Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Integrated cultivation of Euglena gracilis in urban wastewater: pilot-scale evaluation of organic matter removal, carbon capture and biomass valorization

Betina Mariela Barreto
University of Santa Cruz do Sul
Maurício Kersting
University of Santa Cruz do Sul
Carlos Eduardo Flores Santos
University of Santa Cruz do Sul
Francielle Pasqualotti Meinhardt
University of Santa Cruz do Sul
Camille Vitória da Rosa
University of Santa Cruz do Sul
Camila Rafaela Rathke
University of Santa Cruz do Sul
Gabriela Bertol
University of Santa Cruz do Sul
Patrik Gustavo Wiesel
University of Santa Cruz do Sul
Giséle Alves
University of Santa Cruz do Sul
Rosana de Cássia de Souza Schneider
University of Santa Cruz do Sul
Carlos Alexandre Lutterbeck
carlosalexandre@unisc.br

University of Santa Cruz do Sul

Research Article

Keywords: Euglena gracilis, urban wastewater, phycoremediation, biomass valorization, circular bioeconomy, carbon capture

Posted Date: March 9th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-8949330/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: No competing interests reported.

Abstract

This study evaluated the cultivation of *Euglena gracilis* in real urban wastewater as a strategy for organic matter removal, carbon capture, and biomass valorization. Cultivation was conducted at laboratory and pilot scales using different proportions of urban wastewater and a mineral nutrient medium as control. *E. gracilis* successfully adapted to the wastewater from a university wastewater treatment plant, showing growth kinetics at pilot scale comparable to laboratory-scale systems. High removal efficiencies were achieved for chemical oxygen demand (up to 98.0%) and ammoniacal nitrogen (up to 91.6%), demonstrating the potential of this organism for organic load reduction.

Cultivation in undiluted wastewater enhanced carbon incorporation into the biomass, reaching values above 43%, indicating strong carbon assimilation under mixotrophic conditions. The biochemical composition of the biomass varied according to the culture medium, with a clear metabolic shift from protein-rich biomass in mineral medium to carbohydrate accumulation (up to 40.8%) in wastewater-based cultures. These results demonstrate the feasibility of integrating *E. gracilis* cultivation into urban wastewater systems at pilot scale, combining organic matter removal with carbon capture and the generation of biomass with biotechnological potential.

1. Introduction

The advance of urbanization and industrialization, combined with population growth, has led to a significant increase in the generation of urban wastewater (Sadigov 2022). When inadequately managed, these effluents cause severe environmental impacts, such as the eutrophication of water bodies and the degradation of ecosystems (Li et al. 2023b; Hait et al. 2024). This scenario highlights the urgent need to develop efficient and sustainable methods for wastewater treatment, making this issue a global priority due to the necessity of mitigating environmental impacts and preserving water resources (Khan et al. 2022; Obaideen et al. 2022).

Recent studies have demonstrated the effectiveness of physicochemical and hybrid approaches based on iron-derived materials for wastewater treatment. Falyouna et al. (2022) reported that zero-valent iron nanoparticles deposited on titanium nanowires achieved high adsorption efficiency for the removal of ciprofloxacin from aqueous solutions, highlighting the importance of nanostructured supports. Eljamal et al. (2024) showed that the combined use of iron nanoparticles and *Aloe vera* biomass significantly enhanced biogas production and process stability during anaerobic digestion of waste sludge. In addition, Maamoun et al. (2023) demonstrated effective removal of phosphorus and nitrate from aqueous solutions through statistical optimization of zero-valent iron nanoparticle synthesis. Collectively, these studies indicate that iron-based technologies can achieve high contaminant removal efficiencies, although they rely on externally supplied materials and focus primarily on pollutant removal.

Other emerging approaches, integrated urban wastewater treatment systems associated with the cultivation of photosynthetic microorganisms have proven to be a promising technological alternative, both for the removal of organic load and for the production of high-value biomass (Morais et al. 2022;

Cheirsilp et al. 2023). Considering the microorganisms studied, *E. gracilis* stands out as a unicellular euglenoid which, although not belonging to the true microalgae group, exhibits similar photosynthetic metabolism and physiological behavior, being able to grow under autotrophic, heterotrophic, or mixotrophic conditions. This adaptive capacity expands its potential for applications in different types of effluents, making it an ideal candidate for the remediation of contaminated urban environments (Alves et al. 2024; Nezbrytska et al. 2022).

Photosynthetic microorganism-based systems have been increasingly explored within a biorefinery approach for wastewater treatment, as they enable simultaneous nutrient removal and biomass production. These systems are effective in reducing nitrogen and phosphorus concentrations, which are key drivers of eutrophication in aquatic environments, by assimilating dissolved nutrients during biomass growth (Butzke et al. 2024; Nezbrytska et al. 2022). Technologies such as photobioreactors and high-rate algal ponds (HRAPs) provide favorable conditions for biomass accumulation and wastewater polishing and have demonstrated promising performance at pilot and semi-industrial scales across different climatic regions (Sutherland and Ralph 2020). When integrated with physical and biological processes, including sedimentation and activated sludge, these systems can further enhance treatment efficiency while reducing operational costs (González-Camejo et al. 2021).

Within this biorefinery framework, *E. gracilis* has received particular attention due to its multifunctional role in wastewater treatment. In addition to contributing to nutrient removal, this euglenophyte is capable of biofixing carbon dioxide during growth, converting inorganic carbon into biomass enriched in compounds of high economic value, which supports greenhouse gas mitigation efforts (Molazadeh et al. 2019). Moreover, biomass produced from wastewater-grown *E. gracilis* has been reported as a suitable feedstock for biofuel production, including biodiesel, biogas, and bio-oil, offering a renewable alternative to fossil energy sources (Toyama et al. 2018). The use of microalgal biomass for biofuel production presents advantages such as high biomass productivity and the ability to grow in non-arable or saline environments without the need for irrigation, herbicides, or pesticides (Deviram et al. 2020).

E. gracilis exhibits unique characteristics that make it particularly attractive for biotechnological applications, being an excellent source of dietary proteins, vitamins, lipids, and β-1,3-glucan (paramylon), a polysaccharide exclusive to euglenoids (Khatiwada et al. 2020) Its high rate of bioproduct synthesis under different culture conditions positions it as a viable competitor to microalgae such as those of the Chlorella genus, whose products are already commercially available (Sayar and Eryalçın 2023).

Regarding the use of this biomass, it is noteworthy that *E. gracilis* has traditionally been used as food in some Asian countries (Mou et al. 2024). In others, it is already recognized as a novel food (Nuin Garciarena et al. 2025) or generally recognized as safe (GRAS) (Guo et al. 2025). However, when cultivated in urban wastewater, the use of *E. gracilis* biomass faces restrictions due to the potential presence of contaminants. In such cases, its use is more viable in non-food applications, such as agriculture, biomaterial production, and biofuels (Braga et al. 2022; Okeke et al. 2022).

The application of *E. gracilis* in wastewater treatment has been associated with efficient nutrient removal while enabling the production of biomass enriched in compounds such as proteins, lipids, and pigments, thereby increasing its biotechnological relevance (Butzke et al. 2024). Its integration into effluent treatment systems has been proposed as a sustainable strategy to mitigate the environmental impacts of wastewater discharge while supporting resource recovery (Nezbrytska et al. 2022). Within this context, the use of *E. gracilis* has been framed under a biorefinery concept, in which wastewater serves not only as a treatment target but also as a cultivation medium for generating value-added bioproducts (Khatiwada et al. 2020). The incorporation of *E. gracilis* cultivation into wastewater treatment processes may also contribute to improved process efficiency and reduced operational constraints, while enabling effluent valorization through biomass utilization (Toyama et al. 2018; Barsanti et al. 2020). Overall, this multifunctional approach combines environmental remediation with the sustainable production of high-value bioproducts, reinforcing the importance of developing integrated and efficient technologies for water and resource management (Wu et al. 2021; Molinuevo-Salces et al. 2019).

Previous studies have demonstrated the feasibility of cultivating *Euglena* spp. in domestic wastewater, primarily at laboratory scale or under controlled conditions. At lab scale, *Euglena* sp. has been shown to grow mixotrophically in untreated domestic sewage, achieving efficient removal of organic matter and nutrients such as nitrogen and phosphorus, while producing biomass with relevant lipid content for biorefinery applications (Mahapatra et al. 2013). More recent investigations have explored mixed cultures, showing that *E. gracilis* combined with other microalgae, such as *Selenastrum* sp., can enhance wastewater treatment performance. In aquaculture wastewater systems, mixed cultures of *E. gracilis* and *Selenastrum* achieved near-complete ammonium and phosphate removal, along with high reductions in total nitrogen and phosphorus and increased biomass and lipid productivity compared to monocultures (Tossavainen et al. 2019). In addition, the ability of *Euglena* to adapt to real wastewater streams has been demonstrated in engineered systems, such as sequencing batch membrane photobioreactors fed with secondary effluents, where native *Euglena* strains achieved high nutrient removal efficiencies while producing lipid-containing biomass under mixotrophic conditions (Sheng et al. 2017).

At pilot scale, studies involving *Euglena* in domestic wastewater have been more limited and have largely focused on its natural occurrence within complex algal–bacterial communities rather than on its targeted application. In pilot-scale attached-growth pond systems treating raw domestic wastewater, *Euglena* was identified as one of the dominant algal groups contributing to organic matter and nutrient removal (Phyu et al. 2024). Similarly, long-term pilot-scale multimodal algal–bacterial processes treating municipal wastewater have consistently detected members of the Euglenophyceae, including *Euglena* spp., particularly under mixotrophic conditions, where they contributed to system resilience and nutrient assimilation despite not being the dominant taxa (Mahapatra and Murthy 2021). Although these studies highlight the ecological relevance and adaptability of *Euglena* in pilot-scale wastewater treatment systems, they also reveal a clear gap in the literature: systematic investigations involving the deliberate inoculation of *E. gracilis* into raw domestic wastewater at pilot scale, coupled with an integrated evaluation of growth kinetics, treatment performance, carbon assimilation, and biomass composition,

remain scarce. This gap contrasts with the recognized biotechnological potential of *E. gracilis* within biorefinery-oriented wastewater remediation frameworks (Khatiwada et al. 2020) and directly motivates the need for the present pilot-scale study.

Therefore, this study aimed to assess the potential of *E. gracilis* for the treatment of urban wastewater, evaluating its feasibility for biomass production with biotechnological applications. In this context, it represents an innovative approach by employing *E. gracilis* in urban effluent treatment, simultaneously exploring its capacity for nutrient removal, carbon biofixation, and the generation of biomass with biotechnological value. The use of this microorganism in wastewater represents a significant advancement over conventional systems by transforming an environmental liability into a source of renewable resources. Hence, it reinforces the importance of integrating environmental biotechnology with the principles of the circular economy, highlighting promising pathways for the development of clean and sustainable process technologies.

2. Materials and Methods

2.1 Cultivation

E. gracilis, registered in the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SisGen) under number A863DC8, was used in this study The urban effluent employed was collected from the Wastewater Treatment Plant (WWTP) of the University of Santa Cruz do Sul (UNISC). After sampling, the effluent stood for 10 minutes to allow the sedimentation of suspended solids, and the pH was adjusted using a 5% phosphoric acid (H_3PO_4) solution, considering that *E. gracilis* exhibits optimal growth under acidic conditions (Xie et al. 2023). This adjustment also helped to minimize contamination by other microorganisms, favoring the predominant development of the target species.

