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Scientific Paper

Species-specific effects of *Chlorella vulgaris* and *Scenedesmus obliquus* on algal-bacterial granule formation and stability

Efeitos específicos das espécies Chlorella vulgaris e Scenedesmus obliquus na formação e na estabilidade de grânulos algal-bacterianos

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ABSTRACT

This study evaluated the formation and stability of algal-bacterial granular sludge (ABGS) in sequencing batch photobioreactors treating synthetic wastewater, with a focus on the role of two green microalgae species: *Chlorella vulgaris* and *Scenedesmus obliquus*. Prior to reactor operation, a light intensity and photoperiod screening was performed to define suitable cultivation conditions, which led to the selection of 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and a 14 h light/10 h dark cycle. Three 8-L reactors were operated under 6-h cycles, 50% volumetric exchange ratio, and a settling time reduction strategy (20 to 5 min). R1 was inoculated with *C. vulgaris,* R2 with *S. obliquus*, and R3 (control) with activated sludge only. Granulation was achieved in R1 and R2 after 89 and 71 days, respectively, while R3 remained ungranulated. Microalgae presence significantly improved settleability (sludge volume index $[SVI]_{30}/SVI_5$), granule size, and biomass retention. The *S. obliquus* system showed the best overall performance, with faster granulation and larger granules. These results highlight the role of microalgal species in enhancing the structural development of photogranules and their potential for improving ABGS-based wastewater treatment systems.

Keywords: photogranules; microalgae; wastewater treatment.

RESUMO

Este estudo avaliou a formação e a estabilidade de lodo granular algal-bacteriano em fotobiorreatores operados em batelada sequencial tratando águas residuárias sintéticas, com foco no papel de duas espécies de microalgas verdes: *Chlorella vulgaris* e *Scenedesmus obliquus*. Antes da operação dos reatores, foi realizada uma triagem de intensidade luminosa e fotoperíodo para definir condições adequadas de cultivo, o que levou à seleção de 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$ e de um ciclo de 14 h de luz/10 h de escuro. Três reatores de 8 L foram operados com ciclos de 6 h, taxa de troca volumétrica de 50% e estratégia de redução do tempo de sedimentação (de 20 para 5 min). O R1 foi inoculado com *C. vulgaris*, o R2 com *S. obliquus* e o R3 (controle) apenas com lodo ativado. A granulação foi alcançada em R1 e R2 após 89 e 71 dias, respectivamente, enquanto o R3 permaneceu sem granulação. A presença de microalgas melhorou significativamente a sedimentabilidade (IVL_{30}/IVL_5), o tamanho dos grânulos e a retenção de biomassa. O sistema com *S. obliquus* apresentou o melhor desempenho geral, com granulação mais rápida e grânulos maiores. Esses resultados destacam o papel das espécies microalgais na melhoria do desenvolvimento estrutural de fotogrânulos e seu potencial para aprimorar sistemas de tratamento de águas residuárias baseados em lodo granular algal-bacteriano.

Palavras-chave: fotogrânulos; microalgas; tratamento de esgoto.

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INTRODUCTION

With the industrial revolution and exponential global population growth, human activities have generated large amounts of wastewater. If not properly managed, this wastewater can severely impact the environment (Parsy *et al.*, 2024). Consequently, there is a growing need for more economically, socially, and environmentally sustainable wastewater treatment technologies (Torres-Franco *et al.*, 2024).

Aerobic granular sludge (AGS) is an innovative wastewater treatment technology gaining attention for its efficiency, often outperforming conventional activated sludge (CAS). AGS systems offer benefits such as lower operational and energy costs, reduced space requirements, and a unique structure with distinct redox layers that enable simultaneous removal of various pollutants through oxygen diffusion gradients (Dababat *et al.*, 2024). However, the long formation time for stable aerobic granules remains a limitation. This challenge can be overcome by combining microalgae and bacteria to form algal-bacterial granular sludge (ABGS), also known as photogranules (Zhang *et al.*, 2020; Mohsenpour *et al.*, 2021).

