mechanical treatment, and/or coagulation-flocculation of the wastewater.

(14) The method according to any one of (1) to (13), wherein the treated water of step d. of the method is used for the irrigation of plants, such as crops, for manufacturing processes, such as in semiconductor fabrication plants, for example as cooling water and/or for the air conditioning of semiconductor fabrication plants, and/or for industrial processes, such as for the cleaning of machinery in industrial processes, optionally wherein the microalgae biomass of step d. of the method is used as a crop and/or as a fertilizer.

(15) The method according to any one of (1) to (14), wherein in the removing a part of the culture in step c. of the method, older cells and/or accumulated nutrients are removed from the culture, optionally wherein the removal of older cells increases the light utilization in the culture, and/or enhances the nutrient and pollutant uptake from the wastewater.

(16) The method according to any one of (1) to (15), wherein the method does not generate secondary waste, and/or wherein the method does not comprise the use of chemicals.

(17) The method according to any one of (1) to (16), wherein the method further comprises one or more steps selected from:

e. monitoring the quality of the process and/or the content of pollutants in the water,
f. supplying air and/or carbon dioxide to the culture,
g. drying the microalgae biomass separated in step d. of the method, and
h. extracting metals from the microalgae biomass of step d. of the method.

(18) The method according to any one of (1) to (17), wherein the method further comprises the growing of a crop, and wherein the microalgae-bacteria consortium and the crop are in direct contact or wherein the microalgae-bacteria consortium and the crop are separated by an interlayer (such as a mesh, a membrane, or a semi-permeable membrane), optionally wherein the microalgae and/or the microalgae-bacteria consortium is embedded in the interlayer, or wherein inactivated microalgae cells are used as the interlayer to enhance the binding capacity of the microalgae-bacteria consortium, and/or lower the nutrient requirement of the crop.

(19) The method according to (18), wherein the microalgae-bacteria consortium and the crop are separated by an interlayer, wherein the interlayer prevents the movement of at least one ion and/or at least one pollutant, such as an organic and/or an inorganic pollutant, from the wastewater to the crop, and wherein the interlayer allows the diffusion of at least one crop-growth promoting compound and/or treated water to the crop, optionally wherein the at least one crop-growth promoting compound is released by the microalgae-bacteria consortium, and/or the at least one pollutant is metabolized and/or degraded by the microalgae-bacteria consortium and/or accumulated within their cells.

(20) A device for the treatment of wastewater, wherein the device is in particular for use in a method according to any one of (1) to (19), comprising:

(i) at least one wastewater collection tank;

(ii) optionally, at least one pH preconditioning unit;

(iii) at least one wastewater dosing unit;

(iv) at least one algae starter tank;

(v) at least one wastewater processing tank for combining microalgae with wastewater comprising bacteria, and/or for culturing the microalgae in the wastewater comprising the bacteria under conditions to allow a microalgae-bacteria consortium to be established within the at least one wastewater processing tank, and

(vi) optionally, at least one separation unit for separating the content of the processing tank into treated water and a microalgae biomass,

wherein the wastewater from the at least one wastewater dosing unit is supplied to the at least one wastewater processing tank in a batch modus, a fed-batch modus, a repeated fed-batch and harvesting modus, an adaptive modus, such as a fast-track adaptive evolution modus, and/or in a titration modus, preferably wherein the wastewater from the at least one wastewater dosing unit is supplied to the at least one wastewater processing tank in a repeated fed-batch and harvesting modus, a fast track adaptive evolution modus, or in a titration modus.

(21) The device according to (20), wherein the bacteria are a bacterial monoculture selected from *Aeromonadaceae*; *Alcaligenaceae*; *Bacillaceae*; *Comamonadaceae*; *Enterobacteriaceae*; *Nitrosomonadaceae*; *Nocardiaceae*; *Microbacteriaceae*; *Pseudomonadaceae*; *Shewanellaceae*; *Sphingomonadaceae*; *and Streptococcaceae*; or a multi- and/or mixed culture selected from a culture comprising *Aeromonadaceae*, *Rhodobacteraceae*, and *Chromatiaceae*; *Alcaligenaceae*, and *Burkholderiaceae*; *Brucellaceae* (various strains); *Enterobacteriaceae* (genetically modified *Enterobacteriaceae* types or not genetically modified *Enterobacteriaceae* types); *Methylococcaceae* (various strains); *Mycobacteriaceae* (various strains); *Peronosporaceae*, and *Bacillaceae*; *Pseudomonadaceae* (various strains); *Pseudomonadaceae*, and *Methylococcaceae*; *Pseudomonadaceae*, and *Nocardiaceae*; *Pseudomonadaceae*, and *Sphingomonadaceae*; *Rhizobiaceae*, *Sphingomonadaceae*, *Xanthomonadaceae*, and *Actinomycetaceae*; *Rhodocyclaceae*, *Burkholderiaceae*, *Methylophilaceae*, *Sphingomonadaceae*, and *Pseudomonadaceae*; *Sphingobacteriaceae*, *Flavobacteriaceae*, and *Alcaligenaceae*; *Sphingomonadaceae*, *Pseudomonadaceae*, and *Comamonadaceae*; and *Xanthomonadaceae*, and *Sphingomonadaceae*; optionally wherein the bacterial monoculture, or the multi- and/or mixed culture comprises bacteria selected from *Brevibacillus borstelensis*; *Streptomyces albogriseolus*; *Bacillus subtilis*; *Bacillus amyloliquefaciens*; *Bacillus pumilus SE34*; and an isolated consortium augmented with *Aspergillus versicolor*.

(22) The device according to (20) or (21), wherein the microalgae are a microalgal monoculture selected from *Chlamydomonadaceae*; *Chlorellaceae*; *Chlorococcaceae*; *Chroococcaceae*; *Eustigmataceae*; *Goniaceae*; *Naviculaceae*; *Scenedesmaceae*; *Selenastraceae*, *and Stephanodiscaceae*; or a microalgal and microbial mixed culture selected from a culture comprising *Chlorellaceae (various strains)*; *Chlorellaceae, Chaetophoraceae*, and *Diatoms*; *Chaetophoraceae, Chlorellaceae, Diatoms*, and *Selenastraceae*; *and Chlorophyceae*, and *Cyanobacteria*; optionally wherein the microalgal monoculture, or the microalgal and microbial mixed culture comprises a microalgae selected from *Chlorella vulgaris*; *Chlorella sorokiniana*; *Scenedesmus obliquus*; *Scenedesmus quadricauda*; *Desmodesmus spp.*; *Raphidocelis subcapitata* (*Selenastrum capricornutum*); *Chlamydomonas reinhardtii*; *Phaeodactylum tricornutum*; *Thalassiosira*

spp.; *Skeletonema spp.*; *Thalassiosira spp.*; *Synechococcus spp.*; and *Spirulina* (*Arthrospira platensis*); preferably wherein the microalgal and microbial mixed culture comprises *Chlorella vulgaris*.

(23) The device according to any one of (20) to (22), wherein the device additionally comprises one or more of:

(vii) a sensor for process monitoring;

(viii) a unit for supplying air and/or carbon dioxide;

(ix) a sensor for monitoring the water quality of the process and/or the content of pollutants in the water;

(x) a drying unit, and

(xi) an extraction unit, optionally wherein the extraction unit is for extracting metals.

(24) The device according to any one of (20) to (23), wherein the treated water is used for the irrigation of plants, such as crops, in manufacturing processes, such as in semiconductor fabrication plants, for example as cooling water and/or for the air conditioning of semiconductor fabrication plants, and/or for industrial processes, such as for the cleaning of machinery in industrial processes, optionally wherein the microalgae biomass is used as crop and/or as fertilizer.

(25) A closed system comprising a lower part and an upper part, wherein wastewater and a microalgae and/or a microalgae-bacteria consortium is located in the lower part of the closed system, and a root area of a crop is located in the upper part of the closed system, wherein the microalgae and/or the microalgae-bacteria consortium and the root area of the crop are in direct contact or wherein the microalgae and the root area of the crop are separated by an interlayer (such as a mesh, a membrane, or a semi-permeable membrane), optionally wherein microalgae and/or the microalgae-bacteria consortium is embedded in the interlayer, or wherein inactivated microalgae cells are used as the interlayer to enhance the binding capacity of the microalgae-bacteria consortium, and/or lower the nutrient requirement of the crop, further optionally wherein the bacteria, the microalgae, and/or the microalgae-bacteria consortium is embedded in a protective matrix, such as a biofilm, a membrane matrix, and/or an encapsulation matrix.

(26) The closed system according to (25), wherein the microalgae and the root area of the crop are separated by an interlayer, wherein the interlayer prevents the movement of an ion and/or a pollutant, such as an organic and/or an inorganic pollutant, from the wastewater from the lower part of the closed system to the root area of the crop in the upper part of the closed system, and wherein the interlayer allows for diffusion of a crop-growth promoting compound and/or clean water to the upper part of the closed system, optionally wherein the crop-growth promoting compound is released by the microalgae-bacteria consortium, and/or a pollutant is metabolized and/or degraded by the microalgae-bacteria consortium and/or accumulated within their cells.

(27) The closed system according to (25) or (26), wherein the interlayer and/or the microalgae-bacteria consortium reduces crop toxicity, evaporation, product inhibition, and/or substrate competition, and/or increases light utilization, water utilization, nutrient utilization, and/or carbon fixation of the crop, thereby enhancing the crop growth and/or yield.

(28) The closed system according to any one of (25) to (27), wherein the closed system is a reactor, optionally wherein the reactor is a microalgae-bacteria consortium reactor.

(29) The closed system according to any one of (25) to (28), wherein air, carbon dioxide (CO_2), and/or illumination is supplied artificially, optionally wherein the illumination is an LED illumination.

(30) Use of a device according to any one of (20) to (24), or a closed system according to any one of (25) to (29), in a method according to any one of (1) to (19), or for the treatment of wastewater, for the irrigation of a plant, such as a crop, for manufacturing processes, such as for supplying water for semiconductor fabrication plants, for example cooling water for manufacturing processes and/or water for the air conditioning of semiconductor fabrication plants, and/or for supplying water for industrial processes, such as for the cleaning of machinery in industrial processes.

