

## Treatment of diluted landfill leachate using a granular microalgae–bacteria consortium

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### Abstract

Landfill leachate is a complex wastewater, characterized by high concentrations of organic matter, ammonia nitrogen and refractory compounds, requiring the development of efficient and sustainable treatment technologies. In this study, the performance of a granular microalgae-bacteria consortium was evaluated for the treatment of diluted landfill leachate under controlled phototrophic conditions. The consortium consisted of the microalgal species *Chlorella* sp., *Lobochlamys segnis* and *Phormidium* sp., combined with activated sludge-derived bacterial communities, and was cultivated in a laboratory-scale photobioreactor operated in sequencing batch mode using leachate diluted to 2% (v/v). The treatment process was evaluated by monitoring organic matter, nutrients, biomass development and color removal. The granular consortium achieved high organic matter removal efficiencies, with COD reductions ranging from 74–88% and BOD<sub>5</sub> removal reaching 95%. Ammonium removal increased progressively during operation, reaching 92%, while total phosphorus removal reached 72%. The absence of nitrite accumulation and the presence of nitrate suggested the occurrence of nitrification processes within the consortium. Chlorophyll *a* concentration increased from 8.05 to 24.43 mg L<sup>-1</sup>, indicating increased photosynthetic activity and successful adaptation of the biomass to leaching of deposits. Macroscopic observations revealed a continuous growth of granules, with diameters increasing from approximately 0.4 cm to over 1 cm. Despite the efficient removal of organic matter and nutrients, color removal remained limited (16–56%), highlighting the persistence of refractory humic substances. The results demonstrate that granular microalgae-bacteria biomass represents a promising and sustainable approach for treating landfill leachate, combining pollutant removal with biomass production and nutrient recovery potential.

**Keywords:** landfill leachate, microalgae-bacteria consortium, granular biomass, nutrient removal, colour index

### INTRODUCTION

Landfill leachate represents one of the most problematic wastewaters due to its high content of organic matter, ammoniacal nitrogen, phosphorus and refractory compounds such as humic and fulvic acids, which confer a characteristic dark colour and high turbidity [1]. Its composition varies considerably depending on landfill age, climatic conditions, precipitation regime and waste composition, making the selection of an appropriate treatment technology particularly challenging [2]. Mature landfill leachates are especially difficult to treat because they contain elevated concentrations of ammoniacal nitrogen and poorly biodegradable organic compounds, which reduce the effectiveness of conventional treatment processes and increase operational costs [3]. Reported concentrations may vary widely, with chemical oxygen demand (COD) ranging from 140 to 152,000 mg O<sub>2</sub>/L, biochemical oxygen demand (BOD<sub>5</sub>) from 20 to 57,000 mg O<sub>2</sub>/L, BOD<sub>5</sub>/COD ratios between 0.02 and 0.80, ammonium concentrations from 50 to 2,200 mg/L and total phosphorus concentrations from 0.1 to 23 mg/L [1]. Consequently, the removal of organic matter and nitrogen

compounds is generally considered a prerequisite before leachate discharge into natural water bodies [2].

Conventional leachate treatment strategies include co-treatment with municipal wastewater, aerobic and anaerobic biological processes, as well as physicochemical methods. However, these approaches are frequently associated with high operational costs, significant energy requirements and limited resource recovery potential [4]. Although physicochemical methods can effectively remove certain refractory compounds, they often require chemical reagents and generate secondary waste streams. Likewise, conventional biological processes may be inhibited by high ammonium concentrations and unbalanced nutrient ratios commonly encountered in mature landfill leachates [2, 5÷7]. The presence of emerging contaminants and persistent organic compounds further highlights the need for more robust and sustainable treatment technologies capable of operating under the highly variable conditions characteristic of landfill leachate [8]. Consequently, recent research has increasingly focused on biological approaches that combine pollutant removal with nutrient recovery and biomass production [9, 10].

Among these approaches, microalgae have attracted considerable attention due to their ability to utilize sunlight and dissolved nutrients for biomass growth while simultaneously fixing carbon dioxide and removing nitrogen and phosphorus compounds from wastewater [11, 12]. Depending on the species and cultivation conditions, microalgae can grow under autotrophic, mixotrophic, or heterotrophic modes, which enhances their adaptability to wastewater environments characterized by variable nutrient availability and organic matter content [13, 14]. In addition to wastewater treatment, the resulting biomass can be valorised for the production of biofuels, biofertilizers, bioplastics and other value-added products, making microalgal technologies compatible with circular economy principles [9, 11, 15].

