

Article

Nutrient Removal and Recovery from Dairy Wastewater via Co-Cultivation of *Scenedesmus obliquus* and *Lemna minor*

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Abstract

Dairy wastewater is an agro-industrial effluent characterized by elevated concentrations of organic matter, nutrients and suspended solids, posing environmental risks when inadequately treated. This study assessed the feasibility of a simultaneous *Scenedesmus obliquus*–*Lemna minor* co-cultivation system within a single, shared bioreactor for the treatment of modified dairy wastewater, comparing its performance against the two individual monocultures. Raw dairy wastewater was subjected to sequential heat treatment, ultrasound and filtration, which significantly altered its physicochemical composition ($p < 0.05$) prior to cultivation. Experiments were conducted in parallel laboratory-scale open reactors over 14 days with periodic sampling. The mixed system achieved the highest removal efficiencies (95.3% COD, 91.3% TOC, 97.1% NO₃-N and 94.6% PO₄-P), with rapid pollutant reduction during the first 3–6 days followed by a slower stabilization phase. Despite these high removal efficiencies, the final COD concentration remained relatively high in absolute terms, indicating that the system is best suited as an on-site pretreatment step at the dairy facility, reducing the organic and nutrient load prior to discharge into the municipal sewerage network for further treatment at a municipal WWTP. The recovered microalgal biomass was nutrient-rich and exhibited preliminary biofertilizer potential, as reflected in the positive growth trends observed in two of the three tested *Lactuca sativa* L. cultivars, although these did not reach statistical significance relative to controls. Overall, the integrated system represents an effective, low-impact approach combining pollutant removal, biomass valorization and circular bioeconomy principles.

Keywords: dairy wastewater; *Scenedesmus obliquus*; *Lemna minor*; nutrient removal; biofertilizer; *Lactuca sativa* L.; circular economy



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1. Introduction

The dairy industry is one of the most water-intensive sectors of the global food economy [1]. However, the expansion of large-scale production also creates growing concerns regarding the wastewater generated during these processes. Annual dairy wastewater (DWW) generation in EU countries has been estimated at approximately 192.5×10^6 m³, emphasizing the urgent need for efficient and sustainable treatment strategies [2]. Dairy factories generate substantial volumes of wastewater with complex and highly variable composition during equipment washing, pasteurization, production processes and clean-in-place (CIP) operations [3]. Cheese production alone accounts for nearly half of this

wastewater volume, while six countries (Germany, France, Italy, Poland, Spain and the Netherlands) generate more than 70% of the total load [2]. Depending on the processed product and plant management practices, dairy wastewater is typically characterized by high concentrations of biodegradable organic matter, suspended solids, fats, proteins, lactose, and nutrients such as nitrogen and phosphorus [4]. It also harbors diverse microbial communities, including bacteria such as *Bacillus subtilis*, *Escherichia coli*, *Lysinibacillus sphaericus*, *Bacillus cereus*, *Bacillus thuringiensis* and *Brevibacillus* spp. [5]. Inadequately treated effluents may lead to oxygen depletion, eutrophication, odor formation, and the deterioration of receiving aquatic ecosystems [6]. Therefore, dairy wastewater should be properly treated prior to discharge into natural water bodies in order to reduce the potential for environmental damage.

Conventional treatment of dairy effluents commonly relies on physicochemical and biological processes, including coagulation–flocculation, dissolved air flotation, activated sludge systems, anaerobic digestion, membrane separation and advanced oxidation technologies [3]. Although many of these methods are effective, they are often associated with considerable energy demand, operational complexity, chemical consumption and sludge generation. In addition, the highly variable composition of dairy wastewater, characterized by elevated concentrations of fats, proteins, lactose, suspended solids, nitrogen and phosphorus, often limits the efficiency of single-stage treatment systems and necessitates multi-step or integrated approaches [7]. Membrane-based technologies can achieve high removal efficiency; however, limitations such as membrane fouling, associated cleaning or regeneration requirements and high operational costs remain significant constraints, while conventional aerobic systems require intensive aeration and generate excess biomass [8]. In recent years, increasing emphasis has therefore been placed on treatment concepts that combine pollutant removal with resource recovery and lower environmental impact. Such approaches include nature based and biologically integrated systems capable of simultaneous wastewater polishing, nutrient capture and generation of valuable biomass for downstream applications such as bioenergy, fertilizers or other bio-based products [9].