[00129] The term "wastewater" as used herein, is meant to refer to any water that is discharged in a process and that cannot be reused for certain applications in this "state" or "condition", i.e., that needs to be treated prior allowing the reuse of the water in certain applications. The term "wastewater" shall include domestic wastewater generated in households, such as greywater, and wastewater from manufacturing and/or industrial operations. Some types of wastewaters from manufacturing and/or industrial operations are referred to as industrial (IW) or trade waste (TW), which includes liquid waste from any process (e.g., water used to cool machinery or clean plant and equipment).

[00130] According to the present disclosure, the term „treated water" refers to water separated from the part of the culture of step c. of the method of the present disclosure. In accordance with the present disclosure, the term „treated water" shall also refer to "clean water", and both terms can be used interchangeably. One example of „treated water" or "clean water" is water that can be reused in agricultural applications, such as in irrigation, or in production factories. In a further example, the „treated water" or "clean water" is water that can be reused in agricultural applications, such as in irrigation, or in production factories, compatible with EU standards.

[00131] The term "a", as used herein, is meant to refer to "at least one". For example, the term "a lower part" and "an upper part" shall refer to "at least one lower part" and "at least one upper part".

[00132] The term "bacteria", as used herein, is meant to refer to at least one bacterium. The term "bacteria" shall include bacterial monocultures and mixed cultures.

[00133] The term "microalgae", as used herein, is meant to refer to at least one microalga. The term "microalgae" shall include algal monocultures and mixed cultures, and shall include various media in which the microalgae can be contained, such as in an aqueous solution, or a paste, or a gel, or capsules, or microalgae growing on or in a supportive medium such as a mesh, a membrane, or a foam.

[00134] The term "separating", as used herein, shall refer to taking a culture or a part of the culture into treated water and a microalgae biomass, i.e. dividing a culture or a part of the culture into treated water and a microalgae biomass.

[00135] The term "periodically", as used herein, is meant to refer to performing something at regular intervals and/or occasionally, such as after 6 hours, after 1 day, after 3 days, after 10 days, and so on.

[00136] The term "crop", as used herein, is meant to refer to any plant. The terms "crop" and "plant" are sometimes used interchangeably in the present disclosure. The term "crop", as used herein, includes feed, such as food or supplements for human consumption or feed for animal consumption, and also ornamental plants.

[00137] The term "device", as used herein, shall also refer to an apparatus or a treatment facility. The term "device", "apparatus", and "treatment facility" are sometimes used interchangeably in the context of the present disclosure.

[00138] The term "removing", as used herein, shall refer to taking a part of the culture, such as an effluent, or harvesting a part of the culture. Specifically, the term "removing" as used for step c. of the method, refers

to taking a part of the culture, which can then be harvested or used for inoculation of a different culture, such as for inoculation of a subsequent batch of culture.

[00139] The term "biological treatment" of wastewater, as used herein, shall refer to the bioremediation of wastewater. The terms "biological treatment" and "bioremediation" of wastewater, as used herein, are sometimes used interchangeably.

[00140] The main advantages of the methods and devices for the treatment of wastewater of the present disclosure are as follows:

- efficient and timely wastewater treatment methods compatible with EU standards for reuse in agricultural applications, such as in irrigation, or in production factories
- optimization of cultivation conditions of microalgae and bacteria to maximize wastewater treatment while also promoting algal biomass and crops growth
- environmentally friendly, effective, and sustainable solution for the biological removal of polluting compounds from wastewater (even from wastewater that is otherwise too costly and time consuming to be efficiently and effectively treated and reused)
- maximizing sustainable use of resources by simultaneously regenerating water and enhancing the simultaneous production of microalgal and/or crops biomass
- skipping primary and secondary conventional treatments of wastewater, such as preceding sterilization, filtration, chlorine or mechanical treatment, and/or coagulation-flocculation steps
- overcoming the problem of time-consuming screening procedures and artificially assembling and pairing microalgae and bacteria to enable the stable association of microalgae-bacteria consortia
- enable microalgae and bacteria to withstand new environmental conditions of added wastewater
- overcoming problems caused by the presence of complex mixtures of pollutants in wastewater that can greatly influence algal biomass productivity and therefore the efficiency of wastewater treatment to acceptable standards for its safe re-use
- enable different growth environmental conditions for different members in a microalgae-bacteria consortium, thereby overcoming instability and low efficiency of many coculture systems, as well as strain incompatibility issues

[00141] The methods, devices, systems, as well as their uses disclosed herein fulfill the requirements responsible for the above advantages.

EXAMPLES

EXAMPLE 1:

[00142] In the schematic representation shown in Figure 1, the general method for treatment of wastewater according to the present disclosure is depicted. In Figures 1 to 4, the abbreviation "WW" refers to wastewater, which shall include any kind of wastewater. Examples of wastewater (WW) are greywater (GW) and water comprising industrial waste (IW) or trade waste (TW). Of note, the Wastewater Dosing Unit and Process Tanks could be organized in different ways to enable a batch modus, a fed-batch modus, a repeated fed-batch and harvesting modus (Figure 2), an adaptive modus, such as a fast-track adaptive evolution modus (Figure 3), and/or a titration modus (Figure 4).

EXAMPLE 2:

[00143] In the schematic representation shown in Figure 2, the Repeated Fed-Batch and Harvesting Modus is shown. A fed-batch modus ensures that the effluent is properly treated and reaches the limits for recycling/reuse. Periodic harvesting can increase the wastewater treatment performance because

accumulated nutrients from the system are removed, and also vigorous growth of the microalgal-bacterial consortium in the system is maintained (as older cells are removed). This leaves healthy, productive microalgal-bacterial cultures, where self-shading effects are avoided and therefore light utilization is optimized. Overall, a fed-batch process with period-stepwise increasing harvesting increases the efficiency of the nutrient and pollutant uptake from the wastewater.

EXAMPLE 3:

[00144] In the schematic representation shown in Figure 3, the fast-track adaptive evolution modus is shown. A good microbial consortium design must match ecological and evolutionary principles, which can be achieved by a fast-track adaptive evolution modus, i.e. a gradient increase of wastewater combined with an adaptive evolution approach with optimization of initial inoculation ratios. This implies the application of selective pressure as driving force for the selection of microorganisms with enhanced phenotypes, thus allowing the development of new biological functions and performance. The obtained consortium produces higher microalgal biomass, and also results in better wastewater treatment abilities compared to the original consortium.

EXAMPLE 4:

[00145] In the schematic representation shown in Figure 4, the titration modus is shown. As shown in Figure 4, in this configuration, the microbial consortium is established based on both spatial and temporal "segregation". The wastewater – whose only pretreatment relies on pH adjustment – is supplied in a titration-like modus, in order to give sufficient time for the microalgal-bacterial consortium to adapt and react. At the same time, also the algae cells are optimally provided, so that the initial density inside the Microalgal-Bacteria Consortium-Reactor is high enough to withstand the new environmental conditions of the added wastewater. Thus, the titration-like addition of wastewater and the provision of algae cells at an ideal density enables a fast consortium response against wastewater inactivation.

EXAMPLE 5:

[00146] In the schematic representation shown in Figure 5, hydroponic cultures and integrated hydroponic systems according to the present disclosure are shown, which allow simultaneous carbon fixation, wastewater treatment and crops growth bio-stimulation. When coculture systems are difficult to operate due to different growth features of strains, product inhibition, substrate competition, and so on, a spatially segregated design (Figure 5, left side) with semi-permeable submerged interlayer can overcome the issues of the incompatibility of members of the consortium, while also helping the exchange of beneficial metabolites only.

EXAMPLE 6:

[00147] Figure 6 shows experimental results of greywater filtration without pH pre-adjustment, where different percentages of synthetic greywater (0%, 25%, 50%, or 75%) were cultured with the microalgal species *Chlorella vulgaris* (precultured in 3N-BBM algal cultivation medium) for 8 days at a temperature of 25°C. Samples were continuously mixed at 145 rpm and illuminated with white LED at 60 µmol/m2xs (light:dark = 24:0). Visual inspection of the treated greywater at day 0, after 24 h, after 96 h and after 192 h indicates that the treatment using 25% greywater + microalgae was the best option, because these samples contained the clearest water and the most dense and healthy algae surplus. After 8 days, water and microalgae cells were separated by centrifugation and the final biomass density reached was determined. Table 1 shows additional measurements of the experiment.

Table 1:

	Grey water	Algae medium	Microalgae (Chlorella vulgaris) – without pH pre-adjustment			Microalgae (Chlorella vulgaris) – with pH pre-adjustment		
	100%	0%	25% GW	50% GW	75% GW	25% GW	50% GW	75% GW
Time Needed	7 days	7 days	7 days			7 days		
Initial Algae concentration % [v/v]	0	0	10			10		
TSS (Total Suspended Solids) [mg/L]	0.333	0.094	0.077	0.448	0.486	0.151	0.408	0.590
BOD (Biochemical Oxygen Demand) [mg/L]	26.57	7.78	11.5	23.91	26.86	20.5	4	19.5
TSS reduction %	40	0	86	59	70	79	69	70
BOD reduction %	+10	0	+30	0	0	+39	+38	+19
Turbidity reduction %	87	N/A	90	80	75	97	90	89
Nitrates reduction %	4	6	15	0	N/A	45	8	6
Phosphates reduction %	14	29	25	7	0	50	20	12
Total Iron reduction %	100	0	96	96	93	100	100	100
Zinc reduction %	25	0	30	40	42	100	33	68
Molybdenum reduction %	0	0	26	20	40	60	20	0.27
pH range	9.0 ÷ 9.5	7.0	9.0 ÷ 9.5			7.0 ÷ 7.4		
Final Biomass density reached [cells/ml]	-	1.67E06	4.38E06			5.3E06		

TSS of Raw Greywater (non treated == Day 0) = 2.544 g/L

TSS of Raw Algal Medium (non treated == Day 0) = 0.122 g/L

BOD of Raw Greywater (non treated == Day 0) = 29.73 mg/L

BOD of Raw Algal Medium (non treated == Day 0) = 0 mg/L

EXAMPLE 7:

[00148] Figure 7 shows experimental results of greywater filtration, where different percentages of synthetic greywater (0%, 25%, 50%, 75%, or 100%) were cultured with the microalgal species *Chlorella vulgaris* (precultured in 3N-BBM algal cultivation medium) for 14 days at a temperature of 25°C. Samples were continuously mixed at 145 rpm and illuminated with white LED at 60 µmol/m2xs. The pH in some of the cultures was preadjusted with 1M HCl to pH 7. No further pretreatment step was performed. Visual inspection of the treated greywater at day 1, day 3, day 4, day 7, day 11 and day 14 indicates that the treatment using 25% greywater + microalgae was the best option, because these samples contained the clearest water and the most dense and healthy algae surplus.