ABGS is a promising approach for wastewater treatment, integrating the autotrophic capacity of algae with the heterotrophic capacity of bacteria into a highly efficient symbiotic system. This synergistic relationship enhances organic matter and nutrient removal while facilitating resource recovery (Zhang *et al.*, 2023; Wang *et al.*, 2024). Within these systems, microalgal growth and activity are highly sensitive to various factors, with light intensity and photoperiod being particularly critical (Kim *et al.*, 2014). The reactor operational mode is also a key factor for cultivating (photo)granules. Sequencing batch reactors (SBRs) are the most used and effective configuration for such a purpose, promoting their formation by enabling precise control over sludge retention time, applying strong selection pressures through short settling periods, and supporting feast–famine cycles (Billery *et al.*, 2025). This configuration also favors the growth of filamentous cyanobacteria, which are implicated in granulation (Kong *et al.*, 2023).

Despite the considerable potential of ABGS in wastewater treatment, significant knowledge gaps persist regarding the precise factors that influence the formation and long-term stability of these aggregates. Specifically, the impact of microalgal species selection and the optimal operational configurations remain critical areas requiring further investigation.

Previous studies have explored ABGS formation. Liu *et al.* (2018) evaluated ABGS development by co-cultivating mature aerobic granules with pure cultures of *Chlorella vulgaris* and *Scenedesmus obliquus*. However, a limitation of their work was the simultaneous introduction of both microalgal species into a single reactor, which hindered the assessment of their individual contributions to granule development. Moreover, using pre-formed aerobic granules as the bacterial inoculum restricted insights into the initial stages of photogranule formation from activated sludge. In another relevant study, Guo *et al.* (2020) examined the behavior of three green microalgae (*C. vulgaris*, *S. obliquus*, and *Neochloris oleoabundans*) in separate reactors, allowing for a more detailed evaluation of species-specific performance. Nevertheless, that study focused on swine wastewater and did not include comparisons with conventional AGS systems, thus limiting its direct relevance to wastewater treatment and the broader assessment of ABGS as an alternative technology.

To address these identified limitations and advance the understanding of ABGS systems, the present study adopts a systematic and comparative approach. It focuses on the formation and stability of ABGS in sequential batch photobioreactors. Each photobioreactor was inoculated with activated sludge and pure cultures of either *C. vulgaris* or *S. obliquus*, and operated separately under identical conditions. Furthermore, a control system based on conventional AGS was included to provide a critical reference for evaluating the relative performance of the photogranular systems. This experimental design aimed to clarify the specific role of each microalgal species in the granulation process and to identify the most efficient and feasible inoculum combination for future full-scale implementation of ABGS technology in wastewater treatment plants.

MATERIAL AND METHODS

Lighting condition screening for microalgae cultivation

The growth of the green microalgae species *C. vulgaris* and *S. obliquus* was evaluated under different light intensities and photoperiods. The pure strains were provided by the Planktology Laboratory of the Department of Fisheries Engineering at the Universidade Federal do Ceará.

This stage followed a 2^2 full factorial design with three central point replicates and was carried out in transparent glass bottles with a working volume of 1 L, in an incubator chamber (Tecnal model TE-401) at a controlled temperature of 25 ± 2°C. The bottles were inoculated with microalgal cells previously cultured in Guillard f/2 standard medium. The cells were centrifuged (10,000 rpm for 10 min) and then resuspended in synthetic wastewater, prepared according to Argenta *et al.* (2021). The bottles were continuously aerated at an airflow rate of 2.5 $L \cdot min^{-1}$ by air compressors and illuminated by light-emitting diode (LED) strips.

The photoperiod values were defined according to Ji (2022), as follows: 10 h light/14 h dark, 12 h light/12 h dark (central point), and 14 h light/10 h dark. The luminescence values were set according to Bohutskyi *et al.* (2016), as follows: 150, 200 (central point), and 250 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Optical density (OD) was the response variable for growth evaluation and was monitored daily by spectrophotometry at λ = 680 nm (Shimadzu UV-1280).