Several studies have demonstrated the potential of microalgae for landfill leachate treatment. A recent study published in 2024 reported that a mixed microalgal consortium dominated by *Chlorella* achieved removal efficiencies of 98.81% for  $\text{NH}_4^+\text{-N}$ , 88.62% for total nitrogen and 99.55% for total phosphorus within seven days under mixotrophic conditions. The process generated 1.18 g/L biomass and exhibited a favourable carbon balance, highlighting the potential of microalgae-based systems as environmentally sustainable treatment alternatives [10]. A *Chlorella pyrenoidosa*-based algal–bacterial consortium required an initial acclimation period when exposed to landfill leachate, after which high ammonium removal efficiencies were achieved at optimal concentrations [16]. Similarly, *Chlorella* sp. showed successful growth in landfill leachate while contributing to organic matter and ammonium removal [17]. These studies indicate that microalgae can tolerate and treat landfill leachate under a wide range of operating conditions, although treatment efficiency remains strongly dependent on leachate composition and cultivation conditions. The performance of microalgal treatment systems is strongly influenced by environmental and operational parameters, including light intensity, photoperiod, temperature, pH, carbon dioxide availability and inoculum concentration [18]. These factors directly affect biomass growth, photosynthetic activity and nutrient removal efficiency. Their importance becomes even more pronounced in landfill leachate treatment, where nutrient ratios are often unbalanced and phosphorus frequently acts as a limiting nutrient. Consequently, optimizing cultivation conditions is essential to ensure process stability and maximize treatment performance [19].

In recent years, increasing attention has been directed toward algal–bacterial systems that exploit the complementary metabolic activities of both microbial groups. Compared with microalgal monocultures, these systems can improve pollutant removal efficiency, reduce aeration requirements and enhance biomass recovery [20].

Their effectiveness relies on the synergistic interaction between bacteria and microalgae, where bacteria contribute to organic matter degradation and nutrient transformation, while microalgae assimilate nutrients and provide oxygen through photosynthesis [20÷22]. A symbiotic system composed of *Chlorella vulgaris* and activated sludge achieved removal efficiencies exceeding 96% for COD and 97% for ammonium nitrogen, highlighting the potential of algal–bacterial consortia for sustainable landfill leachate treatment [20]. In another recent study conducted using undiluted landfill

leachate, a locally adapted microalgae–bacteria consortium was able to grow under conditions of very high ammonium concentrations, with phosphate availability rather than ammonia toxicity identified as the main limiting factor. The inoculated cultures also exhibited significant reductions in phosphate, nitrite, and nitrate concentrations, suggesting a functional interaction between microalgae and nitrifying bacteria. [19, 23].

An additional advantage of algal–bacterial systems is the possibility of developing granular biomass structures. Granular algal–bacterial aggregates offer improved biomass retention, enhanced settling properties and more efficient solid–liquid separation compared with dispersed suspended cultures. Such characteristics may contribute to increased operational stability and facilitate biomass harvesting, which remains one of the major challenges associated with microalgal wastewater treatment technologies [3, 19].

In this context, the present study aimed to evaluate the performance of a granular microalgae–bacteria consortium for the treatment of diluted landfill leachate under phototrophic conditions. Particular attention was given to the removal of organic matter, ammoniacal nitrogen and phosphorus, as well as to biomass development, chlorophyll-a dynamics and colour removal. In addition, the study investigated the evolution of granular biomass structure during operation and the persistence of humic substances responsible for the characteristic colour of landfill leachate. The results contribute to the development of sustainable biological treatment strategies that combine pollutant removal, nutrient recovery and biomass production.

## MATERIALS AND METHODS

### *Experimental setup*

Experiments were conducted in a laboratory-scale stirred photobioreactor (Sartorius Stedim Biotech) with a total volume of 4 L and a working volume of 1.5 L. The reactor was equipped with a Rushton turbine impeller and online monitoring probes for pH, temperature, and dissolved oxygen (DO). Continuous mixing was maintained at 150 rpm to ensure homogeneous conditions throughout the reactor. The reactor was operated in sequencing batch mode throughout the entire experimental period, including biomass cultivation, granule formation and landfill leachate treatment phases. The reactor operated with a 24 h cycle, including a reaction phase of 23 h and 35 min, while feeding, settling and discharge phases were carried out during the remaining 25 min of each cycle.

Temperature was controlled at  $25 \pm 1$  °C, while illumination was provided under a 12 h light/12 h dark photoperiod. Light intensity was adjusted between 360 and 467  $\mu\text{mol m}^{-2} \text{s}^{-1}$  depending on the operational phase. The pH was maintained within the range of 6.5–8.5 using 0.5 N KOH and 9.66%  $\text{H}_3\text{PO}_4$ . During the leachate treatment phase, 1 N  $\text{H}_2\text{SO}_4$  was additionally used when required to maintain stable operating conditions.