EXAMPLE 8:

[00149] To prove the effect of the microalgal-bacteria consortium established according to the present disclosure on algae biomass growth, Figure 8 shows the comparison of algae biomass growth results for *C. vulgaris* grown in 25% greywater mixtures without pH pre-adjustment (pH ~ 9) and with pH pre-adjustment to pH 7 by addition of HCl 1M. Of note, initially there was no significant differences between the cultures. However, at pH 9 cultures "struggled" for a relatively long period of time to overcome potentially toxic effects. On the contrary, cultures maintained at pH 7 not only did not experience toxicity effects but also grew to reach significantly higher biomass densities (~$4.4 \cdot 10^6$ cells/ml at pH = 9 vs. $7.5 \cdot 10^6$ cells/ml at pH = 7, after 1 week of cultivation).

EXAMPLE 9:

[00150] To prove the effect of the microalgal-bacteria consortium established according to the present disclosure on the wastewater treatment, Figure 9 shows the comparison results for different concentrations of greywater mixtures without pH pre-adjustment (pH ~ 9) and with pH pre-adjustment to pH 7 by addition of HCl 1M. Figure 9 A shows the comparison of Total Suspended Solids results at pH 9 and pH 7. Figure 9 B shows the comparison of the Biochemical Oxygen Demand results at pH 9 and pH 7. Figure 9 C shows the comparison of Nitrates consumption results at pH 9 and pH 7. Figure 9 D shows the comparison of Phosphates consumption results at pH 9 and pH 7.

[00151] To prove the effect of the microalgal-bacteria consortium established according to the present disclosure on the wastewater treatment, Figure 10 shows comparison results for different concentrations of greywater mixtures. Figure 10 A shows the comparison of Zinc consumption results without pH pre-adjustment (pH ~ 9) and with pH pre-adjustment to pH 7. Figure 10 B shows the comparison of Molybdenum consumption results without pH pre-adjustment (pH ~ 9) and with pH pre-adjustment to pH 7. Figure 10 C shows the comparison of total Iron consumption results without pH pre-adjustment (pH ~ 9) and with pH pre-adjustment to pH 7. Figure 10 D shows the pH trend in different concentrations of greywater mixtures on day 0 and day 7 after pH pre-adjustment to pH 7.

[00152] Thus, the foregoing discussion discloses and describes merely exemplary embodiments of the present disclosure. As will be understood by those skilled in the art, the present disclosure may be embodied

in other specific forms without departing from the spirit or essential characteristics thereof. Accordingly, the disclosure of the present disclosure is intended to be illustrative, but not limiting of the scope of the disclosure, as well as other claims. The disclosure, including any readily discernible variants of the teachings herein, define, in part, the scope of the foregoing claim terminology such that no inventive subject matter is dedicated to the public.

Claims

1. A method for the treatment of wastewater, the method comprising the following steps:
    a. contacting the wastewater with microalgae, wherein the wastewater comprises bacteria, optionally wherein the microalgae is a suspension of microalgae in an aqueous solution, a paste, a gel, or capsules, or wherein the microalgae are microalgae growing on or in a supportive medium, such as a mesh, a membrane, or a foam;
    b. culturing the microalgae in the wastewater;
    c. removing a part of the culture from step b., and
    d. separating the part of the culture of step c. into treated water and a microalgae biomass,

    wherein the conditions during the contacting in step a. and/or the culturing in step b. allow a microalgae-bacteria consortium to be established, and wherein the contacting of the wastewater with the microalgae in step a. and/or the removing of the part of the culture in step c. is carried out in form of a batch modus, a fed-batch modus, a repeated fed-batch and harvesting modus, an adaptive modus, such as a fast track adaptive evolution modus, and/or in a titration modus, preferably wherein the contacting of the wastewater with the microalgae in step a. and/or the removing of the part of the culture in step c. is carried out in form of a repeated fed-batch and harvesting modus, a fast track adaptive evolution modus, or in a titration modus.

2. The method according to claim 1, wherein the culturing in step b. of the method is carried out in at least two separate batches, optionally wherein the contacting in step a. of the method and/or the removing of the part of the culture in step c. is carried out periodically and/or stepwise increasingly from one batch to a subsequent batch, such as from a first batch to a second batch, from the second batch to a third batch, from the third batch to a fourth batch, and so on.

3. The method according to claim 1 or 2, wherein in the contacting in step a. of the method, the ratio of wastewater to microalgae is 1% to 75% wastewater to 25% to 99% microalgae, preferably 10% to 40% wastewater to 60% to 90% microalgae, more preferably 20% to 30% wastewater to 70% to 80% microalgae, even more preferably 22.5% to 27.5% wastewater to 72.5% to 77.5% microalgae, and most preferably around 25% wastewater to around 75% microalgae.

4. The method according to any one of claims 1 to 3, wherein when the contacting of the wastewater with the microalgae in step a. of the method and/or the removing of the part of the culture in step c. of the method is carried out in form of the repeated fed-batch and harvesting modus, an amount of a liquid is added to the culture comprising wastewater and microalgae in the contacting of step a. of the method of a subsequent batch, such as the second batch, the third batch, or the fourth batch, that equals the amount of the part of the culture that is removed in step c. of the method of the previous batch, such as the first batch, the second batch, or the third batch, optionally wherein the liquid is

wastewater, further optionally wherein the amount of the liquid that is added to the culture comprising wastewater and microalgae in the contacting in step a. of the subsequent batch, such as the second batch, the third batch, or the fourth batch, that equals the part of the culture that is removed in step c. of the method of the previous batch is increasing from batch to batch, such as is doubled from one batch to the subsequent batch.

5. The method according to any one of claims 1 to 3, wherein when the contacting of the wastewater with the microalgae in step a. of the method and/or the removing of the part of the culture in step c. of the method is carried out in form of the fast track adaptive evolution modus, the part of the culture that is removed in step c. of the method of a previous batch, such as the first batch, the second batch, or the third batch, is added in the contacting of step a. to the wastewater of the subsequent batch, such as the second batch, the third batch, or the fourth batch, wherein the ratio of wastewater to microalgae in the culture that is added in the contacting of step a. to the wastewater of the subsequent batch is increasing compared to the ratio of wastewater to microalgae of the previous batch, such as the first batch, the second batch, or the third batch.

6. The method according to any one of claims 1 to 3, wherein when the contacting of the wastewater with the microalgae in step a. of the method and/or the removing of the part of the culture in step c. of the method is carried out in form of the titration modus, the wastewater is titrated to a culture comprising the microalgae in step a. of the method.

7. The method according to any one of claims 1 to 6, wherein the bacteria are a bacterial monoculture selected from *Aeromonadaceae*; *Alcaligenaceae*; *Bacillaceae*; *Comamonadaceae*; *Enterobacteriaceae*; *Nitrosomonadaceae*; *Nocardiaceae*; *Microbacteriaceae*; *Pseudomonadaceae*; *Shewanellaceae*; *Sphingomonadaceae*; *and Streptococcaceae*; or a multi- and/or mixed culture selected from a culture comprising *Aeromonadaceae*, *Rhodobacteraceae*, and *Chromatiaceae*; *Alcaligenaceae*, and *Burkholderiaceae*; *Brucellaceae* (various strains); *Enterobacteriaceae* (genetically modified *Enterobacteriaceae* types or not genetically modified *Enterobacteriaceae* types); *Methylococcaceae* (various strains); *Mycobacteriaceae* (various strains); *Peronosporaceae*, and *Bacillaceae*; *Pseudomonadaceae* (various strains); *Pseudomonadaceae*, and *Methylococcaceae*; *Pseudomonadaceae*, and *Nocardiaceae*; *Pseudomonadaceae*, and *Sphingomonadaceae*; *Rhizobiaceae*, *Sphingomonadaceae*, *Xanthomonadaceae*, and *Actinomycetaceae*; *Rhodocyclaceae*, *Burkholderiaceae*, *Methylophilaceae*, *Sphingomonadaceae*, and *Pseudomonadaceae*; *Sphingobacteriaceae*, *Flavobacteriaceae*, and *Alcaligenaceae*; *Sphingomonadaceae*, *Pseudomonadaceae*, and *Comamonadaceae*; and *Xanthomonadaceae*, and *Sphingomonadaceae*; optionally wherein the bacterial monoculture, or the multi- and/or mixed culture comprises bacteria selected from *Brevibacillus borstelensis*; *Streptomyces albogriseolus*;

Bacillus subtilis; *Bacillus amyloliquefaciens*; *Bacillus pumilus SE34*; and an isolated consortium augmented with *Aspergillus versicolor*.

8. The method according to any one of claims 1 to 7, wherein the microalgae are a microalgal monoculture selected from *Chlamydomonadaceae*; *Chlorellaceae*; *Chlorococcaceae*; *Chroococcaceae*; *Eustigmataceae*; *Goniaceae*; *Naviculaceae*; *Scenedesmaceae*; *Selenastraceae*, *and Stephanodiscaceae*; or a microalgal and microbial mixed culture selected from a culture comprising *Chlorellaceae (various strains)*; *Chlorellaceae, Chaetophoraceae*, and *Diatoms*; *Chaetophoraceae, Chlorellaceae, Diatoms*, and *Selenastraceae*; *and Chlorophyceae*, and *Cyanobacteria*; optionally wherein the microalgal monoculture, or the microalgal and microbial mixed culture comprises microalgae selected from *Chlorella vulgaris*; *Chlorella sorokiniana*; *Scenedesmus obliquus*; *Scenedesmus quadricauda*; *Desmodesmus spp.*; *Raphidocelis subcapitata (Selenastrum capricornutum)*; *Chlamydomonas reinhardtii*; *Phaeodactylum tricornutum*; *Thalassiosira spp.*; *Skeletonema spp.*; *Thalassiosira spp.*; *Synechococcus spp.*; and *Spirulina (Arthrospira platensis)*; further optionally wherein in the contacting in step a. of the method, the microalgae are present in an algal cultivation medium, such as 3N-BBM + V medium, BBM + V medium, Spirul medium, or F/2 medium, further optionally wherein the microalgae are supplied at an optimal starting density, such as a starting density within a range of between 1 and 3 g/L.