Cultivation of algal-bacterial granular sludge

Experimental setup

As mentioned before, SBRs are the most used and effective configuration for cultivating (photo)granules (Billery *et al.*, 2025). Hence, three 8-L SBRs (R1, R2, and R3) (Figure 1) were inoculated with activated sludge (5 g volatile suspended solids [VSS]$\cdot L^{-1}$, sludge volume index $[SVI]_{30}$ of 164 $mL \cdot g^{-1}$) obtained from a University of Cape Town (UCT) system, located in a domestic wastewater treatment plant of a residential complex (Fortaleza, Ceará, Brazil).

R1 and R2 were also inoculated with 10^{10} cells of *C. vulgaris* and *S. obliquus*, respectively, for ABGS cultivation, whereas R3 served as the control reactor for AGS cultivation, i.e., it was operated under conditions identical to R1 and R2, except for the absence of microalgae (Liu *et al.*, 2018). The illumination for the microalgal-bacterial reactors was provided by LED strips with an average intensity of 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$, and the photoperiod was 14 h of light and 10 h of dark. This light intensity was chosen as some microalgae species grown on thin flat panels obtain photosaturation at irradiances above 150 µmol·m^{-2}·s^{-1} (Sforza *et al*., 2012). Additionally, Gris *et al*. (2014) evaluated the growth of *S. obliquus* in photobioreactors at intensities from 10 to 1,000 µmol·m^{-2}·s^{-1}, noting a maximum growth rate at 150 µmol·m^{-2}·s^{-1}, with growth inhibition observed above this value for the species. Aeration was provided by mini air compressors, ensuring an upflow velocity of 2.5 cm·s^{-1} and a minimum dissolved oxygen concentration of 5 mg·L^{-1}.

Synthetic wastewater

The SBRs were fed with synthetic wastewater, which included propionic acid (850 mg chemical oxygen demand [COD]·L^{-1}) as the carbon source and specific macronutrient and micronutrient solutions. The macronutrient solution (7.4 mL·L^{-1}) comprised NH_4Cl (30 g·L^{-1}), KH_2PO_4 (6.18 g·L^{-1}), $MgSO_4$ (10 g·L^{-1}), and $CaCl_2$ (1 g·L^{-1}). The micronutrient solution (1 mL·L^{-1}) contained H_3BO_3 (50 mg·L^{-1}), $ZnCl_2$ (50 mg·L^{-1}), $FeCl_3 \cdot 6H_2O$ (2,720 mg·L^{-1}), $CuCl_2$ (38 mg·L^{-1}), $MnCl_2 \cdot 4H_2O$ (500 mg·L^{-1}), $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ (50 mg·L^{-1}), $AlCl_3$ (90 mg·L^{-1}), $CaCl_2 \cdot 6H_2O$ (2,000 mg·L^{-1}), $NiCl_2$ (50 mg·L^{-1}), $Na_2SeO_3 \cdot 5H_2O$ (162 mg·L^{-1}), and EDTA (1,000 mg·L^{-1}). Additionally, $NaHCO_3$ was directly added to the final solution at 2.5 g·L^{-1} as a buffering agent.

This wastewater composition, including the chosen organic load, was adapted from previous successful granular sludge cultivation studies, conducted by the authors' research group, which utilized synthetic wastewater concentrations ranging from 500 to 1,000 mg COD·L^{-1} (Argenta *et al*., 2021; dos Santos *et al*., 2022). The selection of this specific concentration aimed to provide a consistent and proven operational baseline to effectively assess the isolated impact of microalgal inoculation on the granulation process.

Experimental procedure

The SBRs were operated in 6-h cycles (20 min of filling, 100 min of anaerobic reaction, 219–234 min of aerobic reaction, 5–20 min of settling, and 1 min of decanting) with a volumetric exchange ratio (VER) of 50% at a room temperature of 28 ± 2°C. The experiment was divided into four phases (I to IV), during which the settling time was gradually reduced from 20 to 5 min to impose selection pressure on biomass and promote granulation. To maintain a constant 6-h cycle duration, the time reduced from the settling period was added to the aerobic reaction period.

Granulation assessment

The granulation process was assessed through physical aspects (granulometry, settleability, and granule stability) and microbiological aspects (production of extracellular polymeric substances [EPS] and chlorophyll *a*).