### *Characteristics of the microbial inoculum and landfill leachate*

The phototrophic inoculum consisted of three microalgal species: the unicellular green microalgae *Chlorella* sp. and *Lobochlamys segnis*, and the filamentous cyanobacterium *Phormidium* sp. *Chlorella* sp. and *Phormidium* sp. were obtained from the culture collection of the National Research and Development Institute for Industrial Ecology (ECOIND, Romania), while *Lobochlamys segnis* was obtained from the culture collection of the Norwegian Institute of Bioeconomy Research (NIBIO, Norway).

Landfill leachate was collected from a municipal solid waste landfill in Romania, specifically from the Integrated Municipal Waste Management Center (CMID) Bălteni, Olt. The raw leachate exhibited high organic and nitrogen loads, with COD values of approximately 4000 mg  $\text{O}_2 \text{L}^{-1}$ , ammonium concentrations of approximately 1515 mg  $\text{L}^{-1}$ , total Kjeldahl nitrogen concentrations of approximately 2015 mg  $\text{L}^{-1}$ , and a color index (CN) of 3.4. The elevated CN value reflects the presence of humic and fulvic substances typically associated with mature landfill leachates. Considering the high ammonium concentration, dark colour and elevated organic load of the raw landfill leachate, a dilution of 2% (v/v) with standardized synthetic municipal wastewater was selected based on previously reported microalgae-based leachate treatment studies. The selected

dilution aimed to reduce inhibitory effects while maintaining sufficient nutrient availability for biomass development and treatment performance. Activated sludge collected from the municipal wastewater treatment plant of Buftea (Romania) was used as bacterial inoculum for the establishment of the algal-bacterial consortium.

#### *Biomass cultivation and granulation*

The granulation procedure was performed according to the Romanian patent RO 130247 B1 [24], with the aim of obtaining a stable microalgae–bacteria consortium characterized by self-sustained O<sub>2</sub>/CO<sub>2</sub> exchange and high settling capacity. Initially, the microalgal culture was grown under controlled phototrophic conditions in standardized synthetic municipal wastewater to establish a stable biomass concentration. Initially, the microalgae were cultivated in standardized synthetic municipal wastewater prepared with distilled water supplemented with macro- and micronutrients, including NH<sub>4</sub>Cl, K<sub>2</sub>HPO<sub>4</sub>, MgSO<sub>4</sub>·7H<sub>2</sub>O, CaCl<sub>2</sub>, FeSO<sub>4</sub>·7H<sub>2</sub>O, KNO<sub>3</sub>, NaHCO<sub>3</sub>, and trace elements. After 14 days of operation, activated sludge was introduced into the reactor to establish a mixed microalgae–bacteria consortium. The reactor continued to operate using the same standardized synthetic municipal wastewater, supplemented with sodium acetate as an organic carbon source to support the development of the microbial consortium. Granular biomass formation was promoted through repeated settling–selection cycles, which acted as a hydrodynamic selection pressure favoring the retention of compact and fast-settling aggregates. The settling time was gradually reduced from 30 min to 5–10 min as granule stability improved, resulting in the progressive enrichment of dense microalgal–bacterial granules.

Following activated sludge inoculation, the reactor continued to operate for an additional two weeks using the same standardized synthetic municipal wastewater supplemented with sodium acetate as an organic carbon source. This period allowed the establishment and stabilization of the microalgae–bacteria consortium, granule formation, and gradual adaptation of the microbial community before the introduction of diluted landfill leachate.

#### *Leachate treatment operation*

After stabilization of the microalgae-bacteria granules, diluted landfill leachate treatment was initiated. A total of 30 treatment cycles were performed. Diluted landfill leachate (2%, v/v), prepared using standardized synthetic municipal wastewater, was introduced into the reactor after stable operating conditions had been established. The resulting influent exhibited COD concentrations ranging from 430 to 570 mg O<sub>2</sub> L<sup>-1</sup>, ammonium concentrations between 50 and 60 mg L<sup>-1</sup>, and phosphate concentrations of up to 16 mg L<sup>-1</sup>.

Throughout operation, periodic analyses were performed to evaluate treatment performance, biomass growth, biomass harvesting efficiency, and the morphological evolution of the algal-bacterial granules. In addition, color index reduction and nutrient removal were monitored to assess reactor stability and treatment efficiency.