9. The method according to any one of claims 1 to 8, wherein the wastewater comprises greywater and/or wastewater from manufacturing operations or production factories and/or wastewater from industrial operations, such as food processing, metal refining, semiconductor wafer production, water used to cool machinery, or clean plant and equipment, optionally wherein the greywater includes water from baths, washing machines, dishwashers, and kitchen sinks.

10. The method according to any one of claims 1 to 9, wherein the wastewater comprises at least one of a heavy metal, such as Zinc (Zn), Iron (Fe^{2+} or Fe^{3+}), Copper (Cu), or Manganese (Mn), a xenobiotic compound, a pathogenic contaminant, an organic contaminant, an inorganic contaminant, an ion, a nitrate, a phosphate, Molybdenum (Mo), Boron (B), Chloride (Cl), Sodium (Na), Silicon, or an Ammonium compound, such as a quaternary Ammonium compound originating from a body lotion, a hair dye or a make-up product, optionally wherein the wastewater is greywater.

11. The method according to any one of claims 1 to 10, wherein the treatment reduces at least one of Zinc (Zn), Molybdenum (Mo), Iron (Fe^{2+} or Fe^{3+}), Copper (Cu), Manganese (Mn), Boron (B), Chloride (Cl), Sodium (Na), nitrate/nitrogen (NO_3/N), phosphate/phosphorus (PO_4/P), the turbidity, the biochemical oxygen demand (BOD), the total suspended solids (TSS), and/or the Sodium Adsorption Ratio (SAR) in the wastewater.

12. The method according to any one of claims 1 to 11, wherein a pretreatment of the wastewater is performed, optionally wherein the pretreatment is an adjustment of the pH, and wherein the pH is adjusted to a pH of 6.0 to 8.0, preferably to a pH of 6.5 to 7.5, more preferably to a pH of 6.8 to 7.2, even more preferably to a pH of 6.9 to 7.1, and most preferably to a pH of around 7.0, further optionally wherein the method does not comprise a preliminary sterilization, filtration, chlorine treatment, mechanical treatment, and/or coagulation-flocculation of the wastewater.

13. The method according to any one of claims 1 to 12, wherein the treated water of step d. of the method is used for the irrigation of plants, such as crops, for manufacturing processes, such as in semiconductor fabrication plants, for example as cooling water and/or for the air conditioning of semiconductor fabrication plants, and/or for industrial processes, such as for the cleaning of machinery in industrial processes, optionally wherein the microalgae biomass of step d. of the method is used as a crop and/or as a fertilizer.

14. The method according to any one of claims 1 to 13, wherein in the removing a part of the culture in step c. of the method, older cells and/or accumulated nutrients are removed from the culture, optionally wherein the removal of older cells increases the light utilization in the culture, and/or enhances the nutrient and pollutant uptake from the wastewater.

15. The method according to any one of claims 1 to 14, wherein the method further comprises one or more steps selected from:
    e. monitoring the quality of the process and/or the content of pollutants in the water,
    f. supplying air and/or carbon dioxide to the culture,
    g. drying the microalgae biomass separated in step d. of the method, and
    h. extracting metals from the microalgae biomass of step d. of the method.

16. The method according to any one of claims 1 to 15, wherein the method further comprises the growing of a crop, and wherein the microalgae-bacteria consortium and the crop are in direct contact or wherein the microalgae-bacteria consortium and the crop are separated by an interlayer (such as a mesh, a membrane, or a semi-permeable membrane), optionally wherein the microalgae and/or the microalgae-bacteria consortium is embedded in the interlayer, or wherein inactivated microalgae cells are used as the interlayer to enhance the binding capacity of the microalgae-bacteria consortium, and/or lower the nutrient requirement of the crop.

17. The method according to claim 16, wherein the microalgae-bacteria consortium and the crop are separated by an interlayer, wherein the interlayer prevents the movement of at least one ion and/or at least one pollutant, such as an organic and/or an inorganic pollutant, from the wastewater to the crop, and wherein the interlayer allows the diffusion of at least one crop-growth promoting

compound and/or treated water to the crop, optionally wherein the at least one crop-growth promoting compound is released by the microalgae-bacteria consortium, and/or the at least one pollutant is metabolized and/or degraded by the microalgae-bacteria consortium and/or accumulated within their cells.

18. A device for the treatment of wastewater, wherein the device is in particular for use in a method according to any one of claims 1 to 17, comprising:
    (i) at least one wastewater collection tank;
    (ii) optionally, at least one pH preconditioning unit;
    (iii) at least one wastewater dosing unit;
    (iv) at least one algae starter tank;
    (v) at least one wastewater processing tank for combining microalgae with wastewater comprising bacteria, and/or for culturing the microalgae in the wastewater comprising the bacteria under conditions to allow a microalgae-bacteria consortium to be established within the at least one wastewater processing tank, and
    (vi) optionally, at least one separation unit for separating the content of the processing tank into treated water and a microalgae biomass,
    wherein the wastewater from the at least one wastewater dosing unit is supplied to the at least one wastewater processing tank in a batch modus, a fed-batch modus, a repeated fed-batch and harvesting modus, an adaptive modus, such as a fast track adaptive evolution modus, and/or in a titration modus, preferably wherein the wastewater from the at least one wastewater dosing unit is supplied to the at least one wastewater processing tank in a repeated fed-batch and harvesting modus, a fast track adaptive evolution modus, or in a titration modus.

19. The device according to claim 18, wherein the device additionally comprises one or more of:
    (vii) a sensor for process monitoring;
    (viii) a unit for supplying air and/or carbon dioxide;
    (ix) a sensor for monitoring the water quality of the process and/or the content of pollutants in the water;
    (x) a drying unit, and
    (xi) an extraction unit, optionally wherein the extraction unit is for extracting metals.

20. A closed system comprising a lower part and an upper part, wherein wastewater and microalgae and/or a microalgae-bacteria consortium are located in the lower part of the closed system, and a root area of a crop is located in the upper part of the closed system, wherein the microalgae and/or the microalgae-bacteria consortium and the root area of the crop are in direct contact or wherein the microalgae and the root area of the crop are separated by an interlayer (such as a mesh, a membrane,

or a semi-permeable membrane), optionally wherein microalgae and/or the microalgae-bacteria consortium is embedded in the interlayer, or wherein inactivated microalgae cells are used as the interlayer to enhance the binding capacity of the microalgae-bacteria consortium, and/or lower the nutrient requirement of the crop, further optionally wherein the bacteria, the microalgae, and/or the microalgae-bacteria consortium is embedded in a protective matrix, such as a biofilm, a membrane matrix, and/or an encapsulation matrix.

21. The closed system according to claim 20, wherein the microalgae and the root area of the crop are separated by an interlayer, wherein the interlayer prevents the movement of an ion and/or a pollutant, such as an organic and/or an inorganic pollutant, from the wastewater from the lower part of the closed system to the root area of the crop in the upper part of the closed system, and wherein the interlayer allows for diffusion of a crop-growth promoting compound and/or clean water to the upper part of the closed system, optionally wherein the crop-growth promoting compound is released by the microalgae-bacteria consortium, and/or a pollutant is metabolized and/or degraded by the microalgae-bacteria consortium and/or accumulated within their cells, further optionally wherein the interlayer and/or the microalgae-bacteria consortium reduces crop toxicity, evaporation, product inhibition, and/or substrate competition, and/or increases light utilization, water utilization, nutrient utilization, and/or carbon fixation of the crop, thereby enhancing the crop growth and/or yield.

22. The closed system according to claim 20 or 21, wherein the closed system is a reactor, optionally wherein the reactor is a microalgae-bacteria consortium reactor.

23. The closed system according to any one of claims 20 to 22, wherein air, carbon dioxide (CO_2), and/or illumination is supplied artificially, and/or naturally, optionally wherein the illumination is an LED illumination.

24. Use of a device according to claim 18 or 19, or a closed system according to any one of claims 20 to 23, in a method according to any one of claims 1 to 17, or for the treatment of wastewater, for the irrigation of a plant, such as a crop, for manufacturing processes, such as for supplying water for semiconductor fabrication plants, for example cooling water for manufacturing processes and/or water for the air conditioning of semiconductor fabrication plants, and/or for supplying water for industrial processes, such as for the cleaning of machinery in industrial processes.

