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Assessing different microalgae species on the cultivation of microalgal-bacterial granular sludge

Freire, R. C.*, Cordeiro, G. L.*, dos Santos, A. B.*, Silva, M. E. R.** and Firmino, P. I. M.*

* Department of Hydraulic and Environmental Engineering, Federal University of Ceará, Ceará, Brazil
** Department of Civil Construction, Federal Institute of Education, Science and Technology of Ceará, Fortaleza, Ceará, Brazil.

Highlights:

- MBGS granulated earlier than the AGS.
- The removal of COD and N was greater in MBGS.
- MBGS with *Scenedesmus obliquus* granulated 18 days before that with *Chlorella vulgaris.*

Keywords: MBGS; microalgal-bacterial consortium; aerobic granules; AGS.

INTRODUCTION

In order to mitigate the negative impact of economic activities on aquatic ecosystems, effluents must be treated before disposal to properly remove pathogens, nutrients, and organic matter. Emerging sewage treatment technologies, such as aerobic granular sludge (AGS) and microalgal-bacterial granular sludge (MBGS), have been identified as some of the most promising solutions (Guo et al., 2024; Liu et al., 2023).

Unlike conventional activated sludge (CAS), AGS offers several advantages, such as a compact microbial structure, excellent settleability, resistance to high organic loads and toxic substances, and the ability to remove organic carbon and nutrients simultaneously (Guo et al., 2024). Additionally, granular sludge requires fewer unit operations for nutrient removal, leading to lower costs, reduced area requirements, lower greenhouse gas emissions, and decreased energy consumption. Meanwhile, MBGS technology possesses all these advantages along with additional benefits, including oxygen production, enhanced nutrient removal through algal absorption, pathogen elimination due to increased temperature, pH, and dissolved oxygen concentration, and the potential use of biomass as fuel (Liu et al., 2023). Therefore, these emerging technologies present favorable conditions for use in compact and decentralized systems.

These technologies have been extensively studied in recent years, however there are few studies comparing traditional aerobic granulation with that associated to microalgae, particularly considering different inoculated species. Therefore, this study compared aerobic granulation in the absence and presence of two species of microalgae, *Chlorella vulgaris* and *Scenedesmus obliquus*, to define the most efficient and viable conditions for designing wastewater treatment plants.

METHODOLOGY

Three 8-L sequencing batch reactors (R1, R2, and R3) were inoculated with sludge (5 g VSS/L) from a UCT system. R1 and R2 were also inoculated with 10^{10} cells of *Chlorella vulgaris* and *Scenedesmus obliquus*, respectively, for MBGS cultivation, whereas R3 was the control reactor for AGS cultivation (Liu et al., 2018). The illumination for the microalgal-bacterial reactors was provided by LED with an

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average intensity of 150 µmol·m^2/s, and the photoperiod was 14 h of light and 10 hours of dark. Aeration was provided by mini air compressors, ensuring a minimum dissolved oxygen concentration of 5 mg/L.

The reactors were fed with synthetic sewage containing 1 g COD·L^{-1} of propionic acid, 50 mg NH_4^+-N/L, 10 mg PO_4^{3-}-P/L, 5 mg Mg^{2+}/L, 10 mg Ca^{2+}/L, 1 mL/L of a micronutrient solution (Rollemberg et al., 2019), and 1 g $NaHCO_3$/L. They 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%. The settling time was gradually reduced from 20 to 5 minutes to impose selection pressure on biomass and promote granulation.

COD, pH, ammonia, and volatile suspended solids (VSS) were determined according to APHA (2012). Nitrite, nitrate, and phosphate were determined by ion chromatography (Rollemberg et al., 2019). Granulometry was determined using sieves with mesh openings of 0.2, 0.6, and 1.0 mm. Settleability was evaluated through volumetric sludge index at 5 (SVI_5) and 30 min (IVL_{30}). Extracellular polymeric substances (EPS) in the granular biomass were assessed by measuring the content of proteins (PN) and polysaccharides (PS) as described elsewhere (Long et al., 2014). Microalgal cell concentration was determined by chlorophyll a concentration (Tang et al., 2016).

RESULTS AND CONCLUSIONS

Regarding chlorophyll a, as shown in Figure 1, the reactor inoculated with *C. vulgaris* (R1) reached the peak of microalgal growth (on day 82) before the reactor with *S. obliquus* (R2) (on day 96). However, the maximum value of chlorophyll a in R1 was lower than that in R2. Furthermore, at the end of the maturation period, both MBGS presented similar values for this parameter (6 mg/L of chlorophyll a). Zhang et al. (2023), operating 6-L MBGS reactors in 4-hour cycles with a 50% VER and a light intensity of 150 µmol·m^2/s, observed a maximum chlorophyll a concentration of 5 mg/L during the maturation phase, which was lower than the 6 mg/L observed in the present study.