As a reference medium, an NPK (12:11:18) solution at a concentration of 3 g L^{-1} was used, which had been previously employed in the maintenance cultures of the strain and served both for inoculum production and as the control condition in the experiments (Alves et al. 2024).

Laboratory-scale cultures were carried out in photobioreactors with a working volume of 4 L, inoculated with *E. gracilis* at 20% of the total volume. The system was maintained for up to three weeks under a photoperiod of 12 hours of light and 12 hours of darkness, with artificial illumination provided by 20.5 W tubular LED lamps (average of 148 µmol photons m^{-2} s^{-1}), in an environment without temperature control. Aeration was provided through a bubble column generated by an air compressor (VigoAr 200 Plus), using a porous stone to ensure homogeneous air distribution.

Different proportions of real raw urban wastewater were evaluated, corresponding to 10, 20, 30, 40, and 100% (v/v) of the culture medium. Considering the use of 20% inoculum and the addition of effluent percentages, the final volume was completed with NPK solution (3 g L^{-1}) according to the effluent proportion tested in each experimental condition. At the pilot scale, cultivation was conducted using

100% raw urban wastewater in 100 L plastic bags equipped with a bubble column, maintained indoors under artificial illumination and a 12:12 photoperiod, as illustrated in Fig. 1.

The cultivation system consisted of plastic-bag photobioreactors made of low-density polyethylene (LDPE) smooth plastic bags (220 × 40 cm; Crudo Plast), arranged vertically and illuminated by LED panels that uniformly illuminated the entire set of bags.

Aeration was provided by a high-flow air compressor (Air 140 compressor for ornamental ponds, 220 V, 140 L min^{-1}), generating a continuous bubble column, with airflow limited by the size of the air inlet orifice (1 cm) into the bag. Temperature and irradiance were recorded using a HOBO data logger (UA-002-64, Onset Computer Corporation, USA).

2.2 Monitoring of Cell Growth

The development of the cultures was monitored weekly through cell counting in a hemocytometer using a microscope and spectrophotometric analyses performed with a UV–Vis spectrophotometer, with readings at 680 nm — the wavelength corresponding to the maximum absorption of chlorophyll — used as an indicative parameter of cell growth. The equipment was calibrated using distilled water as a blank. In parallel, the pH of the samples was measured with a benchtop digital pH meter, allowing the monitoring of variations in this parameter throughout the experiment. At each monitoring interval, samples were collected to determine biomass, which was separated by centrifugation and subsequently dried to constant weight.

The calculation of cells per mL was performed based on Eq. 1, using counts obtained with a Sedgewick–Rafter counting chamber, where **N** is the number of cells counted, **A** is the field area in mm^2 (1), **D** is the chamber depth in mm (1), and **F** is the number of fields counted (10). For the calculation of kinetic data referring to the specific growth rate (μ), the generation rate (k), and the doubling time (td), Equations 2 to 4 were used, where x_1 and x_2 are the initial and final cell counts (cells mL^{-1}) at times t_1 and t_2 (days).

$$CellD\left(cellmL^{-1}\right) = \frac{Nx1000mm^3}{AxDxF} \quad \text{Eq. 1}$$

$$\mu = \frac{lnx2 - lnx1}{t2 - t1} \quad \text{Eq. 2}$$

$$k = \frac{\mu}{ln2} \quad \text{Eq. 3}$$

$$t_d = \frac{ln2}{\mu} \quad \text{Eq. 4}$$

Culture monitoring was also carried out through physicochemical parameters, including Dissolved Oxygen (DO), Electrical Conductivity (EC), and Total Suspended Solids (TSS), measured using a multiparameter meter (AK-88, Akso, Italy); turbidity, measured with a digital turbidimeter (AK410 – Turbidez Max, Akso, Italy).

2.3 Effluent Parameter Testing

Samples were previously centrifuged and filtered through 0.45 µm membranes to remove suspended particles and subsequently diluted in ultrapure water to ensure the accuracy of chemical component measurements. The methods described for Chemical Oxygen Demand (COD), SMEWW 5220 B, and Ammoniacal Nitrogen ($N–NH_3$), SMEWW 4500–NH_3 B, were employed according to Standard methods for the examination of water and wastewater (APHA 2017).

The parameters of Total Organic Carbon (TOC), Total Inorganic Carbon (TIC), and Total Nitrogen (TN) were determined using a TOC/TN elemental analyzer (TOC-LCPH/CPN, Shimadzu, Japan).

2.4 Biomass Characterization

Final biomass separation was performed by centrifugation using a refrigerated benchtop centrifuge operating at 3200 rpm for 5 minutes. After this procedure, the final supernatant was collected and reserved for physicochemical analyses, while the biomass pellet was subjected to lyophilization in a benchtop freeze dryer (LS3000, Terroni, Brazil) for preservation and subsequent characterization.

The dried biomass was evaluated for gravimetric yield and biochemical composition, focusing on biomolecules of interest, including lipids, proteins, and carbohydrates. Lipid content was determined using the method adapted from Bligh and Dyer (1959), as recently applied by Butzke et al. (2024). Protein content was estimated from nitrogen content determined by elemental analysis (N × 4.78), while the fixed carbon content corresponded to the percentage of total carbon measured in the biomass (Martini et al. 2019; Templeton and Laurens 2015). Carbohydrate quantification followed the protocol described by Sluiter et al. (2010), using the concentrated acid hydrolysis method followed by analysis by high-performance liquid chromatography with refractive index detection (HPLC/RID, 20A, Shimadzu, Japan).

2.5 Statistical Analysis

Results were expressed as mean ± standard deviation, based on descriptive data analysis. Statistical analysis was performed using GraphPad Prism software (version 10.5.0). Data were initially tested for normality and homogeneity of variances to assess the suitability of parametric analyses. When these assumptions were met, differences among cultivation conditions were evaluated using one-way analysis of variance (ANOVA), followed by appropriate post hoc tests, with statistical significance set at $p < 0.05$. Statistically significant differences between means are indicated by different letters.

When normality assumptions were not met, data were analyzed using the non-parametric Kruskal–Wallis test followed by Dunn’s post hoc test. In cases where graphical complexity or limited replication precluded the inclusion of statistical lettering in figures or tables, statistically significant differences ($p < 0.05$) are reported directly in the text.

For infrared spectral analysis of the biomass, the ChemoStat 4 software was used. Principal component analysis (PCA) was applied to the mean of the normalized spectra, with data preprocessed by mean-centering.

3. Results and Discussion

3.1 *E. gracilis* Growth

As shown in Table 1, all conditions with the addition of urban effluent exhibited cellular growth. After 21 days, the following order of cell accumulation was observed: 40% > 30% ≈ 20% > 10%. During the first week of cultivation, the greatest increase in biomass was recorded, particularly in the condition with 100% effluent addition, indicating higher nutrient bioavailability at the beginning of cultivation. This behavior is in line with that described by Liao et al. (2024), who reported that higher nutrient loads during the initial stages favor nitrogen and phosphorus assimilation by microalgae, promoting more intense exponential growth rates until the gradual depletion of available nutrients.

The use of 100% effluent allowed for biomass production within 14 days equivalent to that obtained in 21 days under conditions with 10–40% effluent, due to a faster growth kinetic (shorter cell doubling time). However, in denser or deeper cultures, factors such as self-shading, light limitation, optical interference of the effluent (color, turbidity), possible inhibitors present in the medium, and aeration constraints may attenuate this kinetic advantage and limit effective growth (Saccardo et al. 2022).

Even considering factors that may interfere with growth, among the dilutions tested, the 40% concentration showed the highest increase in cell concentration. These results highlight the feasibility of using effluent as a cultivation medium for *E. gracilis*, as also reported by Butzke et al. (2024), who achieved similar results using swine effluent with the same *E. gracilis* strain. However, higher concentrations of swine effluent did not yield better results, unlike what was observed with 100% urban effluent.

The use of effluent concentrations between 10% and 40% resulted in biomass yields ranging from 0.43 to 0.69 g L^{-1}. The dry biomass yield varied according to the different cultivation conditions, demonstrating the adaptability of *E. gracilis* to urban effluent. In the 100% effluent condition, the biomass obtained was closer to the value achieved using NPK as the nutrient source. However, in the pilot-scale system, harvesting must occur earlier—or the culture medium must be partially renewed—since after 11 days the biomass density had already entered a decline phase.

Table 1
Values of μ in day⁻¹, td in days, k in divisions per day for *E. gracilis* in medium with effluent compared to NPK.

Scale	Wastewater content	μ (d^{-1})	Maximum cultive days	t_d	k (d^{-1})	Biomass ($g\ L^{-1}$)
Bench	10%	0.039	21	17.76	0.056	0.43
	20%	0.051	21	13.57	0.074	0.69
	30%	0.052	21	13.32	0.075	0.55
	40%	0.056	21	12.43	0.080	0.56
	NPK	0.162	14	4.29	0.230	0.77
	100%	0.108	15	6.39	0.156	0.65
Pilot	NPK	0.118	14	4.53	0.225	0.93
	100%	0.156	11	6.12	0.222	0.40

* The data were calculated considering experiments conducted during the same weeks; therefore, they were not performed in triplicate.

The growth of *E. gracilis* in raw effluent was monitored through cell density (CellD) and optical density, as shown in Fig. 2, for the culture in 100% effluent. The results indicate that *E. gracilis* was able to assimilate the available nutrients without the need for dilution, demonstrating its potential for the bioremediation of wastewater. Monitoring carried out with the multiparameter probe also revealed significant changes ($p < 0.05$) in medium quality: color, measured by absorbance at 660 nm, increased from 0.229 ± 0.105 to 0.904 ± 0.099, following the rise in biomass density; EC decreased from 699.6 ± 2.7 to 162.4 ± 0.4 mS; TSS decreased from 350.3 ± 1.3 to 81.9 ± 0.2; and DO increased from 2.8 ± 1.0 to 6.2 ± 0.9 mg L^{-1}.

At the pilot scale, the kinetic parameters indicated behavior similar to that observed at the laboratory scale, though with some variations associated with environmental and operational conditions. During the exponential phase, cultures in 100% effluent and in the NPK medium exhibited specific growth rates (μ) of 0.156 and 0.118 day^{-1}, respectively—values comparable to those obtained at the laboratory scale (0.108 and 0.162 day^{-1}). The observed differences can be attributed to factors such as air bubble distribution, temperature, light incidence, and biomass accumulation at the bottom of the bags, which directly influence the photosynthetic efficiency of *E. gracilis*.

The cell doubling time (td) remained within a similar range between the two scales, being 6.12 days in 100% effluent and 4.53 days in NPK medium, compared to 6.39 and 4.29 days at the laboratory scale, respectively. These results suggest that the pilot system was able to maintain yield comparable to bench scale, even without strict temperature control, as shown by the temperature and irradiance data in Fig. 3.

The cell division coefficient (k) also showed little variation between scales, indicating that the adaptation of *E. gracilis* to the larger-volume system was similar.

Figure 3 presents the monitoring of irradiance and temperature throughout the cultivation in the pilot system. Temperature (Fig. 3a) ranged from 24 to 35°C during the experiment, which was conducted between the spring and summer of 2024–2025, a period characterized by high temperatures in southern Brazil. Although the experiments were carried out indoors, the space was not climate-controlled, resulting in significant thermal fluctuations. *E. gracilis* was able to tolerate this gradient; however, previous studies, including those conducted in our laboratory, indicate that its growth is more favorable under controlled conditions at 25°C. In this context, the lack of temperature control may have acted as a limiting factor in the results obtained. For instance, Wang et al. (2018) observed that *E. gracilis* reaches its peak specific growth rate between 27 and 30°C, indicating that significant deviations from this range may reduce its cell multiplication efficiency.