Among nature-based approaches, microalgae have attracted significant attention due to their capacity to assimilate nutrients, utilize inorganic carbon and improve wastewater quality while simultaneously producing valuable biomass, which can potentially be reused in agriculture, bioenergy production or other circular economy applications [10]. Species belonging to the genus *Scenedesmus* are considered particularly promising because of their rapid growth, adaptability to wastewater environments and relatively high tolerance to fluctuating physicochemical conditions [11]. The green microalga *Scenedesmus obliquus* has shown extraordinary vitality in urban wastewaters, registering growth rates similar to those reported for complete synthetic media. This freshwater microalga tolerates a wide range of temperature and pH, making it versatile for sewage purification [10,11]. Biomass of wastewater-grown *S. obliquus* has also demonstrated biostimulant potential in plant bioassays. Extracts increased the germination index of watercress seeds by up to 40% and exhibited cytokinin-like bioactivity (up to 187.5% relative to control) in cucumber bioassays [12], supporting its integration into circular bioeconomy frameworks.

Floating aquatic macrophytes such as *Lemna minor* from the family *Lemnaceae* also represent an attractive biological treatment option. These plants are recognized as the smallest flowering plants in the world and are characterized by very rapid growth and reproduction rates [13]. *Lemna minor* is frequently applied as a final polishing stage in the treatment of secondary domestic wastewater, where it can effectively remove nutrients, generate biomass and reduce concentrations of toxic organic as well as inorganic micropollutants [14]. In recent years, the use of aquatic macrophytes such as *Lemna minor* in wastewater management has gained increasing attention as an efficient option for ni-

trogen and phosphorus removal because of their high nutrient uptake capacity and rapid vegetative propagation [15]. Importantly, *L. minor* has also demonstrated tolerance to and remediation capacity in agro-industrial effluents, including those generated through food processing operations [16–18], reinforcing its relevance as a treatment organism for dairy wastewater. The protein rich biomass of duckweed, accumulating up to 35–44% dry matter depending on nutrient availability, further supports its valorization as an animal feed supplement within a circular resource management approach [18,19].

Over the past decade, treatment strategies that combine microalgae with aquatic plants or constructed wetlands have been increasingly recognized as an effective alternative to single organism systems. Previous studies demonstrated that integrating microalgal processes with wetland vegetation can significantly improve wastewater purification [20]. However, in most of the systems reported to date, microalgae and macrophytes have been cultivated in separate, physically distinct bioreactors operated either in series or as consecutive treatment stages, rather than co-cultivated together within a single reactor. For instance, Kotoula et al. [21] combined *C. sorokiniana* and *L. minor* using two separate bioreactors connected in series for municipal wastewater treatment. Katsara et al. [22] investigated the cultivation of *C. sorokiniana* and *L. minor* separately under batch and sequencing-batch conditions in thermal mineral waters, each organism grown under its own independently optimized nutrient regime, focusing on biomass composition rather than nutrient removal performance. Combinations of *L. minor* with other microalgal or cyanobacterial species have also been reported, including *Chlamydomonas incerta* for palm oil mill effluent treatment and fertilizer production [23], and *Oscillatoria* sp. for heavy metal removal from urban and industrial wastewater [24]. In addition, residual biomass from combined *C. sorokiniana*–*L. minor* cultivation has been valorized into sorbent materials for heavy metal removal [25], and microalgal–duckweed constructed wetlands have been reviewed as an emerging strategy for swine wastewater treatment [26]. Nevertheless, no previous study has reported the simultaneous co-cultivation of *S. obliquus* and *L. minor* within a single, shared bioreactor for the treatment of dairy wastewater, nor has the biomass generated from such a system been assessed for its potential application as a biofertilizer. A simultaneous co-cultivation system combining microalgae and duckweed within a single reactor may represent a promising alternative to conventional sequential treatment configurations. Such an approach has the potential to simplify the treatment process by integrating both biological components into one cultivation system while promoting complementary interactions between the organisms, improving nutrient uptake and enabling the recovery of a combined biomass suitable for further valorization. Despite these potential advantages, this cultivation strategy has received only limited attention, particularly in the treatment of dairy wastewater.

