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Making waves: How to clean surface water with photogranules

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ABSTRACT

Global surface waters are in a bad ecological and chemical state, which has detrimental effects on entire ecosystems. To prevent further deterioration of ecosystems and ecosystem services, it is vital to minimize environmental pollution and come up with ways to keep surface water healthy and clean. Recently, photogranules have emerged as a promising platform for wastewater treatment to remove organic matter and nutrients with reduced or eliminated mechanical aeration, while also facilitating CO_2 capture and production of various bioproducts. Photogranules are microbial aggregates of microalgae, cyanobacteria, and other non-phototrophic organisms that form dense spheroidic granules. Photogranules settle fast and can be easily retained in the treatment system, which allows increased amounts of water and wastewater to be treated. So far, photogranules have only been tested on various "high-strength" wastewaters but they might be an excellent choice for treatment of large volumes of polluted surface water as well. Here, we propose and tested for the first time photogranules on their effectiveness to remove nutrients from polluted surface water at unprecedented low concentrations (3.2 mg/L of nitrogen and 0.12 mg/L of phosphorous) and low hydraulic retention time (HRT = 1.5 h). Photogranules can successfully remove nitrogen (<0.6 mg/L, ~80 % removal) and phosphorous (<0.01 mg/L, 90–95 % removal) to low levels in sequencing batch operation even without the need for pH control. Subjecting photogranules to surface water treatment conditions drastically changed their morphology. While, under "high-strength" conditions the photogranules were spherical, dense and defined, under polluted surface water conditions photogranules increased their surface area by forming fingers. However, this did not compromise their excellent settling properties. Finally, we discuss the future perspectives of photogranular technology for surface water treatment.

1. Introduction

Global surface water quality is deteriorating due to anthropogenic activities (Wolfram et al., 2021; Posthuma et al., 2020). The discharge of untreated wastewater and even treated wastewater, together with the runoff from agricultural activities are the main causes of surface water pollution. The excess of nitrogen and phosphorus in streams, rivers, and lakes, may lead to cyanobacteria blooms, which are detrimental to aquatic ecosystems' health and surface water use (drinking water, recreation) (Peñuelas et al., 2012). To improve surface water quality, countries are enforcing more stringent wastewater treatment plant (WWTP) discharge limits, such as the European Water Framework Directive (WFD) (N-total <2.3 mgN/L, P-total <0.11 mgP/L) (Comm., 2000). However, only improving the WWTP discharge limits will not be sufficient as non-source nitrogen and phosphorus runoff heavily contributes to the pollution. Therefore, technological solutions for reducing the nitrogen and phosphorus concentrations in surface waters are needed, even when point and non-point source pollution are lowered.

The technological solutions for reducing nitrogen and phosphorus in surface water need to be robust, reliable, easy to implement and maintain, have low investment and operational costs, keep high nitrogen and phosphorus removal efficiency under varying nutrient concentrations and environmental conditions, and potentially generate services and/or valuable products for society. Several ecotechnologies have been

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proposed to rejuvenate polluted surface water bodies such as constructed wetlands, sand filtration, microbial technologies, and biopreparations (Simon and Joshi, 2021). However, all of them have disadvantages attributed to the capacity in removing nitrogen and phosphorus or the long treatment period (up to several days) that results in large footprints. Photogranular technology, a novel light-driven wastewater treatment technology, has high nutrient removal capacity and a smaller footprint than the other ecotechnologies, and can be used for cleaning surface water (Brockmann et al., 2020; Abouhend et al., 2018).