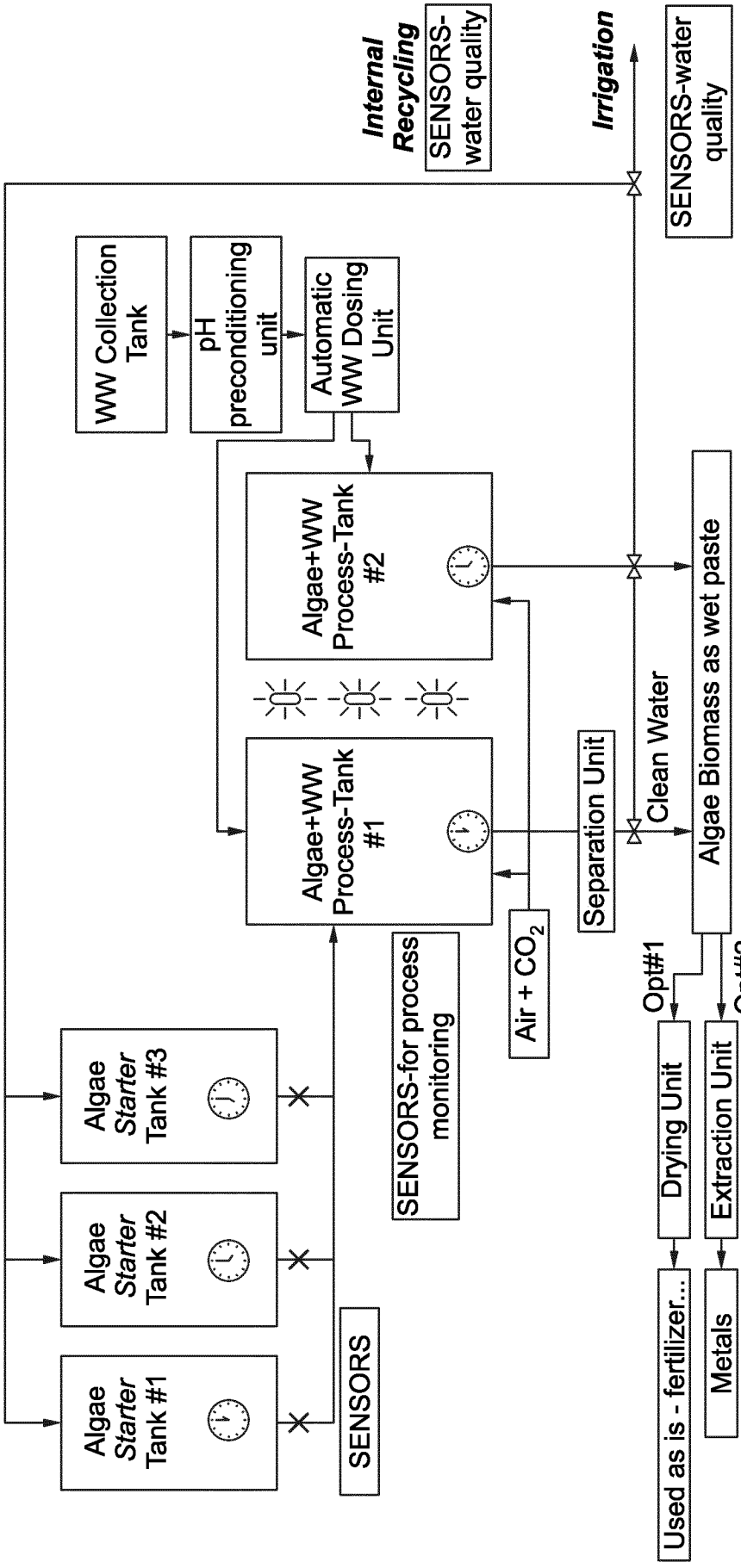

Internal Recycling
SENSORS-water quality
Irrigation
SENSORS-water quality
WW Collection Tank
pH preconditioning unit
Automatic WW Dosing Unit
Algae+WW Process-Tank #2
Algae+WW Process-Tank #1
Separation Unit
Clean Water
Algae Biomass as wet paste
Algae Starter Tank #3
Algae Starter Tank #2
Algae Starter Tank #1
SENSORS
SENSORS-for process monitoring
Air + CO_2
Opt#1
Opt#2
Drying Unit
Extraction Unit
Used as is - fertilizer...
Metals

FIG. 1

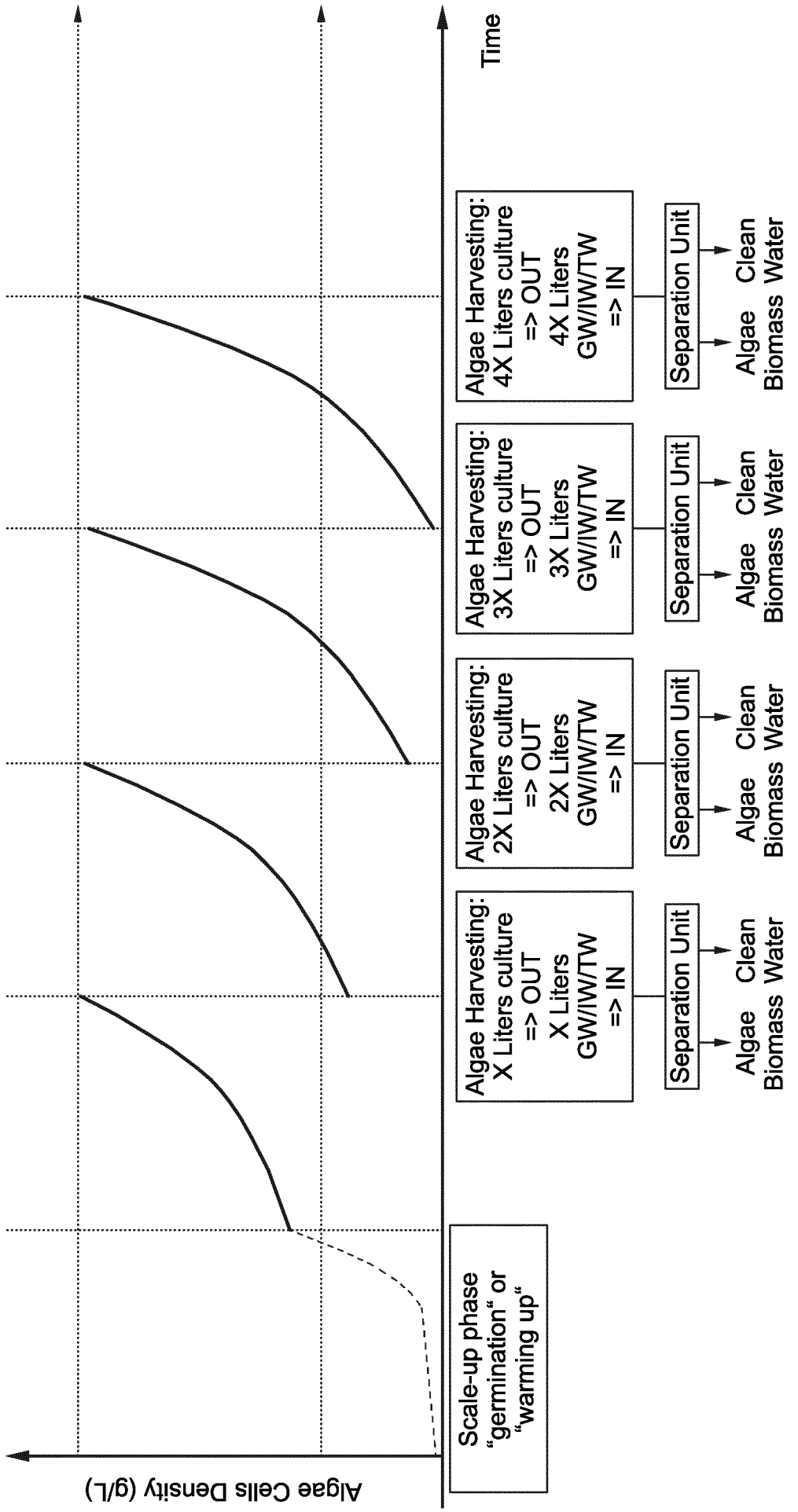

Algae Cells Density (g/L)
Time
Scale-up phase "germination" or "warming up"
Algae Harvesting: X Liters culture => OUT X Liters GW/IW/TW => IN
Algae Harvesting: 2X Liters culture => OUT 2X Liters GW/IW/TW => IN
Algae Harvesting: 3X Liters culture => OUT 3X Liters GW/IW/TW => IN
Algae Harvesting: 4X Liters culture => OUT 4X Liters GW/IW/TW => IN
Separation Unit
Separation Unit
Separation Unit
Separation Unit
Algae Biomass
Clean Water
Algae Biomass
Clean Water
Algae Biomass
Clean Water
Algae Biomass
Clean Water