The recorded irradiance (Fig. 3b) varied from 28 to 40 μmol m^{-2} s^{-1}, a range similar to that used by Butzke et al. (2024) during the light period of a 12:12 photoperiod. These values are also consistent with the light intensities recently reported as typical for microalgae production by Vo et al. (2024) and Adetunji et al. (2025).

3.2 Nutrient Removal in the Cultivation of *E. gracilis* in Urban Effluent

The results of the carbon, nitrogen, and phosphorus analysis in the liquid medium after cell separation are presented in Table 2. The 25.9% reduction in TOC indicates the effective removal of organic compounds present in the effluent, reflecting the ability of *E. gracilis* to assimilate and oxidize organic matter during cultivation. Removal values of this magnitude or higher are typically observed in mixotrophic systems using photosynthetic microorganisms cultivated in urban effluents, especially when good adaptation to organic carbon sources is achieved (Mahapatra et al. 2013; Farjallah et al. 2024). In addition, the synergistic action of heterotrophic bacteria that may be present in the effluent can degrade organic compounds and contribute to TOC removal (Phyu et al. 2024). However, the efficiency of the removal process can be influenced by factors such as light availability, the presence of essential nutrients, and the carbon-to-nitrogen ratio in the medium (Pang et al. 2019). Indeed, Dang et al. (2022) and Xu et al. (2017) demonstrated that inadequate C/N ratios (either too low or too high) result in lower nitrogen and phosphorus removal efficiency, as well as negatively affecting microalgal growth rates. In mixotrophic cultures, Khanra et al. (2020) observed that adjustments in the C/N ratio modulate the assimilation of organic carbon and the protein metabolism of the cells. Similarly, Pereira et al. (2024) reported that variations in the C/N ratio influence the removal of dissolved organic carbon by microalgae. According to Xie et al. (2023), the optimal C/N ratio was 10, and in our system, whose initial C/N ratio was 1.9, it is likely that this value was outside the optimal range for *E. gracilis*, which may have limited additional TOC removal and the utilization of residual nitrogen. These results indicate that, for the

efficient use of this strain in treatment systems, future studies should evaluate the influence of different nutrient proportions on the growth and cellular metabolism of this species.

The expressive 99.1% removal of inorganic carbon (IC) indicates the fixation of inorganic carbon by *E. gracilis*. This result can be explained by the fact that dissolved inorganic carbon represents the main carbon source for photosynthesis in microalgae (Kusi et al. 2024). Additionally, this reduction may be related to the cultivation of *E. gracilis* as an efficient carbon fixation system. Razzak et al. (2017) highlighted that photobiological systems can remove more than 80% of inorganic carbon from effluents, depending on cultivation conditions such as aeration and light availability. The 69.5% removal of total carbon (TC) reflects the combined contribution of both organic and inorganic carbon assimilation processes.

Similar results have been reported in studies that employed microalgae for wastewater treatment, where TC removal ranged from 50% to 80%, depending on effluent composition and experimental conditions (Lu 2025). However, Toyama et al. (2018) noted that the efficiency of TC removal is affected by the presence of symbiotic bacteria, which assist in the conversion of organic matter and promote the release of CO_2 for fixation into the biomass.

The increase observed in total nitrogen and total phosphorus concentrations at the end of treatment is related to the addition of the *E. gracilis* inoculum previously cultivated in synthetic medium containing NPK, which introduced residual nutrients into the system. This effect, however, was less pronounced when an inoculum previously adapted to the effluent was used, indicating that prior adaptation constitutes an effective strategy to minimize external nutrient input during the treatment process. Nevertheless, considering that the culture was initiated with an isolated *Euglena* strain, supplementation with NPK in the inoculum and pH adjustment were necessary to ensure its predominance in the microbial consortium. In applied systems, longer hydraulic retention times and integration with other nature-based technologies (such as constructed wetlands or high-rate algal ponds) may enhance nutrient removal and reduce the dependence on external input addition (Sutherland and Ralph 2020). It is understood that microalgae can represent one component of a broader treatment system, as they remove emerging contaminants and nutrients, increase dissolved oxygen, reduce chemical oxygen demand, and establish a symbiotic environment with bacteria during the treatment process (Nguyen et al. 2022; Bang Truong et al. 2024; Abdelfattah et al. 2023).

The evaluation of treatment efficiency applied to the effluent generated at the university campus demonstrated that the system employing *E. gracilis* met some of the emission standards established by CONSEMA Resolution No. 355/2017, particularly concerning Chemical Oxygen Demand (COD) and Ammoniacal Nitrogen ($N–NH_3$).

Table 2
Parameters of the effluent before and after treatment with *E. gracilis* in 100% effluent.

Parameters		Initial	After 3 weeks	Removal (%)
Carbon	**Organic**	38.92	28.84 ± 2.23	25.9
	Inorganic	53.23	0.47 ± 0.10	99.1
	Total	93.05	28.36 ± 2.26	69.5
Nitrogen	**Total**	39.45	41.31 ± 17.33	-
	Ammoniacal	14.4	1.20 ± 0.99	91.6
Phosphorus	**Total**	2.73	55.96 ± 0.83	-
Eletrical Conductivity (µScm^{-1})		1013	793 ± 179	21.7
Chemical Oxygen Demand (COD) (mg L^{-1})		99,2	2.0	97.9
Turbidity (NTU)		52	3.24 ± 1.9	93.8
Total Suspended Solids (TSS)		350.3	1.3 ± 0.2	99.6
pH		7.6	5*	-
*pH adjusted for cultivation				

COD removal reached 98%, a result superior to that obtained in the study conducted by Butzke et al. (2024), who reported a reduction efficiency of 44% in diluted swine wastewater (25%). This difference may be attributed to the higher nutrient concentration in that type of effluent, which initially presented COD values of 3101 mg L^{-1}—substantially higher than the initial values of the raw urban effluent used in the present study. Kim et al. (2021) demonstrated that the cultivation of *E. gracilis* in tomato processing wastewater under continuous illumination (24 h light) achieved a COD removal of 23% at pH 3. Comparison with these studies indicates that the environmental conditions adopted in the present work —such as the 12:12 light–dark photoperiod and acidic pH—likely favored the optimal utilization of organic carbon fractions present in the wastewater. This emphasizes the importance of environmental conditions for achieving efficient nutrient removal by photosynthetic organisms such as Euglena.

It is also important to highlight the remarkable efficiency of the system in the removal of N–NH_3, reaching values close to 97%, corresponding to a final concentration below 0.5 mg L^{-1}. As expected for *E. gracilis*, ammoniacal nitrogen is primarily removed from the culture medium (He et al. 2025). This performance is comparable to that reported by Silveira et al. (2020) in an integrated system (microalgae associated with constructed wetlands), which achieved 98% nitrogen removal. Kuroda et al. (2018) also demonstrated high nutrient removal potential in primary sedimentation tank effluent, where during 14 days of cultivation *E. gracilis* almost completely removed ammonium (NH_4^+–N), resulting in final concentrations below 0.2 mg L^{-1}.

3.3 Biomass Yield and Composition

The biochemical composition of *E. gracilis* biomass varied significantly according to the proportion of urban effluent in the culture medium (Table 4), reflecting metabolic adjustments to different nutrient availability and physicochemical conditions.

The results indicate that the highest protein synthesis occurred under control conditions (NPK only), both at pilot scale (40.0%) and laboratory scale (49.9%). These higher values are associated with media rich in readily assimilable inorganic nitrogen, which is essential for the synthesis of structural and enzymatic proteins (He et al. 2025; Yaakob et al. 2021). The progressive addition of effluent resulted in a significant reduction in protein content, especially at the 100% effluent concentration, where the value decreased to 23.9%.

Table 3
Biochemical composition of *E. gracilis* dry biomass cultivated at different concentrations of urban effluent.

Wastewater concentration (%)	**Biochemical composition (%)**		
	Lipids	**Protein**	**Carbohydrate**
Pilot-scale			
Control (only NPK)	7.3 ± 0.4	40.7 ± 2.1	13.4 ± 0.0
100	24.4 ± 0.5	27.4 ± 0.7	40.0 ± 0.0
Bench-scale			
Control (only NPK)	15.7 ± 0.7a	49.9 ± 0.1a	13.8 ± 0.8a
10	22.0 ± 0.9b	35.9 ± 0.1b	9.43 ± 0.1b
20	21.8 ± 2.2b	38.6 ± 0.1b	17.1 ± 0.3b
30	23.9 ± 4.8b	37.6 ± 0.1b	20.8 ± 0.8b
40	23.2 ± 0.7b	37.2 ± 0.1b	20.0 ± 0.2b
100	18.5 ± 1.4a	23.9 ± 2.6c	40.8 ± 1.6c

Means followed by the same letter do not differ significantly according to analysis of variance (ANOVA) at $p < 0.05$.

This reduction can be attributed to the higher C/N ratio in urban effluent, characterized by a relative excess of organic carbon and a lower availability of assimilable nitrogen. According to Xie et al. (2023), E. *gracilis* tends to redirect its metabolism as the C/N ratio increases, reducing protein synthesis while favoring the formation of storage compounds. Therefore, mineral media (NPK) or effluent diluted up to 20% are more suitable when the goal is to maximize the protein content of the biomass.

An opposite trend was observed for carbohydrates, whose content increased with the rise in effluent proportion. At the laboratory scale, values ranged from 13.8% (control) to 40.8% (100% effluent),

indicating intense carbohydrate accumulation under high organic load. This increase is related to nitrogen limitation and metabolic stress, which induce *E. gracilis* to accumulate the polysaccharide paramylon, an energy reserve analogous to starch. According to Bakku et al. (2023) and Toyama et al. (2018), this response is typical of mixotrophic or heterotrophic cultures subjected to carbon excess and inorganic nutrient restriction.

In the pilot system, the same trend was observed, although less pronounced, suggesting that physical factors such as light dispersion, thermal gradients, and aeration limitation may modulate the biochemical response of the biomass (Farjallah et al. 2024).

Thus, the presence of urban effluent significantly altered intracellular carbon allocation, redirecting the metabolism of *E. gracilis* from active protein synthesis toward the accumulation of energy reserves. The effluent provides a complex mixture of organic and inorganic compounds that modifies nutrient availability, turbidity, and light penetration in the medium, thereby affecting photosynthetic activity (Abdelfattah et al. 2023). In addition, the possible symbiotic activity of heterotrophic bacteria present in the effluent may contribute to the mineralization of organic matter and the release of assimilable CO_2 (Toyama et al. 2018), reinforcing the mixotrophic nature of the culture.

These findings corroborate the results reported by Alves et al. (2024) for the same strain cultivated in NPK medium supplemented with a high-organic-load residue, which exhibited low lipid (8.4%) and protein (35.9%) contents and a high carbohydrate content (66.5%).

Considering that the same samples used in the laboratory-scale experiments were also analyzed by FTIR, a clear agreement was observed between the results obtained from the different analytical approaches. The characteristic bands of the main compound groups are highlighted in Fig. 4, showing intense signals around 1100 cm^{-1}, attributed to C–O–C and C–O vibrations typical of carbohydrates (Ferro et al. 2019). Bands of lower intensity were also observed near 1650 cm^{-1}, corresponding to the C = O stretching (Amide I) of protein chains, and around 2900 cm^{-1}, associated with the C–H vibrations of methyl and methylene groups present in lipids (Mahapatra et al. 2013; Zhu et al. 2024; Murdock and Wetzel 2009).