Granulometry was determined as described by Bin *et al*. (2011). Settleability was evaluated using the SVI at 5 min (SVI_5) and 30 min (SVI_{30}) (Schwarzenbeck; Borges; Wilderer, 2005). The physical stability of the granules was determined as described by Nor-Anuar *et al*. (2012).

EPS were quantified according to Tay, Liu and Liu (2001). The protein fraction (PN) was determined using a modified Lowry method, and the polysaccharide fraction (PS) was measured using the phenol-sulfuric method (Long *et al*., 2014). To confirm the presence of microalgae in the system, chlorophyll *a* was quantified according to Tang *et al*. (2016).

Statistical analysis

A factorial design with response surface methodology and Pareto analysis was employed to evaluate the influence of light intensity and photoperiod on microalgal growth. Reactor performance comparisons were conducted using one-way ANOVA followed by Tukey's test (α = 0.05) to determine statistically significant differences in granulation-related parameters.

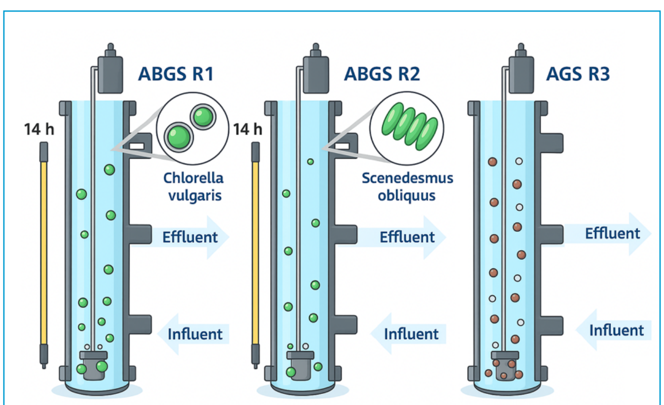
RESULTS AND DISCUSSION

Lighting condition screening for microalgae cultivation

For *C. vulgaris*, the highest OD was observed under a photoperiod of 14 h of light and a light intensity of 250 µmol·m^{-2}·s^{-1}. In contrast, for *S. obliquus*, the best growth conditions, also based on OD, were a photoperiod of 14 h of light and a light intensity of 150 µmol·m^{-2}·s^{-1} (Figure 2).

According to the Pareto chart, the effects of both individual and combined variables were positive for *C. vulgaris*, although not statistically significant. For *S. obliquus*, light intensity had a negative effect, while the photoperiod and combined effects were positive, although also not significant.

Given the negative effect of light intensity on *S. obliquus* (i.e., higher intensities reduced growth) and the low, non-significant impact of this variable on *C. vulgaris*, a light intensity of 150 µmol·m^{-2}·s^{-1} was selected for the



Source: Elaborated by the authors.

Figure 1 – Schematic of the reactors R1 (*C. vulgaris*), R2 (*S. obliquus*), and R3 (control, AGS without microalgae).

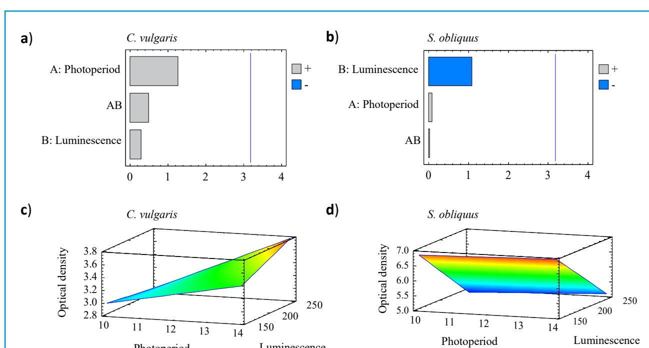


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Figure 2 – Pareto diagram for the independent variables and their combined effects for the microalgae species (a) *C. vulgaris* and (b) *S. obliquus* and response surface for the microalgae species (c) *C. vulgaris* and (d) *S. obliquus* cultivated in synthetic wastewater.

photogranulation experiment. This value aligns with previously reported optimal ranges for photogranule cultivation (Meng *et al.*, 2019; Zhang *et al.*, 2019; Sales *et al.*, 2022).