2. The untapped potential of photogranules for surface water treatment

In the past 5-10 years, photogranular technology has emerged as a promising sunlight-driven wastewater treatment process that significantly reduces energy expenses and greenhouse gas emissions compared to other conventional wastewater treatment systems (Brockmann et al., 2020). Photogranules are spherical microbial aggregates of microalgae, cyanobacteria, and other non-photosynthetic organisms that shows great potential in application for municipal (Abouhend et al., 2018), industrial (dairy, textile) (Tiron et al., 2017), and aquaculture wastewater treatment (Zhang et al., 2023; Santorio et al., 2022) dealing with pollutants such as nitrogen, phosphorous, organic carbon, but also contaminants of organic micropollutants such as tetracycline or sulfamethoxazole (Wang et al., 2020; Hu et al., 2022). Photogranulation occurs under static or hydrodynamic conditions with motile filamentous cyanobacteria playing an important role by forming an interwoven network that acts like a skeleton for other organisms to embed themselves in (Milferstedt et al., 2017; Gikonyo et al., 2021). Further, extracellular polymeric substances excreted by both phototrophic and non-phototrophic organism act like a glue that gives structural support (Trebuch et al., 2020). Due to granular aggregation, stratification occurs within photogranules that allows various microbial functions such as photosynthesis, nitrification, denitrification, and polyphosphate accumulation to take place within the same granule. Additionally, the biomass settles extremely quickly (< 1 min) and is easily retained in the photogranular reactor (Abouhend et al., 2018; Tiron et al., 2017). This allows for very low hydraulic retention times (HRT), where large volumes of water and wastewater pass through the treatment system in the order of minutes while maintaining a high solid retention time (SRT), where the biologically active photogranular biomass stays in the reactor for days or weeks.

Photogranules have proven to be an efficient and versatile wastewater treatment technology under various environmental and operational conditions. Depending on the targeted pollutants and their concentrations, the microbial community composition of the photogranules is purposefully altered and enriched with certain microbial functions that target removal or recovery of specific pollutants. In several studies nitrification and denitrification (Abouhend et al., 2018; Trebuch et al., 2020) or anammox (Kong et al., 2023), were targeted for high nitrogen removal in nitrogen-rich wastewater. Under high phosphorus concentrations, photogranules were successfully enriched with phosphorus accumulating organisms to fully recover phosphorus as polyphosphate in photogranular biomass (Trebuch et al., 2023). When photogranules experienced nitrogen limiting conditions, the cyanobacteria present showed to be able to fix nitrogen from nitrogen gas (N_2)