#### *Analytical methods*

System performance was evaluated through standard physicochemical and biological analyses. Chemical oxygen demand (COD), biochemical oxygen demand (BOD<sub>5</sub>), pH, ammonium (NH<sub>4</sub><sup>+</sup>), nitrate (NO<sub>3</sub><sup>-</sup>), nitrite (NO<sub>2</sub><sup>-</sup>), phosphate (PO<sub>4</sub><sup>3-</sup>), and total Kjeldahl nitrogen (TKN) were determined according to ISO 6060:1996, EN 1899-2:2002, ISO 10523:2012, EN ISO 10304-1:2009 and EN 25663:2000, respectively.

Pollutant removal efficiency was calculated according to Equation (1):

$$RE(\%) = (C_i - C_f) / C_i * 100 \quad (1)$$

where C<sub>i</sub> and C<sub>f</sub> represent influent and effluent concentrations, respectively.

Effluent color was monitored spectrophotometrically at 436 nm, 525 nm and 620 nm after filtration to remove suspended biomass. The color index (CN) was calculated according to [25] using Equation (2):

$$CN = (SAC_{436}^2 + SAC_{525}^2 + SAC_{620}^2) / (SAC_{436} + 5AC_{525} + 5AC_{620}) \quad (2)$$

where SAC represents the spectral absorbance coefficient ( $m^{-1}$ ), calculated as the ratio between absorbance and optical path length.

Biomass concentration was determined gravimetrically as total suspended solids (TSS) and volatile suspended solids (VSS). Chlorophyll-a concentration was determined spectrophotometrically according to ISO 10260:1996 after extraction with 90% ethanol. Microalgal biomass was estimated from chlorophyll-a concentration using a conversion factor of 67 mg algal biomass per mg chlorophyll-a. Bacterial biomass was calculated as the difference between volatile suspended solids and the estimated microalgal biomass. The microalgae-to-bacteria ratio was estimated according to Equation (3):

$$\text{Microalgae/Bacteria ratio} = \text{Microalgal biomass} / \text{Bacterial biomass} \quad (3)$$

The physiological state index (R) was calculated from the absorbance values obtained during chlorophyll-a determination and was used as an indicator of biomass physiological status throughout the experimental period.

The specific growth rate ( $\mu$ ) was calculated according to Equation (4):

$$\mu = [\ln(N_2/N_1)] / (t_2 - t_1) \quad (4)$$

where:  $\mu$  - specific growth rate;  $N_1$  - initial biomass concentration;  $N_2$  - final biomass concentration and  $t$  is time (days).

Macroscopic observations were performed periodically to evaluate granule morphology and structural development throughout the experiment.

As this study represents a laboratory-scale feasibility assessment of a granular microalgae–bacteria consortium for landfill leachate treatment, the reactor was operated as a single experimental unit. Therefore, the reported values correspond to individual measurements obtained during reactor operation, and no statistical analyses were performed. Data processing and graphical representation were carried out using Microsoft Excel.

## RESULTS AND DISCUSSION

### *Cultivation phase performance*

The successful establishment of the microalgae-bacteria consortium during the cultivation phase was essential to ensure process stability before the introduction of leachate. The high COD removal efficiency observed at this stage (93.83% for unfiltered samples and 95.84% for filtered samples) indicates the efficient coupling between heterotrophic bacterial degradation and nutrient assimilation by microalgae. Similar observations have been reported for mixed algal-bacterial systems treating municipal wastewater, where the presence of microalgae increases oxygen availability and reduces aeration requirements [21]. The biomass concentration obtained in the present study ( $0.539 \text{ g L}^{-1}$ ) was within the range reported for algal-bacterial systems grown under nutrient-rich wastewater conditions [11]. Also, the chlorophyll-a concentration ( $4.5235 \text{ mg L}^{-1}$ ) demonstrated that the phototrophic fraction was well established before exposure to leachate, reducing the risk of inhibition after switching to a more complex wastewater matrix.

### *Reactor conditions during leachate treatment*

A clear shift in reactor behavior was observed after transitioning to leachate treatment. Dissolved oxygen concentration dropped to 0% at the beginning of each batch cycle and remained at this level for  $1.45 \div 7.27$  h, depending on the organic loading rate. The subsequent increase in dissolved oxygen concentration above saturation demonstrates that the microalgal fraction remained metabolically active despite the presence of leachate-derived contaminants. This finding suggests that dilution of the leachate successfully reduced inhibitory effects and allowed the establishment of a balanced algal–bacterial consortium. The selection of a 2% (v/v) dilution proved adequate for maintaining photosynthetic activity and granule stability throughout the experiment, while still providing a measurable pollutant load representative of landfill leachate. Stable pH values ranging from 7.4 to 8.5 further indicated good process resilience and effective biological adaptation throughout the experimental period.