FIG. 2

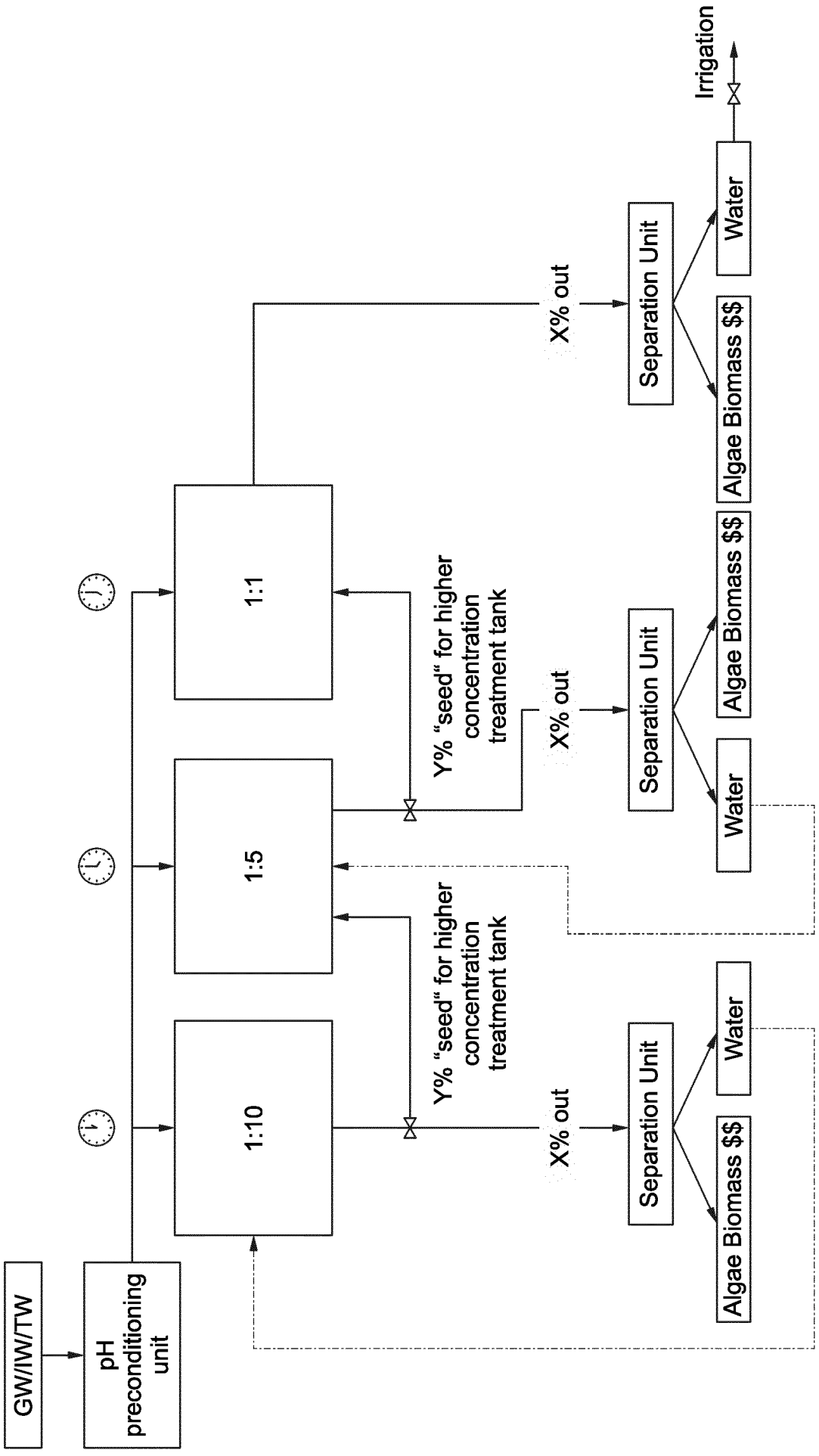

GW/IW/TW
pH preconditioning unit
1:1
1:5
1:10
Y% "seed" for higher concentration treatment tank
Y% "seed" for higher concentration treatment tank
X% out
X% out
X% out
Separation Unit
Separation Unit
Separation Unit
Algae Biomass $$
Algae Biomass $$
Algae Biomass $$
Water
Water
Water
Irrigation

FIG. 3

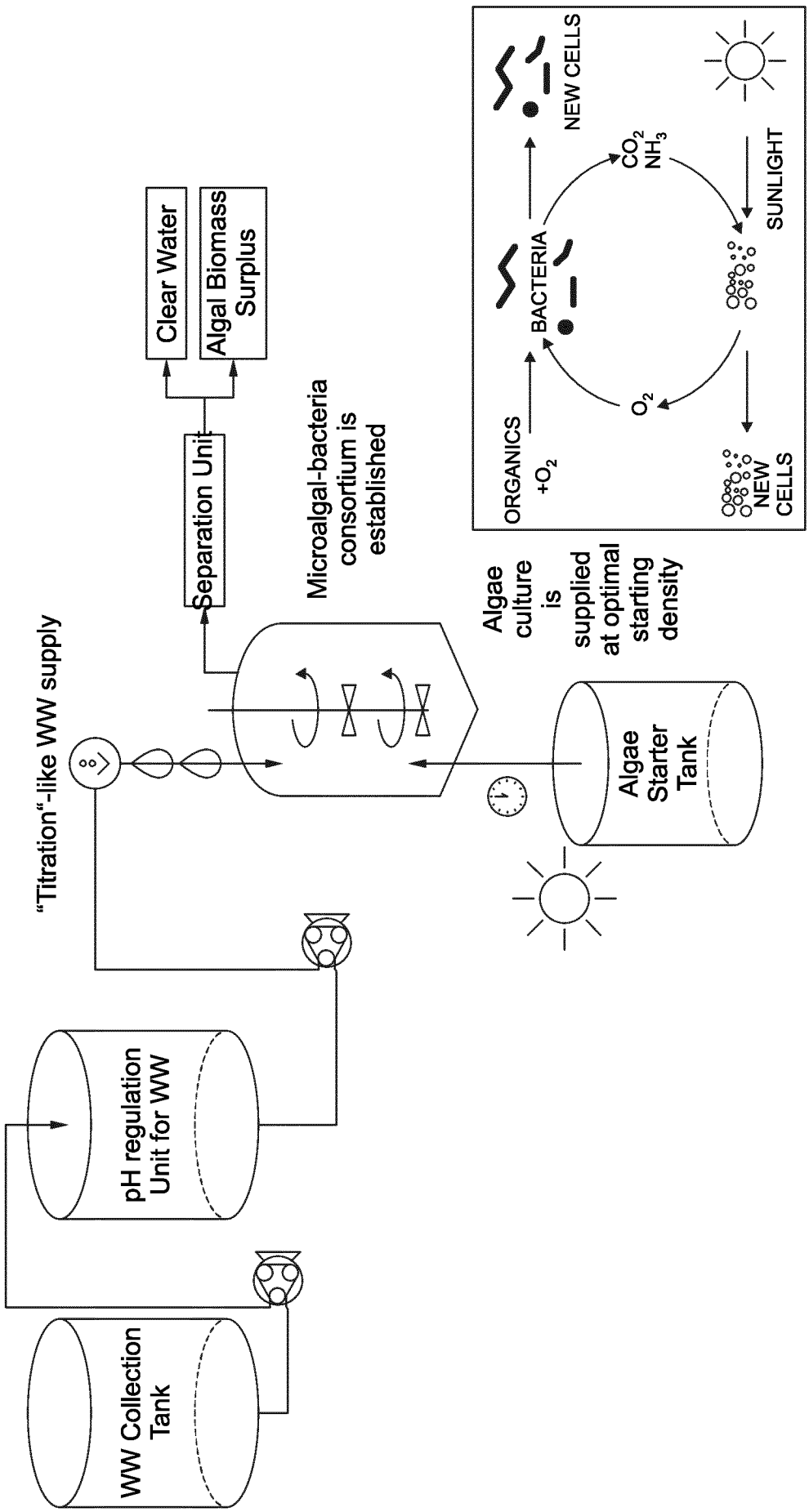

WW Collection Tank
pH regulation Unit for WW
"Titration"-like WW supply
Algae Starter Tank
Algae culture is supplied at optimal starting density
Microalgal-bacteria consortium is established
Separation Unit
Clear Water
Algal Biomass Surplus
ORGANICS
+O2
BACTERIA
NEW CELLS
CO2
NH3
O2
SUNLIGHT
NEW CELLS

FIG. 4

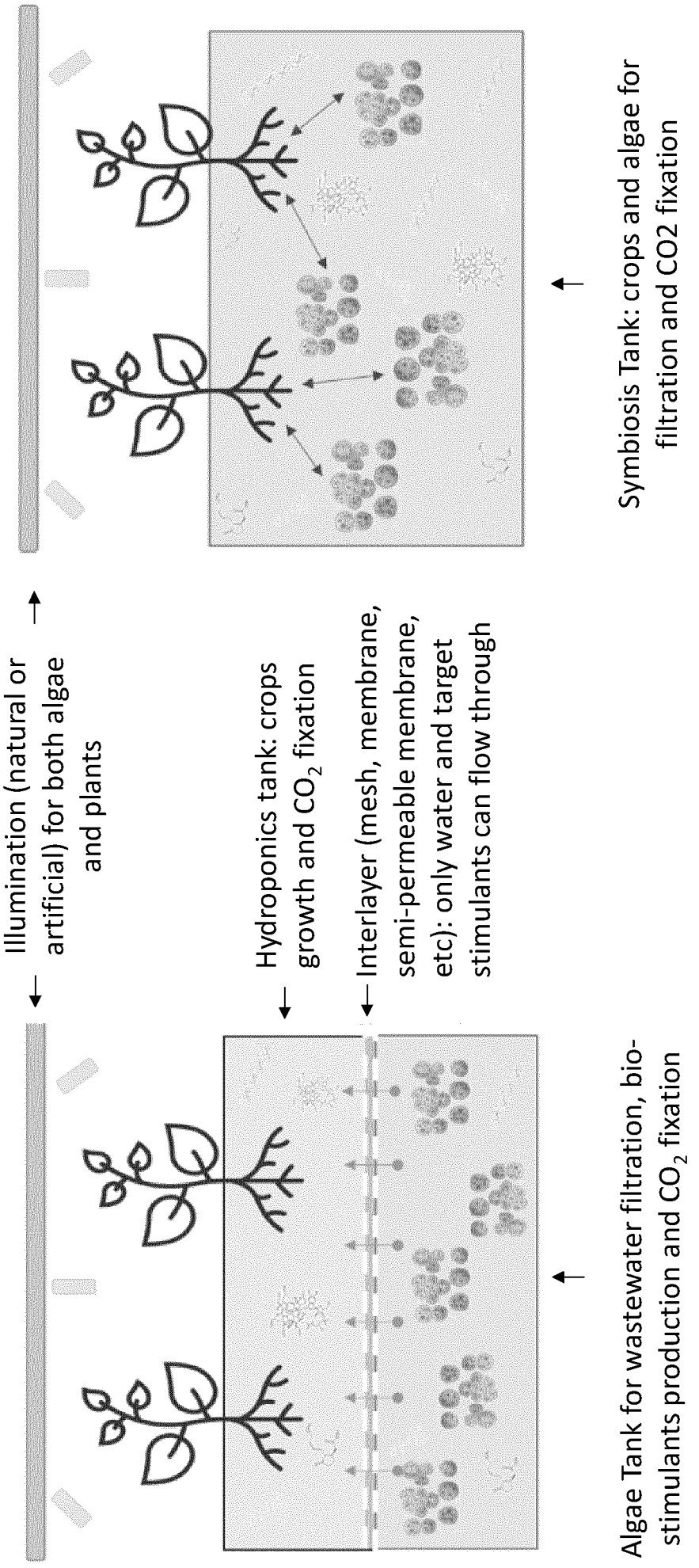

Illumination (natural or artificial) for both algae and plants
Hydroponics tank: crops growth and CO_2 fixation
Interlayer (mesh, membrane, semi-permeable membrane, etc): only water and target stimulants can flow through
Symbiosis Tank: crops and algae for filtration and CO2 fixation
Algae Tank for wastewater filtration, bio-stimulants production and CO_2 fixation