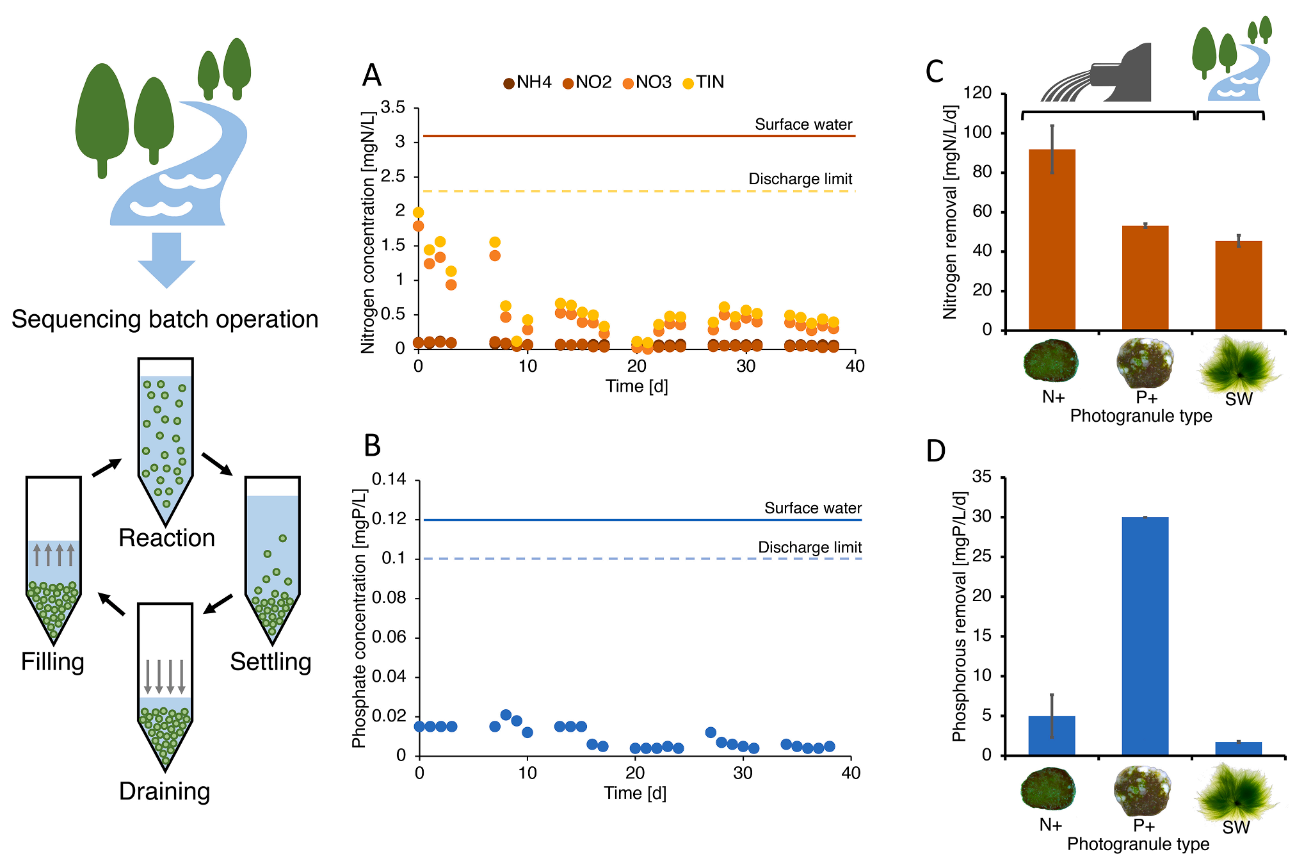

Fig. 1. Photogranular technology used in surface water treatment by applying sequencing batch operation. (A) Effluent concentrations of ammonium (NH_4^+), nitrite (NO_2^-), nitrate (NO_3^-), and total inorganic nitrogen (TIN). The influent concentrations for TIN of the polluted surface water (solid line) and the discharge limits (dashed line) are indicated. (B) Effluent concentration of phosphate (PO_4^{3+}). The influent concentrations for phosphate of the polluted surface water (solid line) and the discharge limits (dashed line) are indicated. (C) Daily nitrogen removal rate of photogranular technology used for three different types of wastewaters. (D) Daily phosphate removal rate of photogranular technology used for three different types of wastewaters. N+ = Trebuch et al., 2020; P+ = Trebuch et al., 2023; SW = surface water treatment with data from this study.

(Trebuch et al., Feb. 2023). Photogranules have also been enriched with methanotrophic bacteria to remove dissolved methane from effluents of anaerobic digesters (Safitri et al., 2021). The flexibility of the microbial community and function redundancy of photogranules suggests that there are still untapped functions that allow for new applications, such as treatment of surface water, where nitrogen and phosphorus are on average 20-100 times lower than in municipal wastewater (Fig. 1, sketch of Photogranules treating surface water/river).

Here, we show for the first time that photogranule technology can be used to treat polluted surface water (from river Reusel, The Netherlands), reducing nitrogen and phosphorus concentrations below the WDF limits (N-total <2.3 mgN/L, P-total <0.11 mgP/L) making this technology future proof (Fig. 1A & B). The process was run in sequencing batch under an HRT of only 1.5 h, which is significantly lower than other proposed technologies which have treatment times of days (Simon and Joshi, 2021). The average daily nitrogen removal of 44 mgN/L/d was comparable to the photogranules enriched with polyphosphate accumulating organisms (P+) but two times lower than photogranules enriched with nitrifiers and denitrifiers (N+). The latter was due to the double function of nitrogen assimilating into biomass and loss of nitrogen gas (Fig. 1C). Daily phosphorus removal was severely limited by phosphorus availability of the river water, which had a high N:P molar ratio of 71 (Fig. 1D). This resulted in a removal rate that is 2x lower compared to N+ and 10x lower compared to P+, which were enriched in polyphosphate accumulating organisms.

In many previous lab studies, the pH was controlled around neutrality to accommodate microorganisms that comprise a photogranule (e.g., polyphosphate accumulating organisms prefer a pH around 7.25) and avoid alkaline conditions that lead to free ammonia formation (> pH 7.5) that is toxic to most microorganisms. However, it is not feasible to control pH when implementing photogranular technology for surface water treatment at large scale. Hence, we decided to not control pH, which resulted in the pH varying from 7.5 during dark periods up to 10.5 during light periods. This clearly indicated that the change in pH is driven by photosynthesis and the consumption of inorganic carbon (HCO_3^- and CO_2). Despite the varying pH during light and dark periods, the performance of the photogranules was not negatively affected and free ammonia formation was not of concern due to the very low ammonium concentrations (<1 mg/L) in the surface water. This shows that pH control will not be necessary at large-scale implementation.