### Organic matter and nutrient removal performance

The COD removal efficiencies obtained in this study ranged from 73.8% to 87.7% for unfiltered samples and 74% to 88.5% for filtered samples. These results demonstrate the ability of the granular microalgae-bacteria consortium to degrade a substantial fraction of the organic load present in the diluted leachate (

Table I). The COD removal efficiencies obtained in the present study were higher than those reported by El Ouaer et al [17], who achieved a maximum COD removal of approximately 60% during the treatment of Tunisian landfill leachate using *Chlorella sp.* cultivated in a medium containing 10% leachate. The higher COD removal observed in the present work may be attributed to the synergistic interactions established within the granular microalgae–bacteria consortium, where heterotrophic bacteria contributed to organic matter degradation while microalgae supported oxygen production and nutrient assimilation.

Ammonium removal efficiency increased progressively from approximately 72% to 92% during operation, indicating a gradual adaptation of the consortium to the nitrogen-rich leachate matrix. Although influent ammonium concentration doubled compared to the cultivation phase (reaching ~60 mg L<sup>-1</sup>), effluent concentrations decreased steadily to 4.75±16.9 mg L<sup>-1</sup>. Similarly, ammonium removal efficiencies reached up to 92.1% in the present study, which is comparable to the 90% NH<sub>4</sub><sup>-</sup>-N removal reported by El Ouaer et al [17]. These findings confirm the capacity of microalgal systems to efficiently remove ammoniacal nitrogen from landfill leachate and suggest that the integration of bacteria within granular structures may further enhance process stability and treatment performance. The absence of nitrite accumulation and the presence of nitrate in the effluent suggest that nitrification occurred within the consortium. Given how quickly the microalgae came to dominate the system, biomass assimilation likely served as the primary nitrogen sink, rather than a complete nitrification-denitrification pathway. In contrast, total nitrogen removal remained relatively limited. This behavior may indicate the presence of refractory organic nitrogen compounds associated with humic substances, which are known to persist during biological treatment.

**Table 1.** Physical-chemical characteristics of diluted landfill leachate before and after treatment using the granular microalgae–bacteria consortium

Parameter, measure unit	Influent (diluted leachate)	Effluent	NTPA 001***	Removal, %
BOD, mg O <sub>2</sub> L <sup>-1</sup>	358÷500	24.5 mg	20.0	95.1
COD, mg O <sub>2</sub> L <sup>-1</sup>	430÷570*	70.4÷150*	125	73.8÷87.7*
	336÷440**	44 ÷114**		74÷88.5**
NH <sub>4</sub> <sup>-</sup> , mg L <sup>-1</sup>	59.7	4.75 (16.9)	2.00 (3.00)	71.7÷92.1
NO <sub>3</sub> <sup>-</sup> , mg L <sup>-1</sup>	0.5 0	0.01÷2.30	75.0	-
NO <sub>2</sub> <sup>-</sup> , mg L <sup>-1</sup>	< 0.10	< 0.10	2.00	-
PO <sub>4</sub> <sup>3-</sup> , mg L <sup>-1</sup>	15.3	8.40	-	45.2
Total Nitrogen, mg L <sup>-1</sup>	69.7÷88.9*	55.9*	10.0	37.0*
	69.1÷83.5**	31.3**		62.5**
Total Phosphorus, mg L <sup>-1</sup>	5.2	1.50	2.00	71.9

\* Filtered samples

\*\* Homogenized samples

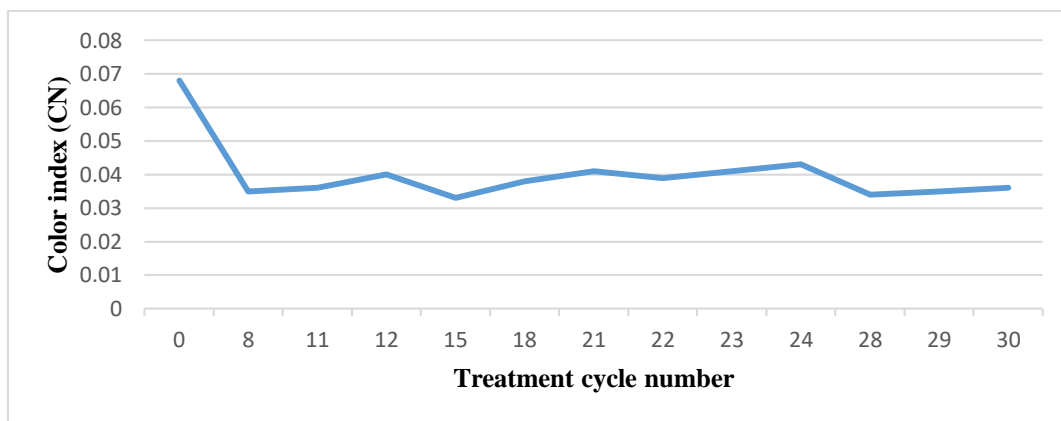
\*\*\*Romanian Regulation on establishing pollutant load limits for industrial and urban wastewater when discharged into natural receptors, NTPA-001/2002, of 28.02.2002.