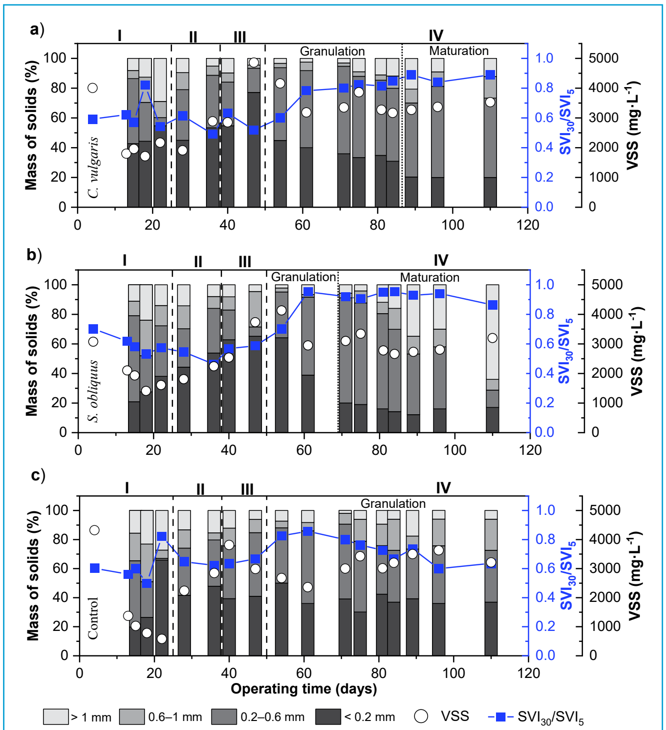
Regarding photoperiod, *C. vulgaris* performed best under prolonged illumination (14 h), whereas this parameter had no significant effect on *S. obliquus*. The response surface plots further supported that the best conditions for *C. vulgaris* occurred at higher light intensity and longer illumination.

Therefore, based on the combined analysis of the Pareto charts, response surface plots, and literature findings, within the experimental design's defined range, the selected lighting conditions for the photogranulation phase were 150 $\mu mol \cdot m^{-2} \cdot s^{-1}$ light intensity and a 14 h light/10 h dark photoperiod for both species.

Cultivation of algal-bacterial granular sludge

During the aerobic photogranulation process, it was observed that, although all systems were inoculated with the same amount of solids (5,000 mg $VSS \cdot L^{-1}$), the solid concentration decreased throughout Phase I, likely due to the washout of more flocculent sludge. By the end of this phase, with a settling time of 20 min, VSS concentrations reached 2,170, 1,610, and 518 mg $VSS \cdot L^{-1}$ in R1, R2, and R3, respectively. Previous studies indicate that the presence of algae in photogranule systems improves settleability (Zhang; Lei; Liu, 2022). Additionally, during Phase I, the granulation markers (granule size and the SVI_{30}/SVI_5 ratio) remained stable (Figure 3).

In Phases II and III, with settling times of 15 and 10 min, respectively, despite fluctuations in total suspended solids (TSS) concentration, there was a trend toward recovery of the biomass lost during Phase I. However, during these phases, the reactors did not reach granulation, suggesting a low selection pressure on the sludge. From Phase IV onward, with the settling time reduced to 5 min, the reactors showed stabilization in VSS concentration, reaching values of 3,185, 2,778, and 2,730 mg $VSS \cdot L^{-1}$ in R1, R2, and R3, respectively. A favorable trend toward granulation was also observed, evidenced by a decrease in the concentration of flocculent sludge with diameters smaller than 0.2 mm and an increase in the SVI_{30}/SVI_5 ratio in R1 and R2, achieving granulation on days 89 and 71, respectively. In contrast, R3 (control, AGS without microalgae) did not achieve granulation, suggesting that the presence of microalgae promoted this process.



Source: Elaborated by the authors.

Figure 3 – Variation of volatile suspended solids (VSS), sludge volumetric index (SVI_{30}/SVI_5), and granulometry of (a) R1 (*C. vulgaris*), (b) R2 (*S. obliquus*), and (c) R3 (control, AGS without microalgae) during the experimental phases.