3. Filamentous photogranules with excellent settling properties

Granules are spherical biofilms, that display the same microbial community complexity and physical chemical phenomena as planar biofilms. In both planar and spherical biofilm systems diffusion transport phenomena dictate the growing conditions of the microbes and thereby the morphology of the biofilm (Flemming and Wingender, 2010). At the low nutrient concentrations of the tested surface water, the diffusion of nutrients into deeper layers of the photogranule was limited, which had a profound effect on photogranule morphology and size. The microbial community responded by forming irregular shapes and filamentous outgrowth (fingers), therefore enlarging its surface area, and ensuring access to nutrients (Fig. 2A). This is a known phenomenon in biofilms (Flemming and Wingender, 2010). The commonly reported dense and confined structure of a photogranule under nutrient-rich wastewater (Abouhend et al., 2020) changed to a photogranule with a dense 0.5 – 1.5 mm core and a fluffy exterior (0.2 – 3 cm) with myriads of filaments extending radically from the core (Fig. 2C). Similar filamentous photogranules were reported by Ouazaite et al. (2021) (Ouazaite et al., 2021) during lab-scale experiments using low-strength municipal wastewater where photogranules increased their specific surface area by forming filamentous outgrowth as well. A similar phenomenon was observed for aerobic granular sludge that exhibited a filamentous nature either under carbon substrate limitation or being exposed to complex carbon substrates that need to undergo hydrolysis before they are available to the microbial community (Pronk et al.,

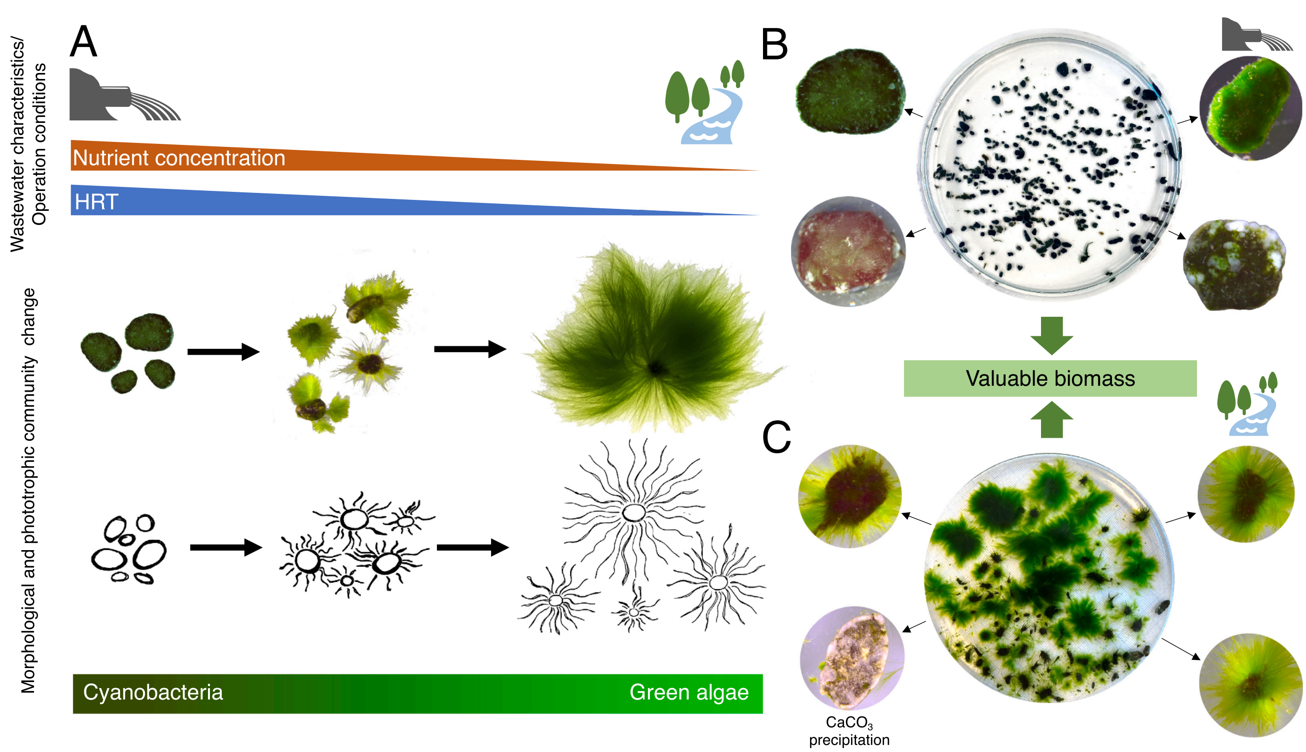

Fig. 2. Morphological and microbial community change of photogranules according to wastewater characteristics and operation conditions. (A) Depiction of various microbial communities and morphologies of photogranules according to the exposed wastewater characteristics and operational conditions. (B) Morphotypes of photogranules cultivated under "high-strength" (municipal) wastewater conditions. (C) Morphotypes of photogranules used for surface water treatment. The photogranular technology generates valuable photogranular biomass that can be used for e.g., organic fertilizer, biopolymers, or pigments.