Phosphate removal was lower than ammonium removal, reaching 45.2%. Such behavior may reflect intracellular phosphorus storage and release mechanisms associated with changes in metabolic activity during light and dark phases.

### Color removal and refractory organics

Although substantial COD reductions were achieved, color removal remained relatively low, indicating that the biological process preferentially targeted the biodegradable organic fraction of the leachate. The color index (CN) was monitored to evaluate the effectiveness of the biological process

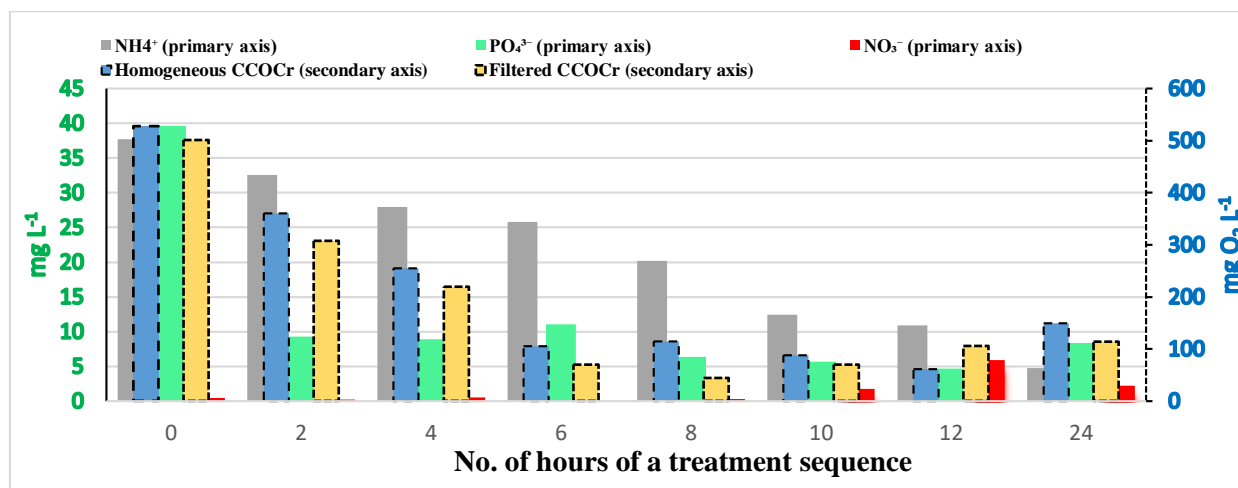
in reducing the color of the diluted leachate (CN = 0.068, Fig. 1), with the CN value in the effluent varying between 0.033 and 0.043, with a resulting CN removal efficiency ranging from 16 to 56%. The persistence of the brown color suggests that humic and fulvic substances, which are major components of mature landfill leachate, were only partially removed. Humic compounds are known to occupy complex aromatic structures that are resistant to biodegradation and can reduce light penetration in phototrophic systems. Consequently, their persistence can negatively affect microalgal productivity and limit the overall treatment performance [2]. The moderate color removal achieved in the present study therefore highlights both the potential and limitations of microalgae-bacterial treatment systems and suggests that additional steps may be required when color removal is a regulatory requirement.



**Fig. 1.** Evolution of the effluent color index (CN) during landfill leachate treatment

#### *Dynamics of a single treatment cycle*

Monitoring a complete treatment cycle provided valuable information on the temporal dynamics of nutrient transformation within the granular consortium. The rapid decrease in COD concentrations, approximately 89% after 12 h, and ammonium during the light phase indicate active degradation of organic matter, coupled with nutrient assimilation (Fig. 2). The temporary increase in nitrate concentration observed during the cycle suggests that nitrification occurred simultaneously with ammonium consumption. However, the subsequent decrease in nitrate concentration may indicate uptake by microalgae and possibly denitrification processes occurring in the oxygen-limited regions of the granules. The oscillatory behavior observed for phosphate is consistent with biological mechanisms of phosphorus storage and further supports the existence of complex microbial interactions within the granules. Despite this variability, total phosphate removal reached approximately 88%.