The spectra were subjected to principal component analysis (PCA), as shown in Fig. 4. The PC1 axis explained 91.27% of the total variance, representing the main source of difference among the samples. This component primarily discriminated the presence of functional groups associated with carbohydrates, since the samples cultivated in medium containing 100% effluent exhibited a higher proportion of these compounds. The PC2 axis explained 5.34% of the variance and reflected variations related to protein content: samples located on the negative side of PC2 and positive side of PC1 exhibited higher protein levels and lower carbohydrate content, except for the sample cultivated in NPK medium, positioned in the positive quadrant of this component, which showed high protein content and lower lipid content. These results corroborate the infrared spectroscopic analyses, confirming through PCA of the spectra the trend observed in the data presented in Table 4.

Regarding the proteins of *E. gracilis* obtained from the different systems tested, it was observed that they are mainly composed of the amino acids arginine, glutamic acid, aspartic acid, and alanine, indicating a protein profile with relevant amounts of essential amino acids, as shown in Fig. 5. These values were similar to those reported by Butzke et al. (2024) for *E. gracilis* cultivated in swine wastewater for the purpose of producing a germination biostimulant. Most amino acids showed a significant difference ($p < 0.05$) between the cultivation conditions. Notably, arginine concentration was markedly higher in the biomass obtained from the bench-scale system cultivated with 100% urban wastewater compared to the other conditions, and this amino acid is of particular importance for plant metabolism. This composition reinforces the potential application of the biomass in agricultural bioinput formulations (Xie et al. 2023).

On the other hand, cultures grown under conditions that favor carbohydrate accumulation—particularly paramylon—make the biomass a promising source of paramylon nanofibers (Azuma and Yamamoto 2024), which can be hydrolyzed into glucose and subsequently used for bioethanol production. Research has demonstrated that, after hydrolysis and fermentation processes, ethanol can be obtained from *E. gracilis* biomass (Mou et al. 2024).

Considering that the biomass obtained from pilot-scale cultivation in 100% effluent presented an average concentration of 0.58 g L^{-1} with 40% total carbohydrates, an equivalent fraction of 0.23 g L^{-1} of carbohydrates was estimated. Of this total, 63.9% correspond to glucose derived from paramylon hydrolysis, resulting in approximately 0.148 g L^{-1} of glucose available for fermentation. Based on the stoichiometry of glucose conversion to ethanol ($C_6H_{12}O_6 \rightarrow 2\ C_2H_5OH + 2\ CO_2$) by *Saccharomyces cerevisiae*, the theoretical ethanol yield is 0.51 g g^{-1} of glucose, corresponding to a production potential of 0.075 g L^{-1} of ethanol. Although this value is modest, it demonstrates that the biomass of *E. gracilis* cultivated in effluent represents an alternative source of fermentable substrate.

3.4 Carbon Capture

Carbon capture varied significantly among the evaluated conditions, demonstrating the effect of medium composition and cultivation scale on the metabolism of *E. gracilis* (Table 4). It was observed that the use of 100% urban effluent promoted the highest carbon incorporation into the biomass, both at the laboratory scale (43.66%) and at the pilot scale (43.33%), surpassing the values obtained in the NPK controls. This behavior indicates that the effluent provided not only inorganic carbon sources but also assimilable organic fractions, which is characteristic of mixotrophic systems. Recent studies have reinforced that the presence of dissolved organic carbon in effluents stimulates carbon assimilation by microalgae, resulting in high net fixation rates and potentially carbon-negative systems due to the simultaneous utilization of atmospheric CO_2 and available organic carbon (Abdelfattah et al. 2023; He et al. 2025; Wang et al. 2024).

Table 4
Elemental analysis of carbon captured in the biomass of *E. gracilis* cultivated in urban effluent medium.

Escale	Effluent (%)	Carbon (%) (mean ± SD)
Pilot	100	43.33 ± 0.45a
	NPK	40.74 ± 0.40b
Laboratory	10	38.00 ± 1.91c
	20	40.36 ± 2.01b
	30	39.54 ± 1.97bc
	40	38.84 ± 1.94c
	100	43.66 ± 2.10a
	NPK	38.84 ± 2.19c

Carbon capture in microalgal systems is intrinsically linked to biomass growth and carbon allocation within the cells. In this context, the pilot-scale cultivation of *E.gracilis* in the present study showed stable growth kinetics and consistent incorporation of carbon into the biomass under realistic operational conditions. Similar behavior has been reported in previous pilot and semi-pilot studies with *E. gracilis*, which demonstrated the capacity of this species to sustain growth and metabolic activity in wastewater-based systems subject to environmental variability (Nezbrytska et al. 2022).

Although methodological differences prevent direct quantitative comparison, the growth stability and carbon-rich biomass observed at pilot scale in this study are consistent with trends reported for *E. gracilis* cultivated in real wastewater environments (Butzke et al. 2024; Barsanti et al. 2020). These findings indicate that pilot-scale assessments are essential for capturing realistic growth and carbon incorporation dynamics, which cannot be fully represented under controlled laboratory conditions.

The superior performance observed in cultures with 100% effluent is consistent with the findings of He et al. (2025), who demonstrated that optimized microalgal systems for carbon capture can achieve high fixation rates when cultivated in carbon- and nutrient-rich matrices such as domestic wastewater. These authors emphasized that the use of effluents not only reduces the need for external CO_2 supplementation but also mitigates emissions associated with conventional treatment processes. Similarly, Wang et al. (2024) reported significant carbon capture capacity by microalgae cultivated in urban effluents, coupled with the simultaneous removal of nutrients and organic compounds. The mixotrophic contribution was also highlighted by Li et al. (2023a) and Viswanaathan et al. (2022), who emphasized the importance of balancing inorganic and organic carbon sources to maximize cell accumulation and photosynthetic efficiency.

On the other hand, in effluent dilutions ranging from 10% to 40%, total carbon values were lower and more variable, suggesting the importance of organic carbon availability in biomass production, as it

directly influences carbon assimilation and metabolic performance (Rubiyatno et al. 2021).

The consistency between carbon capture results obtained at laboratory and pilot scales (bag systems) demonstrates that the scaled-up cultivation conditions maintained favorable relationships of light distribution, mixing, and gas transfer for carbon assimilation, comparable to those used at the bench scale. This observation is relevant since efficiency losses are typically expected during the transition from small to large scales, mainly due to light gradients and CO_2 diffusion limitations (Li et al. 2023a).

From an environmental perspective, the use of effluents as a cultivation medium represents an integrated strategy for CO_2 emission mitigation and nutrient recovery (Wang et al. 2024; Viswanaathan et al. 2022), contributing to the carbon neutrality of the processes that generated the effluent (He et al. 2025). In this case, the beneficiary of carbon capture through the use of effluent as a medium for *E. gracilis* cultivation is the university's wastewater treatment plant (WWTP). For future studies, life cycle assessments may demonstrate that the valorization of biomass produced in such systems—particularly when applied as agricultural biofertilizer or as a source of biofuels—can yield a positive environmental balance, with emissions compensated by biogenic carbon (Li et al. 2023a).

3.5 Operational aspects and challenges at pilot and large scale

Despite the promising performance observed at pilot scale, the cultivation of *E. gracilis* in urban wastewater systems involves operational aspects that require careful consideration as cultivation volume increases. Studies investigating *E. gracilis* in wastewater environments have indicated that system performance may be influenced by environmental variability, particularly fluctuations in temperature and light availability, which are inherent to pilot-scale operation under realistic conditions (Barsanti et al. 2020; Nezbrytska et al. 2022). Such variability is commonly recognized as a factor that can affect growth stability, biomass productivity, and carbon assimilation in outdoor or semi-controlled cultivation systems.

As cultivation systems are further scaled up, additional challenges related to light distribution, aeration efficiency, and hydrodynamic mixing become increasingly relevant. These aspects have been broadly discussed in studies involving *E. gracilis* cultivated in real wastewater streams, which emphasize the need for adequate system design to minimize spatial heterogeneity and maintain stable cultivation conditions (Butzke et al. 2024). Moreover, pilot-scale studies have highlighted that limitations associated with biological replication, long-term operational stability, and integration with existing wastewater treatment infrastructure remain important considerations for advancing *E. gracilis*-based processes toward larger-scale implementation (Barsanti et al. 2020; Nezbrytska et al. 2022).

From a broader perspective, the scale-up of microalgal- and euglenoid-based wastewater treatment systems is commonly associated with increased energy demand for aeration and mixing, as well as with challenges related to light distribution, biomass harvesting, and nutrient management. These aspects have been widely recognized in recent studies as key factors influencing the technical, environmental,

and economic performance of large-scale algal cultivation systems integrated with wastewater treatment. In this context, recent reviews emphasize that long-term operational stability, energy efficiency, and effective integration with existing wastewater treatment infrastructure remain critical challenges to be addressed before full-scale implementation (Cheirsilp et al. 2023; González-Camejo et al. 2021; Nagarajan et al. 2020).

4. Conclusions

This study demonstrated that *E. gracilis* can be successfully cultivated in real urban wastewater, exhibiting high adaptability and stable growth at both laboratory and pilot scales. The comparable growth kinetics observed between scales indicate that the cultivation system can be effectively scaled up without significant losses in performance, even under non-controlled temperature conditions. The cultivation of *E. gracilis* in urban wastewater resulted in high removal efficiencies of chemical oxygen demand and ammoniacal nitrogen, confirming its potential for reducing organic load and nitrogenous compounds in wastewater streams. These results highlight the relevance of this microorganism as a biological component in integrated wastewater treatment strategies, particularly for organic matter and ammoniacal nitrogen removal.

Urban wastewater also promoted enhanced carbon assimilation, with carbon content in the biomass exceeding 43% under undiluted effluent conditions. This indicates a strong capacity for carbon capture under mixotrophic cultivation, contributing to the mitigation of carbon emissions associated with wastewater treatment processes. The biochemical composition of the biomass was strongly influenced by the culture medium. Cultivation in mineral nutrient medium favored protein-rich biomass, whereas undiluted wastewater induced a metabolic shift toward carbohydrate accumulation, reaching values above 40%. This metabolic flexibility enables the targeted production of biomass with different biotechnological applications, depending on cultivation objectives.

Although net removal of total phosphorus was not achieved under the experimental conditions due to nutrient carry-over and pH adjustment requirements, the results provide a robust proof-of-concept for integrating *E. gracilis* cultivation into urban wastewater treatment systems. Future studies should focus on optimizing nutrient management, inoculum preparation, and operational parameters to enhance overall nutrient removal and process efficiency. Overall, this work demonstrates the feasibility of pilot-scale *E. gracilis* cultivation in urban wastewater, combining organic matter reduction, carbon capture, and biomass valorization, and supports its potential role in sustainable and integrated wastewater treatment frameworks.

Nevertheless, the large-scale implementation of microalgal-based technologies still requires careful consideration of operational stability, energy demand, and system integration with existing wastewater treatment infrastructure. Addressing these challenges through extended pilot operation and process optimization will be essential for advancing such systems toward full-scale application.