FIG. 5

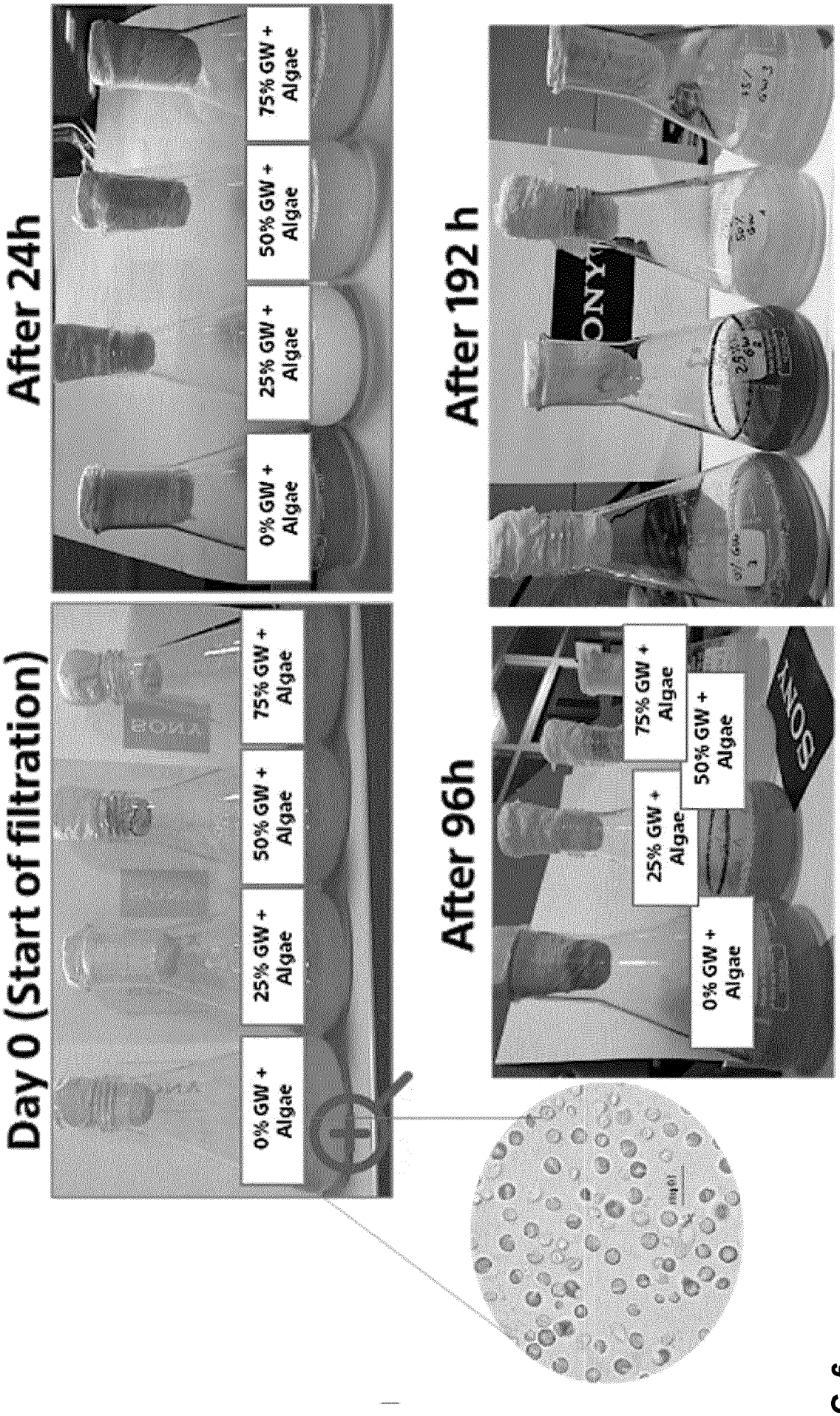

Day 0 (Start of filtration)
After 24h
After 96h
After 192 h
0% GW + Algae
25% GW + Algae
50% GW + Algae
75% GW + Algae

FIG. 6



FIG. 7

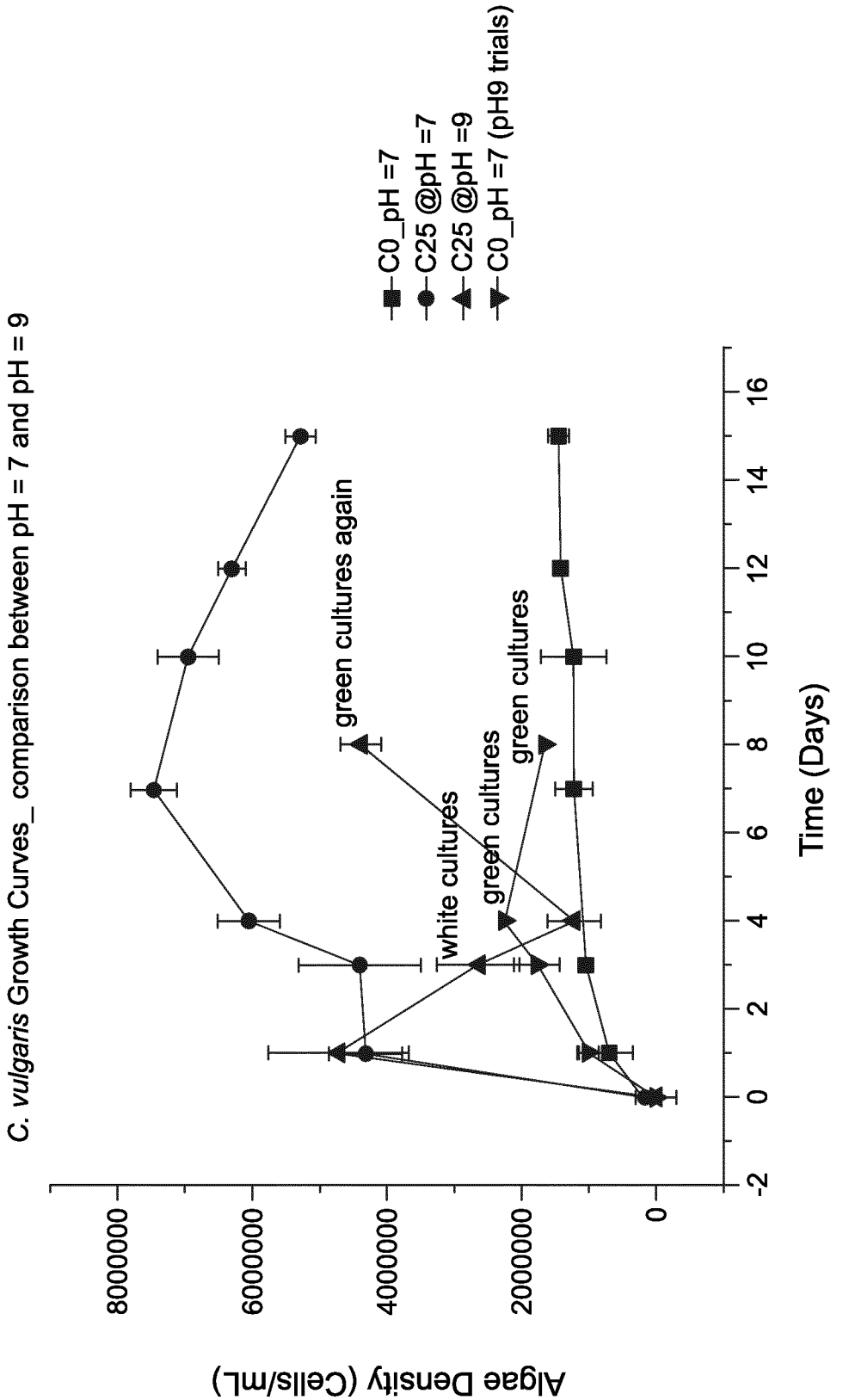

C. vulgaris Growth Curves_ comparison between pH = 7 and pH = 9
C0_pH =7
C25 @pH =7
C25 @pH =9
C0_pH =7 (pH9 trials)
green cultures again
white cultures
green cultures
green cultures
Algae Density (Cells/mL)
8000000
6000000
4000000
2000000
0
-2
0
2
4
6
8
10
12
14
16
Time (Days)