This is further supported during maturation, when R1 and R2 reached percentages above 80% of biomass with a diameter > 0.2 mm and an SVI_{30}/SVI_5 ratio > 0.8. However, R3 remained at 60% of biomass with a diameter > 0.2 mm and an SVI_{30}/SVI_5 ratio < 0.8, values below the expected limit for biomass to be considered granular (de Kreuk; Kishida; van Loosdrecht, 2007).

It can also be noted that R2, inoculated with *S. obliquus*, not only reached granulation in a shorter time but also maintained higher levels of the SVI_{30}/SVI_5 ratio throughout Phase IV. Additionally, in Phase IV, R2 had more than 60% of the granules with a diameter > 1.0 mm, while R1 reached just over 30% and R3 < 10%.

The loss of sludge in the early phases was also reported by Purba *et al.* (2023), who evaluated the performance of ABGS in SBRs for real domestic wastewater treatment with 184 mg $COD \cdot L^{-1}$ in 6-h cycles, initially with 10 min of settling. This behavior can be considered expected due to the washout of the more flocculent sludge with low settleability.

With the same reactor configurations, operational cycles, and selection pressure strategy, Rollemberg *et al.* (2019b) and dos Santos *et al.* (2022), using CAS inoculum and three different carbon sources (acetate, glucose, and ethanol: 600 mg $COD \cdot L^{-1}$; acetate, propionate, glycerol, glucose, and sucrose: 750 to 1,000 mg $COD \cdot L^{-1}$, respectively), achieved success in the granulation process and, therefore, had different results from R3 in this study, which did not granulate. It is suggested that the success of granulation is not only dependent on operational conditions (selection pressure strategies) but also on the inoculum sludge, meaning the variety of the bacterial population is also crucial for the granulation process. Interestingly, under the same conditions, systems inoculated with microalgae strains (R1 and R2) were able to overcome this difficulty and achieve granulation, highlighting their importance in the process.

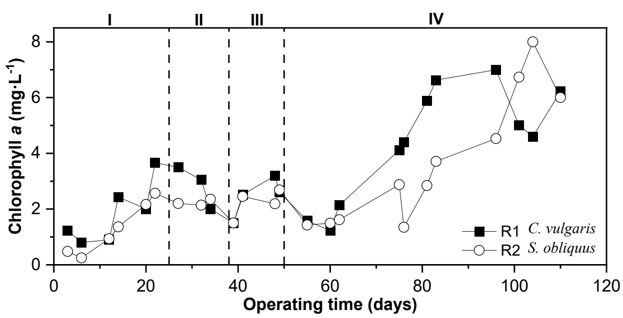
Chlorophyll *a* monitoring was conducted to assess microalgal growth and evaluate the influence of these microorganisms on other processes. During Phase I, there was a trend of growth, followed by stabilization until the end of Phase III. In Phase IV, a 200% increase was observed, from ~2.0 to 6.0 $mg \cdot L^{-1}$ in R1 and R2 (Figure 4).

Other studies have reported microalgal growth associated with photogranulation, such as Liu *et al.* (2023), who cultivated ABGS without algal inoculum, only with light stimulation (80 $\mu mol \cdot m^{2} \cdot s^{-1}$, 12 h light/12 h dark), and reported an increase in chlorophyll *a* concentration along with other granulation markers, such as granule size and SVI_{30}/SVI_5 ratio, corroborating the results presented in this study.

It is known that EPS are composed of polysaccharides, proteins, and other substances, which act as a glue for microbial aggregation, as well as for the formation and stability of granules (Zhang *et al.*, 2018). In the present study, all three reactors showed the same behavior throughout the granulation phases, reaching average EPS concentrations of 515, 512, and 466 mg EPS·g^{-1} VSS in R1, R2, and R3, respectively, with no statistical difference ($p > 0.05$).