The effectiveness of biological treatment is therefore strongly dependent on the initial properties of the wastewater. Dairy effluents frequently contain colloidal particles, residual fats and suspended solids that reduce light penetration and may limit biological performance. For this reason, pretreatment steps such as heating [27–32], ultrasound application [33–38] and filtration [39] have been shown to improve wastewater suitability prior to biological polishing by enhancing nutrient bioavailability and optical transparency of the medium. However, these pretreatment approaches also carry practical limitations. Ultrasound-based pretreatment is associated with substantial energy demand, which remains one of its major drawbacks relative to thermal, chemical or biological alternatives [40]. Moreover, pretreatment of dairy effluents for microalgal cultivation has been comparatively little studied from a physicochemical and cost perspective, indicating a gap between demonstrated laboratory-scale benefits and validated large-scale practice [6]. More broadly, recent reviews identify pretreatment requirements, together with biomass harvesting and

process scalability, as key obstacles to the economic competitiveness of microalgae-based wastewater treatment technologies [41,42].

Therefore, this study aimed to assess the feasibility of a simultaneous *S. obliquus*–*L. minor* co-cultivation system for the treatment of modified dairy wastewater, comparing its performance against individual monocultures to determine whether microalgae–duckweed interactions provide synergistic benefits for organic matter degradation, nutrient assimilation and biomass generation. Given the complementary physiological functions of both organisms, it was hypothesized that the integrated co-culture would outperform the monocultures under identical cultivation conditions. Beyond pollutant removal, the resulting biomass was evaluated for its suitability as an agricultural input through *Lactuca sativa* L. growth assays, exploring its valorization within a circular wastewater management framework. The novelty of this study lies in the simultaneous, single-reactor co-cultivation of *S. obliquus* and *L. minor* for dairy wastewater treatment, integrating remediation, biomass recovery and resource valorization within one system.

2. Materials and Methods

2.1. Characterization of Dairy Wastewater Composition and Pretreatment Conditions

Dairy wastewater used in this study was obtained from a local dairy processing facility in Slovakia. The collected wastewater was transported to the laboratory under controlled conditions to preserve its physicochemical properties.

Prior to each experimental series, the wastewater was characterized to ensure consistency of the influent composition. Characterization was performed once per experimental series, resulting in three independent datasets corresponding to three weekly batches. The analyzed parameters included total nitrogen (TN, mg L^{−1}), nitrate nitrogen (NO₃-N, mg L^{−1}), ammonium nitrogen (NH₄-N, mg L^{−1}), orthophosphate phosphorus (PO₄-P, mg L^{−1}), potassium (K, mg L^{−1}), chemical oxygen demand (COD, mg O₂ L^{−1}), total organic carbon (TOC, mg L^{−1}), pH, electrical conductivity (mS cm^{−1}), total dissolved solids (TDS, mg L^{−1}) and turbidity (NTU).

Following characterization, a pretreatment procedure was applied to reduce turbidity and enhance light penetration during subsequent biological experiments. The wastewater samples were subjected to thermal pretreatment in a laboratory oven (Mettler UFE 400, Schwabach, Germany) set at 100 °C. The samples, placed in glass flasks, were heated for 1.5 h, during which the internal temperature of the wastewater gradually increased and reached approximately 75 °C at the end of the heating period. After thermal treatment, the samples were allowed to cool to room temperature and were subsequently stored at 4 °C until further use in the subsequent experimental procedures. This thermal pretreatment was applied to reduce the microbial load and minimize the potential influence of indigenous microorganisms on the cultivation conditions of the microalgae. Subsequently, the heat-treated wastewater underwent ultrasonic treatment using an Ultrasonicator UP400St (Hielscher, Teltow, Germany). This step was applied to disrupt residual particulate matter, including protein and fat aggregates, as well as microbial flocs. The process was carried out for 3 min per 2.5 L sample at 70% amplitude, corresponding to an output power of approximately 100 W. The pretreated wastewater was then filtered through Whatman® qualitative filter paper (grade 470, 230 × 305 mm²) to remove residual solids. Finally, the processed wastewater was stored at 4 °C and used within 24 h of preparation.