Declarations

Funding Declaration

Carlos Alexandre Lutterbeck acknowledges the Brazilian “Conselho Nacional de Desenvolvimento Científico e Tecnológico” (CNPq) for their financial support (Grant N. 440111/2022-6) and the Fundação de Amparo a Pesquisa do Estado do Rio Grande do Sul (Grant Ns. 300079/2023-0 and 23/2551-0000777-0). Rosana de Cássia de Souza Schneider reports the financial support provided by the “Conselho Nacional de Desenvolvimento Científico e Tecnológico” (CNPq) for their financial support (Grant N. 306216/2022-1).

Author Contribution

Betina Mariela Barreto: Conceptualization, Formal analysis, Investigation, Methodology, Validation, Writing – original draftMaurício Kersting: Formal Analysis, Investigation, MethodologyCamila Rafaela Rathke: Investigation, Formal AnalysisCarlos Eduardo Flores dos Santos: Formal Analysis, Investigation, MethodologyFrancielle Pasqualotti Meinhardt: Investigation, MethodologyCamille Vitoria da Rosa: Investigation, MethodologyGabriela Bertol: Investigation, MethodologyPatrik Gustavo Wiesel: Investigation, Methodology, Writing – original draftGiséle Alves: Investigation, MethodologyRosana de Cassia de Souza Schneider: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing Carlos Alexandre Lutterbeck: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing

Data Availability

Data will be provided by request

References

1. Abdelfattah A, Ali SS, Ramadan H, El-Aswar EI, Eltawab R, Ho SH, Elsamahy T, Li S, El-Sheekh MM, Schagerl M, Kornaros M, Sun J (2023) Microalgae-based wastewater treatment: Mechanisms, challenges, recent advances, and future prospects. Environ Sci Ecotechnol 13:100205. doi:https://doi.org/10.1016/j.ese.2022.100205
2. Adetunji AI, Gumbi SfT, Erasmus M (2025) Harnessing the potential of microalgae in sequestration of CO2 emissions: Removal mechanisms, optimization strategies, and bioenergy production. J Hazard Mater Adv 18:100722. doi:https://doi.org/10.1016/j.hazadv.2025.100722
3. Alves G, Rizzetti TM, Linton MAO, Hoeltz M, Dupont A, da Rocha VC, Ritzel L, Souza Schneider RdC (2024) Biomass of Euglena gracilis Produced in Mineral Medium Supplemented with Craft Brewery

Residue. Waste Biomass Valor 15 (7):4175–4189. doi:https://doi.org/10.1007/s12649-024-02464-w

4. APHA (2017) Standard Methods for the Examination of Water and Wastewater. 23th edn. APHA Press, Washington DC
5. Azuma Y, Yamamoto K (2024) Effects of destruction of Euglenagracilis by ultrasonic cavitation. Jpn J Appl Phys 63 (2):02SP89. doi:https://doi.org/10.35848/1347-4065/ad1e05
6. Bakku RK, Yamamoto Y, Inaba Y, Hiranuma T, Gianino E, Amarianto L, Mahrous W, Suzuki H, Suzuki K (2023) New insights into raceway cultivation of Euglena gracilis under long-term semi-continuous nitrogen starvation. Sci Rep 13 (1):7123. doi:https://doi.org/10.1038/s41598-023-34164-1
7. Bang Truong H, Nguyen THT, Ba Tran Q, Son Lam V, Thao Nguyen Nguyen T, Cuong Nguyen X (2024) Algae-constructed wetland integrated system for wastewater treatment: A review. Bioresour Technol 406:131003. doi:https://doi.org/10.1016/j.biortech.2024.131003
8. Barsanti L, Ciurli A, Birindelli L, Gualtieri P (2020) Remediation of dairy wastewater by Euglena gracilis WZSL mutant and β-glucan production. J Appl Phycol 33 (1):431–441. doi:https://doi.org/10.1007/s10811-020-02314-x
9. Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37 (8):911–917. doi:https://doi.org/10.1139/o59-099
10. Braga MQ, Magalhães IB, Silveira CF, Couto E, Calijuri ML, Assemany P (2022) Transforming wastewater treatment plants in sustainable units coupled with local economies: Microalgae as resource recovery agents. J Clean Prod 377:134551. doi:https://doi.org/10.1016/j.jclepro.2022.134551
11. Butzke VLL, Ferreira A, de Oliveira Corrêa D, Furlan JM, Gouveia L, de Cassia de Souza Schneider R, Corbellini VA (2024) Unlocking the potential of Euglena gracilis cultivated in piggery wastewater: biomass production, nutrient removal, and biostimulant potential in lettuce and tomato plants. J Appl Phycol 36 (5):2681–2702. doi:https://doi.org/10.1007/s10811-024-03286-y
12. Cheirsilp B, Maneechote W, Srinuanpan S, Angelidaki I (2023) Microalgae as tools for bio-circular-green economy: Zero-waste approaches for sustainable production and biorefineries of microalgal biomass. Bioresour Technol 387:129620. doi:https://doi.org/10.1016/j.biortech.2023.129620
13. Dang B-T, Nguyen T-T, Ngo HH, Pham M-D-T, Le LT, Nguyen N-K-Q, Vo T-D-H, Varjani S, You S-J, Lin KA, Huynh K-P-H, Bui X-T (2022) Influence of C/N ratios on treatment performance and biomass production during co-culture of microalgae and activated sludge. Sci Total Environ 837:155832. doi:https://doi.org/10.1016/j.scitotenv.2022.155832
14. Deviram G, Mathimani T, Anto S, Ahamed TS, Ananth DA, Pugazhendhi A (2020) Applications of microalgal and cyanobacterial biomass on a way to safe, cleaner and a sustainable environment. J Clean Prod 253. doi:10.1016/j.jclepro.2019.119770
15. Eljamal O, Eljamal R, Falyouna O, Maamoun I, Thompson IP (2024) Exceptional contribution of iron nanoparticle and aloe vera biomass additives to biogas production from anaerobic digestion of waste sludge. Energy 302:131761. doi:https://doi.org/10.1016/j.energy.2024.131761

16. Falyouna O, Maamoun I, Bensaida K, Tahara A, Sugihara Y, Eljamal O (2022) Chemical deposition of iron nanoparticles (Fe0) on titanium nanowires for efficient adsorption of ciprofloxacin from water. Water Pract Technol 17 (1):75–83. doi:https://doi.org/10.2166/wpt.2021.091
17. Farjallah A, Fillion M, Gueguen C (2024) Metabolic responses of Euglena gracilis under photoheterotrophic and heterotrophic conditions. Protist 175 (3):126035. doi:https://doi.org/10.1016/j.protis.2024.126035
18. Ferro L, Gojkovic Z, Gorzsás A, Funk C (2019) Statistical Methods for Rapid Quantification of Proteins, Lipids, and Carbohydrates in Nordic Microalgal Species Using ATR–FTIR Spectroscopy. Molecules 24 (18). doi:https://doi.org/10.3390/molecules24183237
19. González-Camejo J, Ferrer J, Seco A, Barat R (2021) Outdoor microalgae-based urban wastewater treatment: Recent advances, applications, and future perspectives. WIREs Water 8 (3):e1518. doi:https://doi.org/10.1002/wat2.1518
20. Guo B, Zhang W, Zou J, Sun L, Dong N, Liu B (2025) beta-1,3-glucans from Euglena Gracilis protects against ulcerative colitis via modulating the gut barrier, T-cell immunity and gut microbiota. Int J Biol Macromol 305 (Pt 2):141288. doi:https://doi.org/10.1016/j.ijbiomac.2025.141288
21. Hait M, Kashyap NK, Bhardwaj AK (2024) Emerging pollutants from urbanization and industrialization in the global south. In: Biomonitoring of Pollutants in the Global South. Springer, pp 39–87
22. He Q, Zhang X, Xu H, Wang X, Zhang X, Hao Y, Song X, Cao X (2025) Cultivation of high-protein Euglena gracilis for enhanced protein production under inorganic nitrogen sources: mechanisms revealed by proteomics. Bioresour Technol 430:132560. doi:https://doi.org/10.1016/j.biortech.2025.132560
23. Khan SAR, Ponce P, Yu Z, Golpira H, Mathew M (2022) Environmental technology and wastewater treatment: Strategies to achieve environmental sustainability. Chemosphere 286 (Pt 1):131532. doi:https://doi.org/10.1016/j.chemosphere.2021.131532
24. Khanra A, Vasistha S, Kumar P, Rai MP (2020) Role of C/N ratio on microalgae growth in mixotrophy and incorporation of titanium nanoparticles for cell flocculation and lipid enhancement in economical biodiesel application. 3 Biotech 10 (8):331. doi:https://doi.org/10.1007/s13205-020-02323-0
25. Khatiwada B, Sunna A, Nevalainen H (2020) Molecular tools and applications of Euglena gracilis: From biorefineries to bioremediation. Biotechnol Bioeng 117 (12):3952–3967. doi:https://doi.org/10.1002/bit.27516
26. Kim S, Wirasnita R, Lee D, Yu J, Lee T (2021) Enhancement of Growth and Paramylon Production of Euglena gracilis by Upcycling of Spent Tomato Byproduct as an Alternative Medium. Appl Sci 11 (17). doi:https://doi.org/10.3390/app11178182
27. Kuroda M, Horino T, Inoue D, Morikawa M, Ike M (2018) Biomass production and nutrient removal through cultivation of Euglena gracilis in domestic wastewater. Jap J Water Treat Biol 54:105–113. doi:https://doi.org/10.2521/jswtb.54.105

28. Kusi PA, McGee D, Tabraiz S, Ahmed A (2024) Bicarbonate concentration influences carbon utilization rates and biochemical profiles of freshwater and marine microalgae. Biotechnol J 19 (8):e2400361. doi:https://doi.org/10.1002/biot.202400361
29. Li G, Xiao W, Yang T, Lyu T (2023a) Optimization and Process Effect for Microalgae Carbon Dioxide Fixation Technology Applications Based on Carbon Capture: A Comprehensive Review. C-J Carbon Res 9 (1):35. doi:https://doi.org/10.3390/c9010035
30. Li Y, Mi W, Ji L, He Q, Yang P, Xie S, Bi Y (2023b) Urbanization and agriculture intensification jointly enlarge the spatial inequality of river water quality. Sci Total Environ 878:162559. doi:https://doi.org/10.1016/j.scitotenv.2023.162559
31. Liao Y, Fatehi P, Liao B (2024) A Study of Theoretical Analysis and Modelling of Microalgal Membrane Photobioreactors for Microalgal Biomass Production and Nutrient Removal. Membranes (Basel) 14 (12). doi:https://doi.org/10.3390/membranes14120245
32. Lu Q (2025) A State-of-the-Art Review of Microalgae-Based Food Processing Wastewater Treatment: Progress, Problems, and Prospects. Water 17 (4). doi:https://doi.org/10.3390/w17040536
33. Maamoun I, Eljamal R, Eljamal O (2023) Statistical optimization of nZVI chemical synthesis approach towards P and NO3 – removal from aqueous solutions: Cost-effectiveness & parametric effects. Chemosphere 312:137176. doi:https://doi.org/10.1016/j.chemosphere.2022.137176
34. Mahapatra DM, Chanakya HN, Ramachandra TV (2013) Euglena sp. as a suitable source of lipids for potential use as biofuel and sustainable wastewater treatment. J Appl Phycol 25 (3):855–865. doi:https://doi.org/10.1007/s10811-013-9979-5
35. Mahapatra DM, Murthy GS (2021) Long term evaluation of a pilot scale multimodal algal bioprocess for treatment of municipal wastewater. J Clean Prod 311:127690. doi:https://doi.org/10.1016/j.jclepro.2021.127690
36. Martini FA, Rubert A, de Souza MP, Kist LT, Hoeltz M, Benitez LB, Rizzetti TM, Gressler PD, Schneider RCS (2019) Periphytic biomass composition and exploitation from algae turf scrubber system. SN Appl Sci 1 (7). doi:https://doi.org/10.1007/s42452-019-0802-z
37. Molazadeh M, Ahmadzadeh H, Pourianfar HR, Lyon S, Rampelotto PH (2019) The use of microalgae for coupling wastewater treatment with CO2 biofixation. Frontiers in bioengineering and biotechnology 7:42
38. Molinuevo-Salces B, Riaño B, Hernández D, Cruz García-González M (2019) Microalgae and Wastewater Treatment: Advantages and Disadvantages. In: Alam MA, Wang Z (eds) Microalgae Biotechnology for Development of Biofuel and Wastewater Treatment. Springer Singapore, Singapore, pp 505–533. doi:https://doi.org/10.1007/978-981-13-2264-8_20
39. Morais EG, Amaro Marques JC, Cerqueira PR, Dimas C, Sousa VS, Gomes N, Ribau Teixeira M, Nunes LM, Varela J, Barreira L (2022) Tertiary urban wastewater treatment with microalgae natural consortia in novel pilot photobioreactors. J Clean Prod 378:134521. doi:https://doi.org/10.1016/j.jclepro.2022.134521