2015; de Graaff et al., 2020).

Simultaneously the phototrophic microbial community shifted from a cyanobacteria dominated one, as often observed in photogranules (Trebuch et al., 2020; Milferstedt et al., 2017), to filamentous green algae (Fig. 2A) as shown by pigment analysis. Many filamentous green algae prefer low nutrient concentrations and can tolerate a larger range of pH that could have led to this enrichment (Borowitzka et al., 2016). Another drastic change compared to N+ and P+ photogranules was the severely reduced heterotrophic microbial community and a more simplified photoautotrophic and chemoautotrophic microbial community. This was due to the near absence of organic substrate in the polluted surface water to support an active heterotrophic microbial community. The shift in the microbial community to a filamentous green alga while maintaining granular structure was unprecedented and illustrates the great adaptability of photogranules to changing chemical concentrations of the growth medium and to environmental conditions such as pH, temperature, and light.

Despite the morphological and microbial community changes, the photogranules maintained their excellent settleability and nitrogen and phosphorus removal efficiency (Fig. 1C,D) compared to previous studies (Trebuch et al., 2020; Trebuch et al., 2023). This high efficiency was also not disturbed by the natural fluctuation of the pH between 7.5-10.5. At these alkaline conditions, $CaCO_3$ precipitated on the surface of the photogranules, a phenomenon often reported in natural phototrophic biofilms (STAL, 1995). This precipitate possibly increased the overall density of photogranules and had the positive effect of enhancing their settleability as observed in other studies (Gikonyo et al., 2021; Gikonyo et al., 2022). Irrespective of the filamentous nature of the photogranules, they maintained a sludge volume index <55 mL/g, a settling velocity lower than 0.0075 m/s, which is similar to the average settling velocities of 0.0086 m/s reported from other photogranules (Gikonyo et al., 2022), and excellent biomass retention within the bioreactors (>99.5 %), which facilitates the treatment of high quantities of polluted surface water.

4. The added value of photogranular technology: resource recovery

Besides removing nitrogen and phosphorus to extremely low concentrations, the photogranular technology enables the recovery of these nutrients, and a wide array of other elements, into a photogranular biomass, which is 2-3 times higher than aerobic granular sludge (Trebuch et al., 2023), and that can be used as raw material for the circular economy (Fig. 2B & C). These elements include macro- and microelements (C, N, P, K, Ca, Fe, Mg, etc.), photopigments (i.e., chlorophyll, phycobilin proteins and carotenoids), lipids, proteins, antioxidants (e.g., alkaloids, flavonoids, phenols, tannins, phlorotannins, terpenoids) and storage compounds or biopolymers (e.g., PHA, polyP, cyanophycin) (Borowitzka et al., 2016; Haq et al., 2019; Kehrein et al., 2020). The EU has strong ambitions for a circular bio-based economy and algae plays an important role due to its multi-product generation (Communication from The Commission to The European Parliament 2022). Additionally, the EU aims to reduce the use of artificial fertilizers by 20 % by 2030 and replace them with organic fertilizers from bio-waste and side streams (Parliament, 2019). One of the bio-waste is biomass (sludge) from wastewater treatment, such as photogranular biomass. This will allow getting a significant economic benefit by valorizing the photogranular biomass while treating wastewater and cleaning polluted surface waters.