2.2. *Scenedesmus obliquus* Inoculum and Cultivation System

The microalgal system was inoculated with *Scenedesmus obliquus* (CCALA 451), obtained from the Culture Collection of Autotrophic Organisms (CCALA), Třeboň, Czech Republic.

Initially, the culture was maintained in Z medium with the following composition (mg/L): NaNO₃ (480.0), Ca(NO₃)₂·4H₂O (59.0), K₂HPO₄ (31.0), MgSO₄·7H₂O (25.0), Na₂CO₃ (21.0), Fe-EDTA complex (10.0 mL/L) and micronutrient solution (0.08 mL/L).

The culture was scaled up under controlled laboratory conditions in an open bioreactor system. Cultivation was performed under continuous aeration (24 h day⁻¹) to ensure homogeneous mixing of the culture, prevent biomass sedimentation and provide sufficient gas exchange. The temperature was maintained at 25 °C and illumination was provided by LED light at an intensity of 648 µmol m⁻² s⁻¹ under a 12 h light/12 h dark photoperiod. The pre-cultured biomass was used as inoculum and introduced into the wastewater at the beginning of each experimental series.

2.3. Duckweed (*Lemna minor*) Source

Lemna minor biomass, originally supplied by Lemna Environmental Technologies, Inc. (LET), Vadnais Heights, MN, USA, was subsequently isolated from municipal wastewater treatment effluents in Skýcov, Slovakia. Following isolation, the plant material was thoroughly rinsed with deionized water to remove attached impurities. Cultivation was performed at 25 °C and illumination was provided by LED light at an intensity of 648 µmol m⁻² s⁻¹ under a 12 h light/12 h dark photoperiod. Fresh duckweed biomass was introduced at the beginning of each weekly experimental series.

2.4. Experimental Design

The experiment was conducted in laboratory-scale bioreactors with a diameter of 11.5 cm and a height of 14 cm, each operated at a working volume of 1 L. The reactors were continuously aerated with atmospheric air, supplied via an air pump (EHEIM air400, Deizisau, Germany) connected to each bioreactor through an individual outlet, without external CO₂ supplementation. The reactors were maintained at a controlled temperature of 25 °C. Illumination was provided by LED light at an intensity of 648 µmol m⁻² s⁻¹ under a 12 h light/12 h dark photoperiod.

Three experimental systems were established: (I) a microalgal system, (II) a duckweed system and (III) a hybrid system combining both organisms. In all systems, 550 mL of pretreated dairy wastewater was used as the growth medium. In the microalgal system, the wastewater was supplemented with 450 mL of *Scenedesmus obliquus* culture. In the duckweed system, the wastewater was supplemented with 450 mL of deionized water, and *Lemna minor* was introduced onto the surface of the wastewater without the addition of microalgae. In the hybrid system, 550 mL of wastewater was combined with 450 mL of *Scenedesmus obliquus* culture and *Lemna minor* was subsequently introduced onto the surface of the reactor.

The initial biomass of *Lemna minor* was 15.65 g fresh weight (FW). The microalgal inoculum was introduced at an initial chlorophyll-a concentration of 8.52 µg mL⁻¹ in both the monoculture and hybrid systems to ensure comparable starting conditions.

The experiment was conducted over three consecutive experimental series, each lasting 14 days and representing an independent run. Each biological system was replicated across all three series, resulting in three temporal replicates per system ($n = 3$).