**Fig. 2.** Temporal evolution of organic matter and nutrient concentrations during a 24-h landfill leachate treatment cycle

### *Biomass growth, composition and physiological status*

The biomass accumulation during the treatment phase indicated that the microalgae-bacteria consortium successfully adapted to the diluted landfill leachate and maintained active growth throughout the experiment. The maximum biomass concentration was recorded on day 8, reaching 1.795 g of dry solids, of which 1.6071 g corresponded to volatile solids and only 0.1878 g to the mineral fraction. The calculated specific growth rate of 0.0715 days<sup>-1</sup> further confirmed the consortium's ability to utilize the nutrients present in the leachate and sustain biomass production. Similar to previous findings [20], the present study demonstrated successful adaptation of the algal-bacterial consortium to landfill leachate conditions. The increase in chlorophyll-a concentration and the progressive enlargement of granules confirmed active biomass growth and stable consortium development throughout operation. The increase in chlorophyll-a concentration from 8.05 to 24.43 mg L<sup>-1</sup> demonstrated that the biomass increase was accompanied by increased photosynthetic activity. This observation suggests that, despite the presence of potentially inhibitory compounds, the diluted leachate provided sufficient nutrients to support the growth of the microalgal fraction. The progressive increase in the microalgae-to-bacteria ratio, from 1.2 to 12.9, further indicates a shift towards phototrophic dominance within the consortium, highlighting the ability of the microalgae to successfully compete under the applied operating conditions. Further evidence of consortium adaptation was provided by the physiological condition index (R). The initial decrease from 1.65 to 1.51 in the first week suggests a temporary stress response following exposure to real landfill leachate (Table 2). However, the subsequent recovery of the index indicates successful biomass acclimation and restoration of physiological activity, demonstrating the resilience of the algal-bacterial consortium. Although the biomass increase remained significant, a decrease in the total biomass concentration was observed after day 8. This behavior coincided with the appearance of larger and less compact granules, which probably reduced aggregate density and structural stability. Such morphological changes could have promoted the detachment and washing of the biomass, explaining the increased chlorophyll-a concentrations measured in the effluent during the later stages of operation. The low chlorophyll-a concentrations detected in the effluent during most of the experiment, however, indicate that the granular structure ensured efficient biomass retention and limited losses of phototrophic biomass.

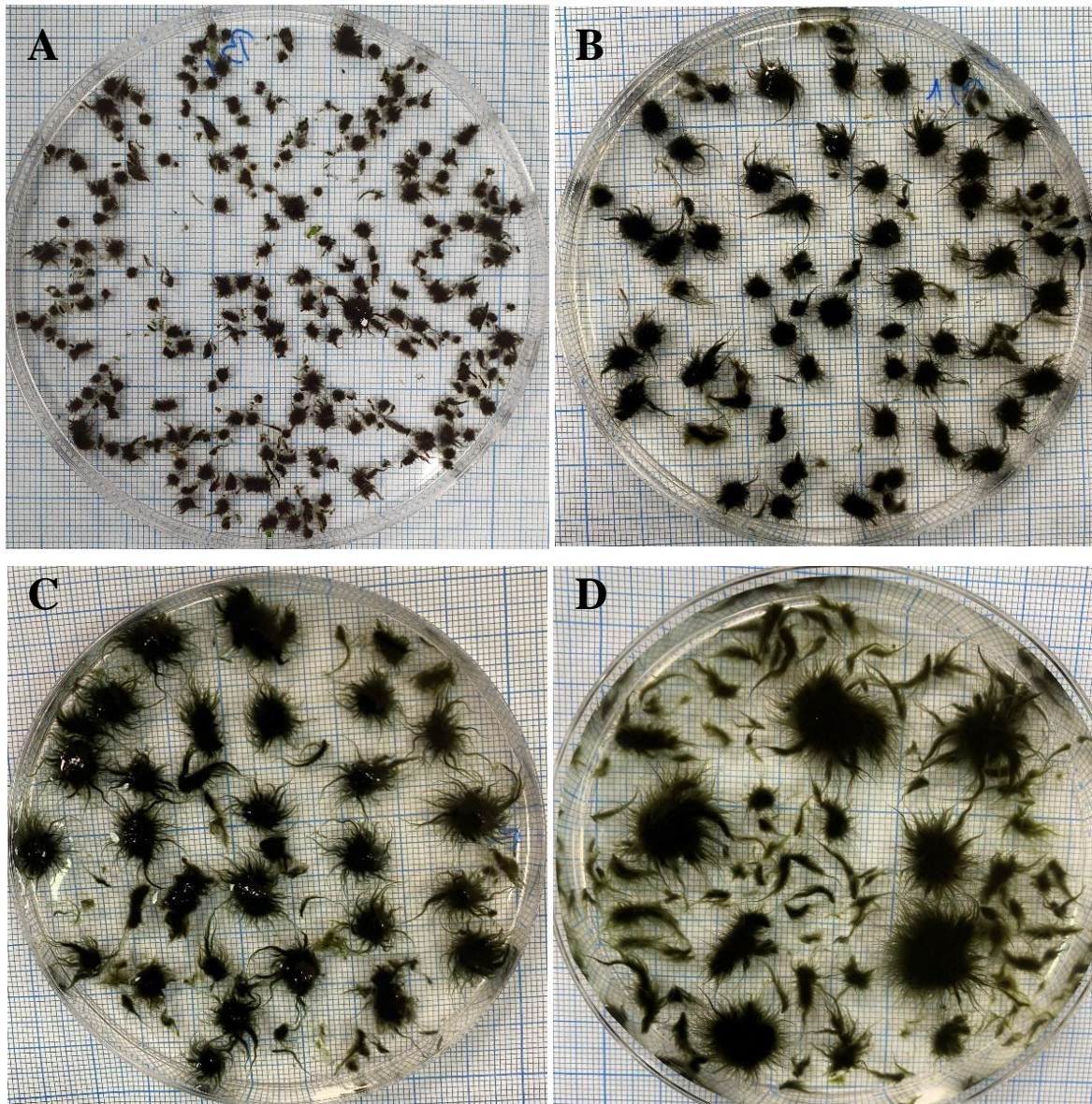
**Table 2.** Dynamics of biomass physiological and compositional parameters during landfill leachate treatment