The application of photogranular biomass as organic fertilizer or bioamendment can nurture soil health and stimulate plant growth (Suleiman et al., 2020), which is required, as globally the reduction of microelements (e.g., Zn, Cu, Fe, Mn) in soil is leading to lower soil biodiversity and therefore poorer soil health (Lal, 2016). Biopolymers and extracellular polysaccharides can be extracted and used for various applications such as coating material for slow-release fertilizers, and paper or fire retardant, as showcased by *Kaumera* Nereda® *Gum* (Kaumera 2024) extracted from aerobic granular sludge. Recently, alginate-like exopolymers and similar other exopolysaccharides found in aerobic granular sludge were described in photogranules, which could have similar application (Chen et al., 2023). Another interesting product of photogranular biomass are pigments that are produced by microalgae, cyanobacteria, and bacteria. These pigments are characterized in chlorophylls, phycobilins, and carotenoids, which have applications as colorant but also as bioactive compounds. For instance, carotenoids such as astaxanthin, lutein, fucoxanthin are applied as antioxidant, anti-inflammatory, immunoprophylactic, antitumor activities (Patel et al., 2022). As photogranular biomass will be grown on wastewater or polluted surface water, it is imperative to assess and remove possible contaminants according to application, therefore ensuring a safe product valorization.

5. How to scale up photogranular technology for surface water treatment

One of the next crucial steps will be to decipher the way of how to bring this technology to application at large scale. In our laboratory experiments, we considered the average nutrient composition and environmental conditions (light intensity, temperature, and pH) from March to October where nutrient concentrations in the river Reusel were above the environmental limits of the WFD. From these experiments, we know that per m^3 of reactor volume about 16 m^3 of surface water can be treated on average per day, which is 6-100 times the capacity of other proposed technologies for surface water treatment (Simon and Joshi, 2021). Extrapolating from this result, a full-scale treatment system for the Reusel river would require a total reactor volume of 1127 m^3 to treat the flow of 0.20 m^3/s of the river. For phototrophic treatment systems the reactor volume alone is not enough to identify, it is also important to take the light exposed surface into account. Calculating with an average light input of 31 $mol_{photons}/m^2/d$ and approximately 0.73 m^2 of required area per m^3 of treated surface water resulting from our lab-scale experiments, a total area of 820 m^2 would be required to capture sufficient light energy to treat the river. These are indicative numbers from lab-scale experiments, and they must be handled with care.

Additionally, it is important to know that the performance of photogranular technology will increase or decrease with available nutrients (N, P, inorganic carbon) in surface water and available light, which will fluctuate in time. Therefore, in future endeavors and when designing such a photogranular treatment system, the seasonal variation in nutrient composition and light availability must be assessed and integrated in the design. For example, when nutrient concentrations are high or light input is low the hydraulic retention time (HRT) can be increased to assure enough time for the photogranules to take up nutrients to the required concentrations. This is only one example on how to deal with seasonal variation and nutrient composition. Changing the solid retention time (the amount of active biomass within the treatment system), or improving photobioreactor design by enhancing light penetration, are amongst other aspects that should be further explored.

There are several types of photobioreactors that could accommodate the photogranular technology, such as tubular, flat panel, or bubble column bioreactors and these systems need to be tested for their suitability (De Vree et al., 2015). The operation under sequencing batch mode seems to be vital for maintaining photogranules. However, the option of operating reactors continuously should be explored as it would improve the amount of water that could be treated per day. Recently, there has been significant progress for continuously operated reactors for aerobic granular sludge with specific design to retain active biomass (Samaei, 2023), which could inspire to do the same for photogranular technology. Since surface water qualities around the globe are decreasing, it is vital to act fast and try to find solutions to safeguard water quality and ecosystem services. Photogranular technology could be a solution and therefore should be explored further for its application at large-scale with a combined effort of scientists, industry, water authorities, and governments.

6. Conclusions and future perspectives

- Photogranular technology can clean surface water to the standard from the European Water Framework Directive (WFD) and shows a great potential for surface water treatment with enhanced performance compared to other biological systems.
- Essential elements can be recovered from polluted surface water and produce valuable biomass that could be used as raw material for chemicals, biopolymers, and organic fertilizers.
- Future endeavors should explore reactor design and operation for large-scale applications and should explore the full potential of photogranular biomass.
- Surface water treatment will be necessary to safeguard water quality and with photogranular technology be an integral part of the future biobased/circular economy.

CRediT authorship contribution statement

Lukas M. Trebuch: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Conceptualization. **Jolieke Timmer:** Methodology, Investigation, Formal analysis, Conceptualization. **Jan van de Graaf:** Writing – review & editing, Funding acquisition, Conceptualization. **Marcel Janssen:** Writing – review & editing, Conceptualization. **Tânia V. Fernandes:** Writing – review & editing, Visualization, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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