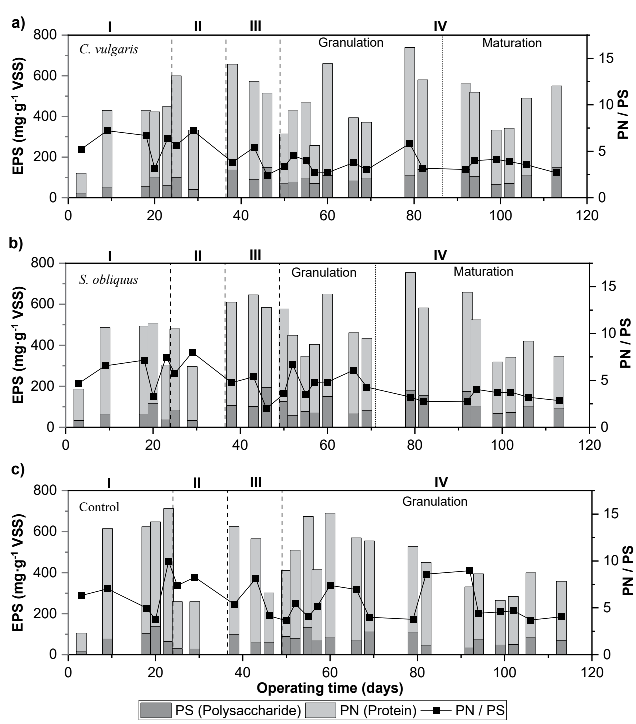
Despite the lack of statistical difference, the photogranule reactors (R1 and R2) had higher EPS concentrations, which may be linked to improved granulation performance (Figure 5). This is corroborated by studies showing that microalgae can produce EPS and that their interaction with bacteria enhances EPS secretion, favoring the formation of aerobic granular biofilms (He *et al.*, 2018).

It is also noted that the PN remained higher than the carbohydrate (polysaccharide) fraction throughout the study in all the three reactors. This behavior is commonly reported for aerobic granules (Silva; Rollemberg; dos Santos, 2021; Oliveira Júnior *et al.*, 2024) and photogranules (Wang *et al.*, 2022; Liu *et al.*, 2023), with the PN being the most abundant EPS component in more stable granules.



Source: Elaborated by the authors.

Figure 4 – Chlorophyll *a* concentration during the photogranulation process in reactors R1 (*C. vulgaris*) and R2 (*S. obliquus*).



Source: Elaborated by the authors.

Figure 5 – Evaluation of extracellular polymeric substances (EPS) concentration in reactors (a) R1 (*C. vulgaris*), (b) R2 (*S. obliquus*), and (c) R3 (control, AGS without microalgae) during the experimental phases.

The PN/PS ratio was also evaluated, and, like EPS production, it did not show any behavior associated with the presence of microalgae or phase changes, reaching values of 3.88, 4.28, and 5.14 in R1, R2, and R3, respectively. EPS production, as well as the PN and PS fractions, is influenced by various factors, such as reactor configurations, inoculum type, aeration time, cycle time, shear stress, and others (Silva; Rollemberg; dos Santos, 2021).

Other studies with ABGS report PN/PS ratios > 4.0 (Wang *et al.*, 2022; Liu *et al.*, 2023; Luo *et al.*, 2024), close to the values found in this study, as well as < 2.0 (Sales *et al.*, 2022; Xiong *et al.*, 2023). He *et al.* (2018) and Rollemberg, Ferreira and dos Santos (2022) report that a PN/PS ratio close to 1.0 results in a system with good stability. On the other hand, Zhang *et al.* (2007) suggest that a higher PN/PS ratio indicates that the granule surface has higher hydrophobicity and lower surface charge, which contributes to structural stability.

However, Rollemberg *et al.* (2019a), evaluating the influence of SBR on AGS production, with 6-h cycles and 700 mg COD·L^{-1}, achieved a PN/PS ratio close to 1.0, but with low granule stability, as indicated by a stability coefficient (S) that ranged from 28.5 to 41.8%. Similarly, Rollemberg *et al.* (2020), investigating the impact of cycle time on the formation, stability, and performance of AGS in SBR with 6-h cycles and a selection pressure strategy varying the settling time from 20 to 5 min, using 550 mg COD·L^{-1}, also achieved a PN/PS ratio close to 1.0, but with S > 20, ranging from 39.4 to 47.2%.

In the present study, with a PN/PS ratio higher than the references cited above, the granules also showed low stability, with S values of 38.1, 45.8, and 38.3% for R1, R2, and R3, respectively, highlighting that there is no consensus between the PN/PS ratio and granule stability.