FIG. 8

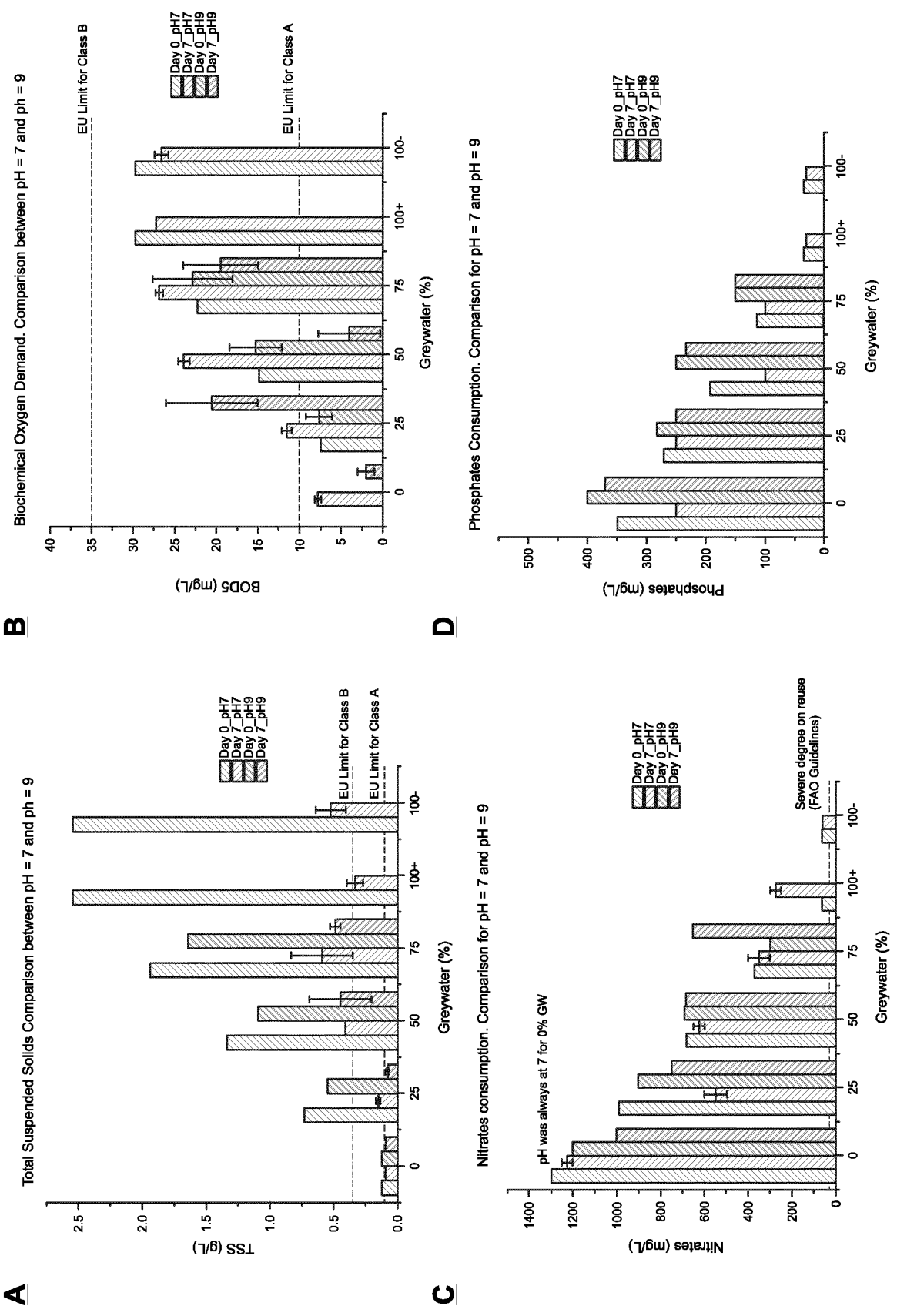

A
Total Suspended Solids Comparison between pH = 7 and ph = 9
TSS (g/L)
Greywater (%)
Day 0_pH7
Day 7_pH7
Day 0_pH9
Day 7_pH9
EU Limit for Class B
EU Limit for Class A
B
Biochemical Oxygen Demand. Comparison between pH = 7 and ph = 9
BOD5 (mg/L)
Greywater (%)
EU Limit for Class B
EU Limit for Class A
C
Nitrates consumption. Comparison for pH = 7 and pH = 9
Nitrates (mg/L)
Greywater (%)
pH was always at 7 for 0% GW
Severe degree on reuse (FAO Guidelines)
D
Phosphates Consumption. Comparison for pH = 7 and pH = 9
Phosphates (mg/L)
Greywater (%)

FIG. 9

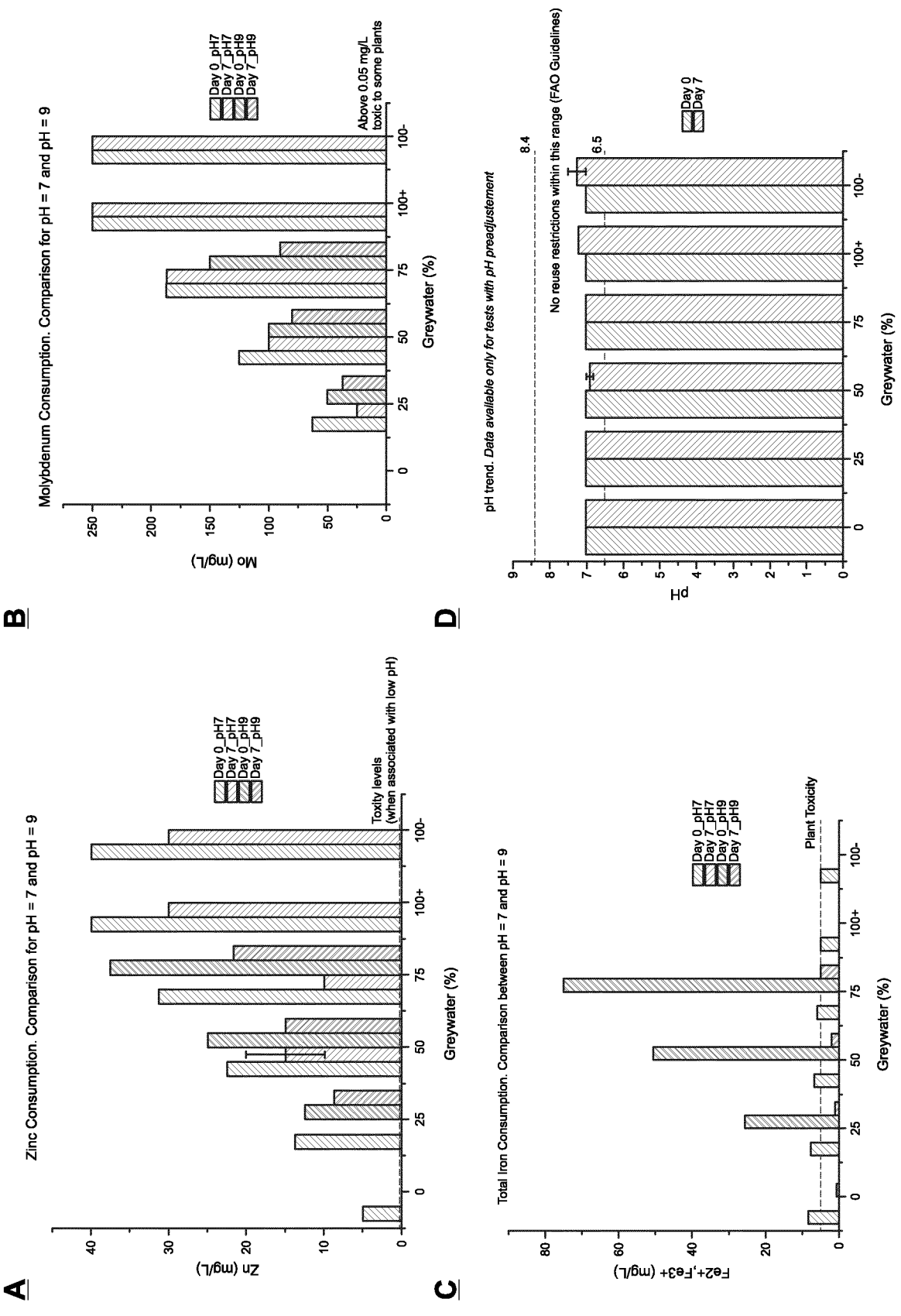

A
Zinc Consumption. Comparison for pH = 7 and pH = 9
Zn (mg/L)
Greywater (%)
Day 0_pH7
Day 7_pH7
Day 0_pH9
Day 7_pH9
Toxity levels (when associated with low pH)
B
Molybdenum Consumption. Comparison for pH = 7 and pH = 9
Mo (mg/L)
Greywater (%)
Day 0_pH7
Day 7_pH7
Day 0_pH9
Day 7_pH9
Above 0.05 mg/L toxic to some plants
C
Total Iron Consumption. Comparison between pH = 7 and pH = 9
Fe2+,Fe3+ (mg/L)
Greywater (%)
Day 0_pH7
Day 7_pH7
Day 0_pH9
Day 7_pH9
Plant Toxicity
D
pH trend. Data available only for tests with pH preadjustement
pH
Greywater (%)
Day 0
Day 7
8.4
6.5
No reuse restrictions within this range (FAO Guidelines)

FIG. 10

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2024/052181

A. CLASSIFICATION OF SUBJECT MATTER

INV. C02F3/32 C02F3/34 C12N1/12 C12N1/20 C12P39/00
ADD. C02F3/10 C02F101/10 C02F101/16 C02F101/20 C02F101/30
C02F103/16 C02F103/40 C02F103/02

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)
C02F

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 599 443 A (YAMASAKI KAZUYUKI [JP] ET AL) 4 February 1997 (1997-02-04) column 1, lines 9-16 column 6, line 36 - column 15, line 61	1-24
X	CN 107 047 429 A (LI QINGHUA; LI GENGHUAN) 18 August 2017 (2017-08-18) figures 2,3 paragraphs [0021] - [0043]	1-24

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[x] Further documents are listed in the continuation of Box C.

[x] 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

"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	Date of mailing of the international search report
28 March 2024	11/04/2024

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Rozanska, Agnieszka

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Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HUO SHUHAO ET AL: "The influence of microalgae on vegetable production and nutrient removal in greenhouse hydroponics", JOURNAL OF CLEANER PRODUCTION, ELSEVIER, AMSTERDAM, NL, vol. 243, 24 September 2019 (2019-09-24), XP085891932, ISSN: 0959-6526, DOI: 10.1016/J.JCLEPRO.2019.118563 [retrieved on 2019-09-24] pages 2-7	1-24
X	AHMED SHAMS FORRUQUE ET AL: "Progress and challenges of contaminate removal from wastewater using microalgae biomass", CHEMOSPHERE, PERGAMON PRESS, OXFORD, GB, vol. 286, 22 July 2021 (2021-07-22), XP086849016, ISSN: 0045-6535, DOI: 10.1016/J.CHEMOSPHERE.2021.131656 [retrieved on 2021-07-22]	1-19
A	pages 3-7	20-24
X	US 2012/091058 A1 (BYRD DEREK [US]) 19 April 2012 (2012-04-19)	18-24
A	figures 1,2,4 paragraphs [0006] - [0024], [0030] - [0038]	1-17
A	ZHANG BO ET AL: "Adaptive Laboratory Evolution of Microalgae: A Review of the Regulation of Growth, Stress Resistance, Metabolic Processes, and Biodegradation of Pollutants", FRONTIERS IN MICROBIOLOGY, vol. 12, 18 August 2021 (2021-08-18), pages 1-8, XP093145839, Lausanne ISSN: 1664-302X, DOI: 10.3389/fmicb.2021.737248 the whole document	1-24
A	Allman Tony: "The Difference Between Batch, Fed-batch and Continuous Processes", , 23 July 2020 (2020-07-23), pages 1-7, XP093145842, Retrieved from the Internet: URL:https://www.infors-ht.com/en/blog/the-difference-between-batch-fed-batch-and-continuous-processes/ [retrieved on 2024-03-26] the whole document	1-24

2

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2024/052181

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5599443 A	04-02-1997	JP 3056361 B2	26-06-2000
		JP H07124581 A	16-05-1995
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