Day	Chlorophyll-a (mg L <sup>-1</sup> )	Chlorophyll-a content (mg g <sup>-1</sup> )	Physiological state index (R)	Microalgae/Bacteria ratio (g g <sup>-1</sup> )
1	8.05	8.07	1.65	1.20
3	17.0	10.3	1.61	2.20
8	24.4	13.6	1.51	10.3
24	11.0	13.9	1.55	12.9
30	10.1	12.5	1.65	-

### *Granule morphology evolution*

Macroscopic observations revealed a continuous increase in granule size throughout the treatment period, indicating successful biomass growth and aggregation under landfill leachate conditions. The initial granules, with diameters of approximately 0.4 cm (Fig. 3A), progressively increased in size, with some aggregates exceeding 1 cm by the end of the experiment (Fig. 3D). This enlargement suggests active biomass accumulation supported by the organic matter and nutrients available in the diluted leachate.

As granule growth progressed, an increasingly heterogeneous size distribution became evident. In addition to larger compact aggregates, elongated filamentous structures were observed attached to several granules. The formation of these structures may reflect changes in microbial community organization and aggregation behaviour during adaptation to the complex composition of landfill leachate.



**Fig. 3.** Macroscopic evolution of granular microalgae–bacteria biomass during landfill leachate treatment: day 1 (A), day 14 (B), day 22 (C) and day 30 (D)

The development of larger granules is advantageous from an operational perspective, as it improves biomass retention and facilitates solid-liquid separation. However, the increasing heterogeneity observed during the later stages of operation may indicate the onset of structural instability, which could contribute to biomass detachment and washout. These observations are consistent with the temporary increase in suspended biomass and chlorophyll-a concentrations detected in the effluent towards the end of the experimental period. The observed morphology confirms the ability of the consortium to maintain a granular structure throughout the treatment period.

## CONCLUSIONS

The results obtained in this study demonstrate that granular microalgae–bacteria consortia can be successfully adapted to diluted landfill leachate and maintained under stable phototrophic operating conditions. The development and persistence of granular biomass throughout the experimental period confirmed the suitability of this biological configuration for the treatment of complex wastewaters characterized by elevated nutrient concentrations.

The observed treatment performance highlights the importance of the synergistic interactions established between microalgae and bacteria within the granules. These interactions supported the transformation and assimilation of nutrients while simultaneously promoting biomass production and

maintaining process stability. The progressive shift towards microalgal dominance further indicates the capacity of the phototrophic fraction to acclimate to landfill leachate and remain metabolically active throughout operation.

Granule formation provided important operational benefits by improving biomass retention and facilitating solid–liquid separation, addressing one of the main limitations associated with conventional suspended microalgal cultures. However, the limited reduction of the color index suggests that humic and fulvic substances remained largely unaffected by the biological treatment and may require complementary treatment processes.

Overall, the study supports the application of granular microalgae–bacteria systems as a sustainable approach for landfill leachate management, combining wastewater treatment with biomass generation. The produced biomass may represent a valuable resource for further valorisation, contributing to circular bioeconomy strategies and resource recovery within wastewater treatment systems.

### AI Use Statement

We used an AI assistant (ChatGPT) to support sentence structure correction, improve grammar, and enhance clarity during manuscript preparation. All AI-generated suggestions were carefully reviewed, and all final editorial decisions were made by the authors. We confirm that the content, interpretations, and any remaining errors remain solely the responsibility of the authors.

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