40. Mou J-H, Liu S-F, Yang L-L, Qin Z-H, Yang Y-F, Wang Z-Y, Li H-Y, Lin CSK, Wang X (2024) Sustainable paramylon production from food waste by Euglena gracilis using a waste-based cell immobilisation technique. Chem Eng J 481:148594. doi:https://doi.org/10.1016/j.cej.2024.148594
41. Murdock JN, Wetzel DL (2009) FT-IR microspectroscopy enhances biological and ecological analysis of algae. Appl Spectrosc Rev 44 (4):335–361. doi:https://doi.org/10.1080/05704920902907440
42. Nagarajan D, Lee DJ, Chen CY, Chang JS (2020) Resource recovery from wastewaters using microalgae-based approaches: A circular bioeconomy perspective. Bioresour Technol 302:122817. doi:https://doi.org/10.1016/j.biortech.2020.122817
43. Nezbrytska I, Shamanskyi S, Pavliukh L, Gorbunova Z (2022) Application of Euglena gracilis in wastewater treatment processes. BioTechnologia (Pozn) 103 (4):323–330. doi:https://doi.org/10.5114/bta.2022.120702
44. Nguyen LN, Aditya L, Vu HP, Johir AH, Bennar L, Ralph P, Hoang NB, Zdarta J, Nghiem LD (2022) Nutrient Removal by Algae-Based Wastewater Treatment. Curr Pollut Rep 8 (4):369–383. doi:https://doi.org/10.1007/s40726-022-00230-x
45. Nuin Garciarena I, Ackerl R, García Ruiz E, Glymenaki M, Mendes V, Muñoz-González A, Noriega Fernández E, Precup G, Rodríguez-Fernández P, Roldán-Torres R, Rossi A, Turla E, Ververis E, Germini A, Kass GEN (2025) The safety assessment of microalgae-derived products as novel foods by the European Food Safety Authority. Future Foods 11:100661. doi:https://doi.org/10.1016/j.fufo.2025.100661
46. Obaideen K, Shehata N, Sayed ET, Abdelkareem MA, Mahmoud MS, Olabi AG (2022) The role of wastewater treatment in achieving sustainable development goals (SDGs) and sustainability guideline. Energy Nexus 7:100112. doi:https://doi.org/10.1016/j.nexus.2022.100112
47. Okeke ES, Ejeromedoghene O, Okoye CO, Ezeorba TPC, Nyaruaba R, Ikechukwu CK, Oladipo A, Orege JI (2022) Microalgae biorefinery: An integrated route for the sustainable production of high-value-added products. Energy Convers Manag: X 16:100323. doi:https://doi.org/10.1016/j.ecmx.2022.100323
48. Pang N, Gu X, Chen S, Kirchhoff H, Lei H, Roje S (2019) Exploiting mixotrophy for improving productivities of biomass and co-products of microalgae. Renew Sustain Energy Rev 112:450–460. doi:https://doi.org/10.1016/j.rser.2019.06.001
49. Pereira ASAdP, Magalhães IB, Silva TA, Reis AJDd, Couto EdAd, Calijuri ML (2024) Municipal and industrial wastewater blending: Effect of the carbon/nitrogen ratio on microalgae productivity and biocompound accumulation. J Environ Manag 370:122760. doi:https://doi.org/10.1016/j.jenvman.2024.122760
50. Phyu K, Zhi S, Liang J, Chang CC, Liu J, Cao Y, Wang H, Zhang K (2024) Microalgal-bacterial consortia for the treatment of livestock wastewater: Removal of pollutants, interaction mechanisms, influencing factors, and prospects for application. Environ Pollut 349:123864. doi:https://doi.org/10.1016/j.envpol.2024.123864

51. Razzak SA, Ali SAM, Hossain MM, deLasa H (2017) Biological CO2 fixation with production of microalgae in wastewater – A review. Renew Sustain Energy Rev 76:379–390. doi:https://doi.org/10.1016/j.rser.2017.02.038
52. Rubiyatno, Matsui T, Mori K, Toyama T (2021) Paramylon production by Euglena gracilis via mixotrophic cultivation using sewage effluent and waste organic compounds. Bioresour Technol Rep 15:100735. doi:https://doi.org/10.1016/j.biteb.2021.100735
53. Saccardo A, Bezzo F, Sforza E (2022) Microalgae growth in ultra-thin steady-state continuous photobioreactors: assessing self-shading effects. Front Bioeng Biotechnol Volume 10–2022. doi:https://doi.org/10.3389/fbioe.2022.977429
54. Sadigov R (2022) Rapid Growth of the World Population and Its Socioeconomic Results. The Scientific World Journal 2022 (1):8110229. doi:https://doi.org/10.1155/2022/8110229
55. Sayar M, Eryalçın K (2023) Investigation of Growth Performance, Proximate and Fatty Acid Composition of Freshwater (Euglena gracilis, Chlorella vulgaris) and Marine (Pavlova lutheri, Diacronema vlkanium) Microalgae. Aquat Sci Eng 0 (0):0–0. doi:https://doi.org/10.26650/ase20241303511
56. Sheng ALK, Bilad MR, Osman NB, Arahman N (2017) Sequencing batch membrane photobioreactor for real secondary effluent polishing using native microalgae: Process performance and full-scale projection. J Clean Prod 168:708–715. doi:https://doi.org/10.1016/j.jclepro.2017.09.083
57. Silveira EO, Lutterbeck CA, Machado ÊL, Rodrigues LR, Rieger A, Beckenkamp F, Lobo EA (2020) Biomonitoring of urban wastewaters treated by an integrated system combining microalgae and constructed wetlands. Sci Total Environ 705:135864. doi:https://doi.org/10.1016/j.scitotenv.2019.135864
58. Sluiter JB, Ruiz RO, Scarlata CJ, Sluiter AD, Templeton DW (2010) Compositional analysis of lignocellulosic feedstocks. 1. Review and description of methods. J Agric Food Chem 58 (16):9043–9053. doi:https://doi.org/10.1021/jf1008023
59. Sutherland DL, Ralph PJ (2020) 15 years of research on wastewater treatment high rate algal ponds in New Zealand: discoveries and future directions. N Z J Bot 58 (4):334–357. doi:https://doi.org/10.1080/0028825X.2020.1756860
60. Templeton DW, Laurens LML (2015) Nitrogen-to-protein conversion factors revisited for applications of microalgal biomass conversion to food, feed and fuel. Algal Res 11:359–367. doi:https://doi.org/10.1016/j.algal.2015.07.013
61. Tossavainen M, Lahti K, Edelmann M, Eskola R, Lampi A-M, Piironen V, Korvonen P, Ojala A, Romantschuk M (2019) Integrated utilization of microalgae cultured in aquaculture wastewater: wastewater treatment and production of valuable fatty acids and tocopherols. J Appl Phycol 31 (3):1753–1763. doi:https://doi.org/10.1007/s10811-018-1689-6
62. Toyama T, Kasuya M, Hanaoka T, Kobayashi N, Tanaka Y, Inoue D, Sei K, Morikawa M, Mori K (2018) Growth promotion of three microalgae, Chlamydomonas reinhardtii, Chlorella vulgaris and Euglena

gracilis, by in situ indigenous bacteria in wastewater effluent. Biotechnol Biofuels 11 (1):176. doi:https://doi.org/10.1186/s13068-018-1174-0

63. Viswanaathan S, Perumal PK, Sundaram S (2022) Integrated Approach for Carbon Sequestration and Wastewater Treatment Using Algal–Bacterial Consortia: Opportunities and Challenges. Sustainability 14 (3):1075. doi:https://doi.org/10.3390/su14031075
64. Vo PHN, Kuzhiumparambil U, Kim M, Hinkley C, Pernice M, Nghiem LD, Ralph PJ (2024) Biomining using microalgae to recover rare earth elements (REEs) from bauxite. Bioresour Technol 406:131077. doi:https://doi.org/10.1016/j.biortech.2024.131077
65. Wang X, Hong Y, Wang Z, Yuan Y, Sun D (2024) High capacities of carbon capture and photosynthesis of a novel organic carbon-fixing microalgae in municipal wastewater: From mutagenesis, screening, ability evaluation to mechanism analysis. Water Res 257:121722. doi:https://doi.org/10.1016/j.watres.2024.121722
66. Wang Y, Seppanen-Laakso T, Rischer H, Wiebe MG (2018) Euglena gracilis growth and cell composition under different temperature, light and trophic conditions. PLoS One 13 (4):e0195329. doi:https://doi.org/10.1371/journal.pone.0195329
67. Wu M, Du M, Wu G, Lu F, Li J, Lei A, Zhu H, Hu Z, Wang J (2021) Water reuse and growth inhibition mechanisms for cultivation of microalga Euglena gracilis. Biotechnol Biofuels 14 (1):132. doi:https://doi.org/10.1186/s13068-021-01980-4
68. Xie W, Li X, Xu H, Chen F, Cheng KW, Liu H, Liu B (2023) Optimization of Heterotrophic Culture Conditions for the Microalgae Euglena gracilis to Produce Proteins. Mar Drugs 21 (10). doi:https://doi.org/10.3390/md21100519
69. Xu J, Wang X, Sun S, Zhao Y, Hu C (2017) Effects of influent C/N ratios and treatment technologies on integral biogas upgrading and pollutants removal from synthetic domestic sewage. Sci Rep 7 (1):10897. doi:https://doi.org/10.1038/s41598-017-11207-y
70. Yaakob MA, Mohamed R, Al-Gheethi A, Aswathnarayana Gokare R, Ambati RR (2021) Influence of Nitrogen and Phosphorus on Microalgal Growth, Biomass, Lipid, and Fatty Acid Production: An Overview. Cells 10 (2). doi:https://doi.org/10.3390/cells10020393
71. Zhu L, Liu M, Wang Y, Zhu Z, Zhao X (2024) Euglena gracilis Protein: Effects of Different Acidic and Alkaline Environments on Structural Characteristics and Functional Properties. Foods 13 (13):2050. doi:https://doi.org/10.3390/foods13132050