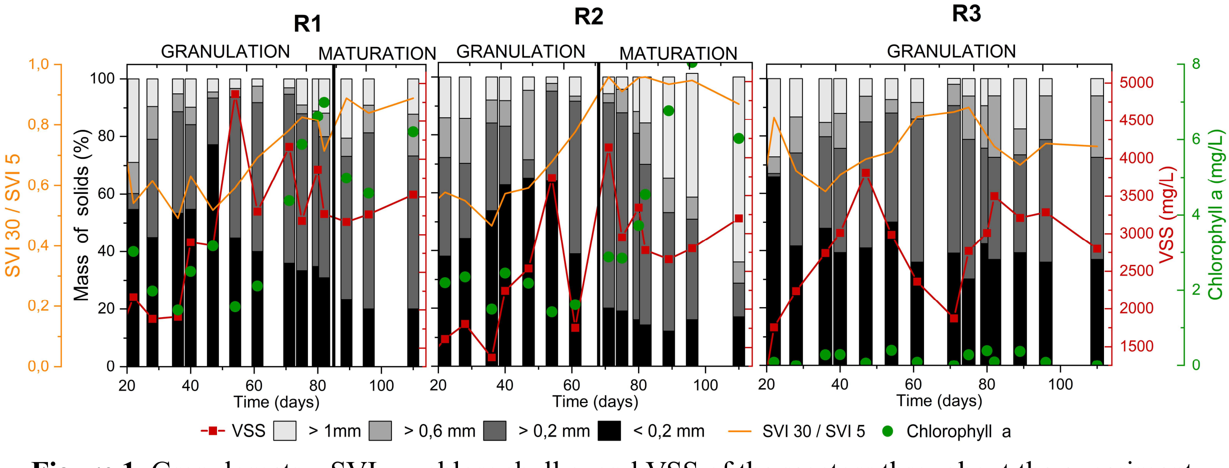

Figure 1. Granulometry, $SVI_{30/5}$, chlorophyll a, and VSS of the reactors throughout the experiment.

Granulation is achieved when the SVI_{30}/SVI_5 ratio is greater than 0.8, and less than 20% of the solid mass is smaller than 0.2 mm (De Kreuk et al., 2007). Accordingly, R1 granulated on day 89 (SVI_{30}/SVI_5: 0.89 and 20% of biomass < 0.2 mm), whereas R2 granulated on day 71 (SVI_{30}/SVI_5: 0.9 and 20% of biomass < 0.2 mm), predominating relatively small granules (diameter between 0.2 and 0.6 mm). Surprisingly, R3 did not granulate until day 110 (end of the study) (Figure 1). In contrast, Liu *et al*

(2018), operating a 2-L MBGS reactor (6-h cycles, 40% VER, and 12 h of light at ~150 µmol·m^2/s) inoculated with both *Chlorella* and *Scenedesmus* (10^8 cells), observed that most granules (~16%) reached a size of 1 mm.

After granulation, the MBGS reactors presented stable VSS values, with R1 reaching the highest average concentration (3.3 g VSS/L). As for the AGS reactor, the VSS values were stable from day 80 to 96 (3.2 g VSS/L). However, there was a decline to 2.8 g VSS/L by the end of the experiment (Figure 1).

Regarding COD removal, in the granulation phase, all systems exhibited high efficiencies, above 96%, with no significant differences between them, and, in the maturation phase, the MBGS systems had their efficiencies slightly improved (Table 1).

Parameter	**R1**		**R2**		**R3**
	Granulation	**Maturation**	**Granulation**	**Maturation**	**Granulation**
COD removal (%)	96.6 ± 3.3	98.1 ± 2.7	96.6 ± 3.1	97.4 ± 1.8	96.8 ± 3.0
N removal (%)	68.5 ± 4.9	74.6 ± 6.5	68.5 ± 6.9	74.2 ± 7.4	67.6 ± 13.8
P removal (%)	33.2 ± 9.8	17.4 ± 2.6	30.2 ± 6.5	20.5 ± 9.1	34.2 ± 12.2
EPS (mg/g VSS)	520 ± 264	508 ± 201	518 ± 229	501 ± 171	465 ± 166
PN/PS ratio	4.0	3.7	4.6	3.6	5.2

Table 1. Operational performance of the reactors throughout the experiment.

Considering N removal, the MBGS systems were similar and more efficient than the AGS system. All reactors showed a small increase in efficiency in the maturation phase (Table 1), whose values were higher than those found by Liu et al. (2018) (50%). Unlike N, P removal decreased with the maturation of the microalgal-bacterial granules (R1: from 33% to 17% and R2: from 30% to 20%), and the AGS reactor exhibited a higher P removal (Table 1). This low removal can be justified by oxygen production through photosynthesis in MBGS reactors. As P removal requires alternating anaerobic and aerobic periods, oxygen production during the anaerobic period may impair the metabolism of polyphosphate-accumulating organisms. Similarly, Liu et al. (2018) found low P removal (~35%) in a MBGS reactor.

Regarding the total EPS production, MBGS presented a higher value than AGS. However, the PN/PS ratio was higher in AGS. Liu et al. (2018) also observed this same trend of EPS production and PN/PS ratio. Despite this, the total EPS and PN/PS ratio in their both AGS and MBGS reactors (average values of ~200 g EPS/g VSS and 3.5, respectively) were lower than the values obtained in the present study (average values of 500 g EPS/g VSS and 3.9, respectively). The lower PS content compared to PN content (as indicated by the higher PN/PS ratio) observed in the present experiment suggests a less porous and denser structure, which may contribute to the formation of more compact granules with greater mechanical resistance (Hou et al., 2021).

In conclusion, microalgae augmentation improved granulation and operational performance of the granular systems, except P removal, with the best results being obtained by the reactor inoculated with *S. obliquus*.

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