Comparative characterization of mature granules with and without microalgae inoculation

Phase IV corresponds to the maturation stage of the granulation process and represents the most stable operational period in this study. To assess the influence of microalgae inoculation on the structural and compositional characteristics of the mature granules, a comparative analysis was performed among the three reactors: R1 (inoculated with *C. vulgaris*), R2 (inoculated with *S. obliquus*), and R3 (control, AGS without microalgae). The main granule parameters are summarized in Table 1.

R1, inoculated with *C. vulgaris*, exhibited slightly higher VSS and chlorophyll *a* concentrations compared to R2, suggesting greater biomass retention and microalgal growth. However, R2 achieved granulation in a shorter time and presented higher mean SVI_{30}/SVI_5 values and a greater proportion of granules > 0.2 mm, indicating more efficient granule formation even at lower algal biomass.

A statistically significant difference ($p \leq 0.05$) was observed between the microalgae-inoculated reactors (R1 and R2) and the control (R3) in terms of SVI_{30}/SVI_5 and the proportion of large granules, reinforcing the role of microalgae in promoting granule formation. Furthermore, the system with *S. obliquus* (R2) exhibited a significantly larger mean granule diameter ($p \leq 0.05$), which likely contributes to the establishment of stratified microenvironments with distinct oxygen concentrations, thereby supporting microbial diversity and the coexistence of different functional groups (Dababat *et al.*, 2024).

Table 1 – Summary of granule characteristics during Phase IV (maturation) in R1 (*C. vulgaris*), R2 (*S. obliquus*), and R3 (control, AGS without microalgae).

Parameter	Reactor		
	R1	R2	R3
VSS ($mg{\cdot}L^{-1}$)	3,271 ± 237	2,875 ± 245	3,111 ± 500
SVI_{30}/SVI_5	0.83 ± 0.11^a	0.90 ± 0.08^a	0.74 ± 0.10^b
Granules > 0.2 mm (%)	77 ± 21^a	84 ± 23^a	61 ± 15^b
Mean diameter (mm)	0.39 ± 0.06^b	0.48 ± 0.16^a	0.38 ± 0.04^b
PN ($mg{\cdot}g^{-1}$ VSS)	378 ± 116	371 ± 100	375 ± 115
PS ($mg{\cdot}g^{-1}$ VSS)	98 ± 27	86 ± 43	72 ± 28
PN/PS	3.7 ± 0.9	3.7 ± 1.2	4.63 ± 1.81
Chlorophyll *a* ($mg{\cdot}L^{-1}$)	4.3 ± 1.9	3.6 ± 2.2	-

Different superscript letters in the same row indicate statistically significant differences ($p \leq 0.05$).
Source: Elaborated by the authors.

CONCLUSIONS

The systems inoculated with *C. vulgaris* (R1) and *S. obliquus* (R2) exhibited enhanced granulation and greater system stability compared to the control system (R3), which was inoculated only with activated sludge and did not achieve granulation.

The presence of microalgae contributed to improved settleability and a higher proportion of larger granules, promoting the granulation process. Among the species tested, *S. obliquus* demonstrated superior performance, reaching granulation faster and producing a larger fraction of granules compared to *C. vulgaris* and the control.

These improvements are likely related to microalgae's production of EPS and their interaction with bacteria, which enhance granule cohesion and stability. This work highlights *S. obliquus* as a promising species to accelerate and improve aerobic photogranulation, contributing valuable knowledge for advancing microalgal-bacterial systems in wastewater treatment.

AUTHORS' CONTRIBUTIONS

Cordeiro, G.L.: Formal analysis, Investigation, Methodology, Writing––original draft. Freire, R.C.: Conceptualization, Formal analysis, Investigation, Writing––original draft. dos Santos, A.B.: Funding acquisition, Project administration, Resources, Writing––review and editing. Silva, M.E.R.: Conceptualization, Supervision, Writing––review and editing. Firmino, P.I.M.: Conceptualization, Funding acquisition, Project administration Supervision, Writing––review and editing.

DATA AVAILABILITY STATEMENT

The datasets generated and analyzed during the current study are presented within the article in the form of tables and figures.

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