Figures

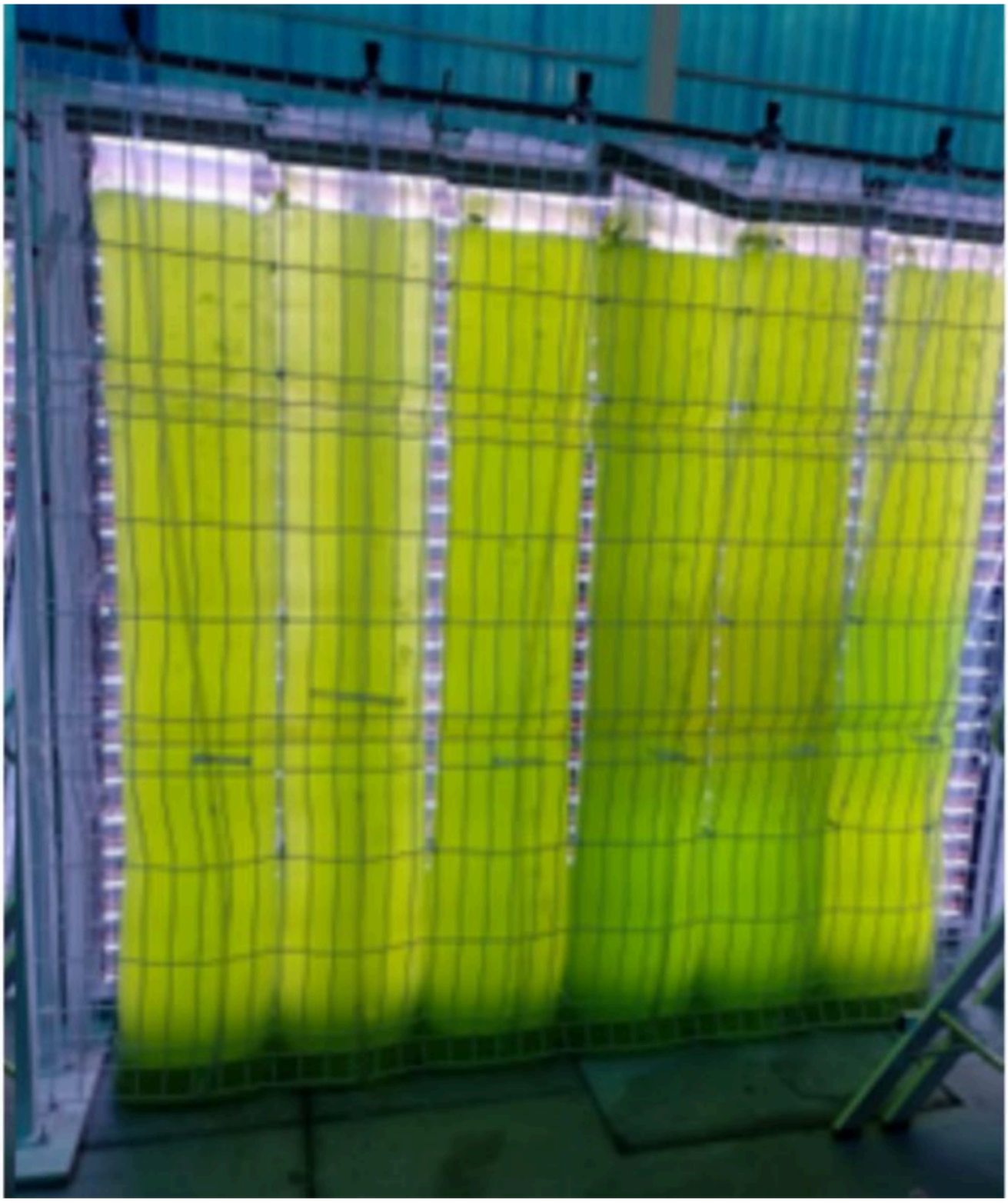


Figure 1

Pilot-scale system for the production of photosynthetic microorganisms consisting of 100 L plastic bags with aeration through a bubble column.

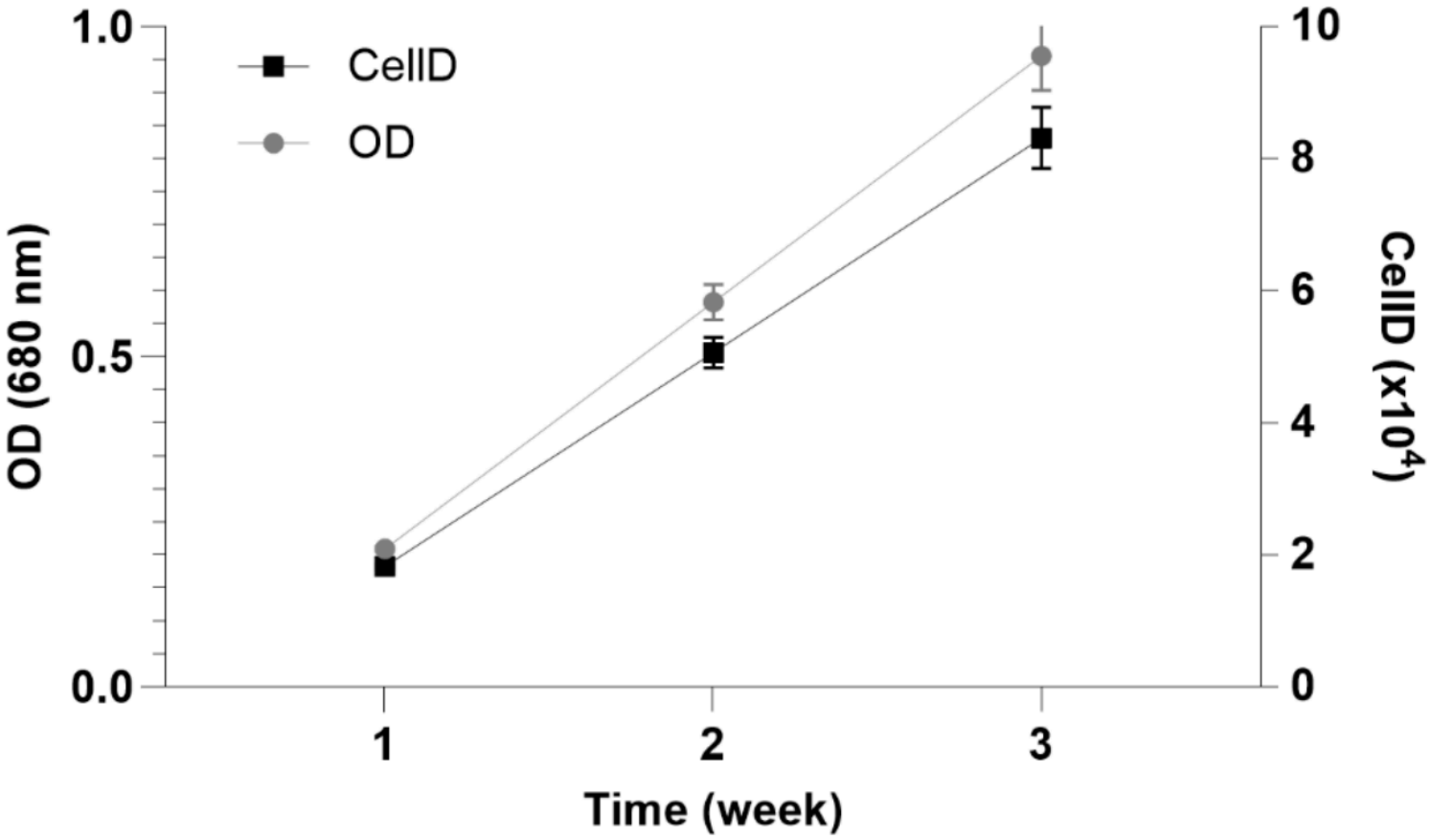


Figure 2

Growth curve by OD and Cell Density (CellD) of *E. gracilis* in 100% urban effluent.

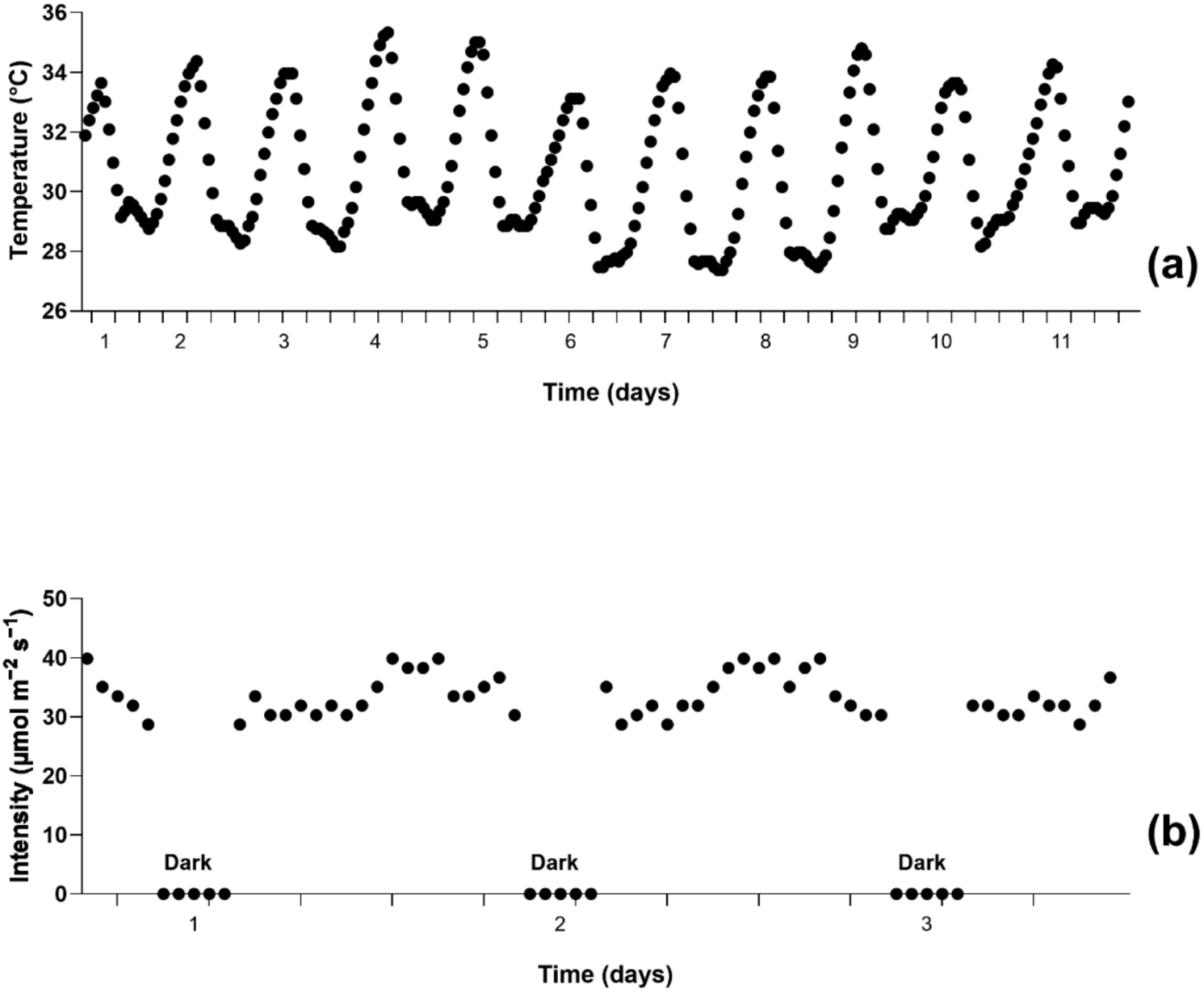


Figure 3

Temperature (a) and Irradiance (b) for readings taken over three consecutive days during the cultivation of *E. gracilis* in an indoor environment, representing the entire cultivation period.

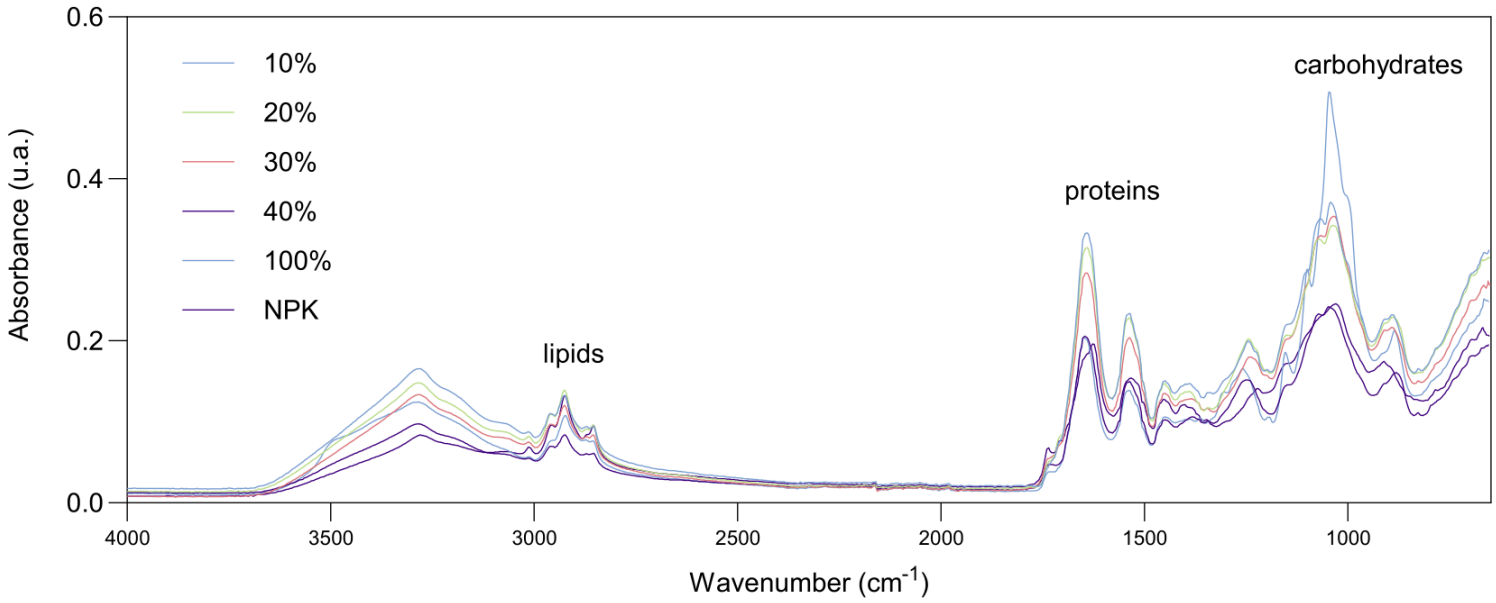


Figure 4

Infrared spectrum of *E. gracilis* biomass samples cultivated in different media containing urban effluent.

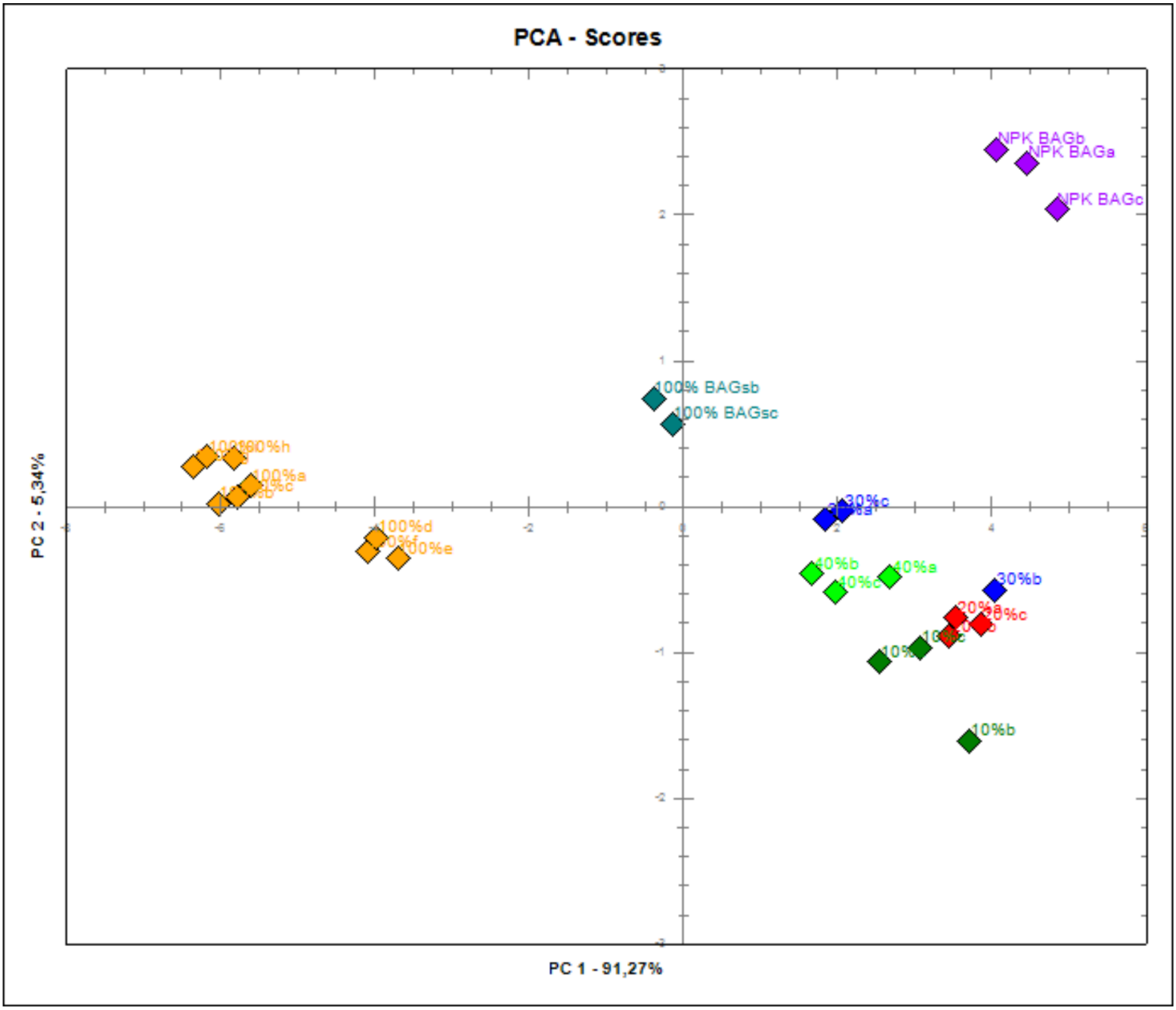


Figure 5

Principal Component Analysis (PCA) obtained from the FTIR spectra of samples cultivated in different proportions of effluent and in NPK medium.

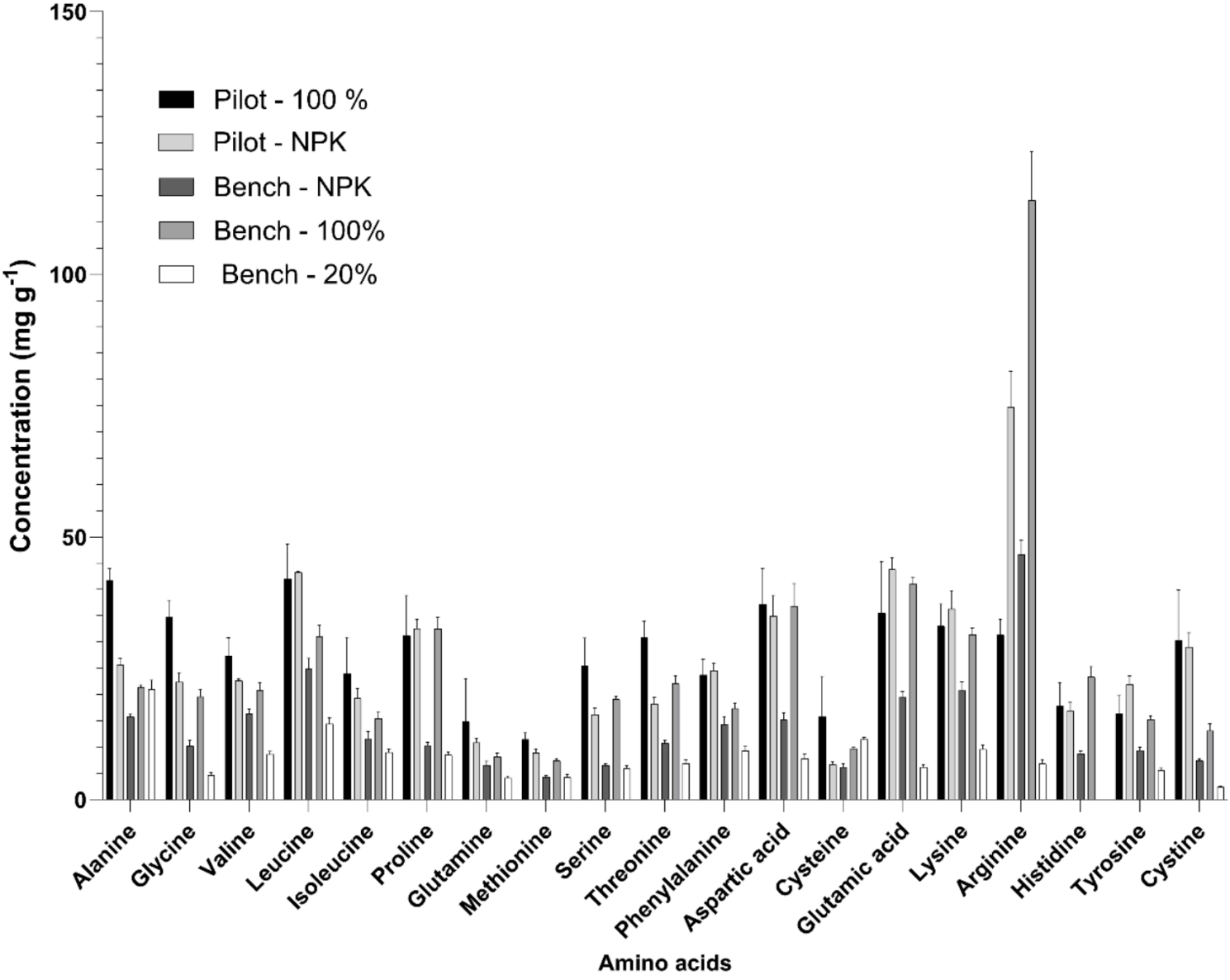


Figure 6

Concentration of amino acids present in the biomass from the analyzed systems.