Sampling was performed on days 1, 3, 7, 11 and 14. At each sampling point, 50 mL of wastewater was collected from each reactor and subsequently filtered prior to physicochemical analysis.

2.5. Chlorophyll-a Analysis

Chlorophyll-a pigment was extracted using ethanol and quantified spectrophotometrically (DR 6000, Hach, Loveland, CO, USA) by measuring absorbance at 665 nm and 649 nm,

according to the method of Lichtenthaler and Wellburn [43]. Chlorophyll-a concentration was calculated using Equation (1):

$$\text{Chl a} = 13.95 A_{665} - 6.88 A_{649} \quad (1)$$

where A_{665} and A_{649} represent the absorbance at 665 nm and 649 nm, respectively. All measurements were performed in triplicate.

2.6. Analytical Methods

Physicochemical parameters of wastewater were analyzed using standardized methods. TN, $\text{NO}_3\text{-N}$, $\text{NH}_4\text{-N}$, $\text{PO}_4\text{-P}$, K, COD and TOC were determined using Hach cuvette test kits (Hach, Loveland, CO, USA) according to the manufacturer's instructions and measured with a DR 6000 spectrophotometer (Hach, Loveland, CO, USA). Electrical conductivity and pH were measured using a Hach Pocket Pro+ multi-parameter meter (Hach, Loveland, CO, USA). Turbidity was determined using a turbidimeter (PCcompact AQUA LYTIC, Dortmund, Germany).

The treatment efficiency of each biological system was evaluated based on the removal of pollutants from the wastewater. Removal efficiency was calculated as the difference between the initial (day 1) and final (day 14) wastewater sample concentrations, while removal percentage was calculated according to Equation (2):

$$\text{Removal (\%)} = ((C_{\text{initial}} - C_{\text{final}})/C_{\text{initial}}) \times 100 \quad (2)$$

where C_{initial} represents the initial concentration of a given parameter in the wastewater and C_{final} represents the concentration measured after biological treatment.

2.7. Biomass Harvesting, Elemental Characterization and Agronomic Evaluation of the Recovered Biomass

At the end of the wastewater treatment experiment, the settled biomass consisting of *Scenedesmus obliquus* and residual *Lemna minor* was collected from the cultivation reactors using a peristaltic pump. The harvested biomass was divided into two portions. The fraction intended for use as a liquid biofertilizer was sieved to remove residual *L. minor* biomass, yielding a suspension composed predominantly of *S. obliquus*, potentially containing trace amounts of dissolved exudates or fine plant-derived particulates originating from *L. minor*. The chlorophyll-a concentration of this suspension was determined as $11.19 \mu\text{g mL}^{-1}$. This portion was used directly in liquid form for subsequent biofertilizer application, while the second portion was dried at 100°C for 1 h in a laboratory oven for elemental characterization.

The elemental composition of the dried biomass was determined through X-ray fluorescence (XRF) spectroscopy using an Olympus Vanta VCA analyzer (Olympus Scientific Solutions Americas, Waltham, MA, USA). The concentrations of macro- and micronutrients and selected trace elements were expressed as mg kg^{-1} dry weight.

The agronomic potential of the recovered biomass was evaluated in a pot experiment using lettuce (*Lactuca sativa* L.) as a model crop. The study was conducted using three lettuce cultivars, including Dubagold, Green Pick and Lollo Rosso. Seeds were sown in a commercial horticultural substrate and cultivated under controlled environmental conditions with constant temperature and humidity, using LED lighting at an intensity of $648 \mu\text{mol m}^{-2} \text{s}^{-1}$ under a 12 h light/12 h dark photoperiod.

The liquid biomass was applied directly to the root zone as a soil drench to maximize the availability of nutrients and bioactive compounds. Three experimental treatments were established: (I) a control treatment irrigated with water only, (II) a single dose treatment

receiving 2 mL of microalgal suspension per plant once per day, and (III) a double-dose treatment receiving 4 mL of microalgal suspension per plant once per day. The first application was performed 13 days after sowing, immediately following the initial measurement of plant height and was subsequently repeated at daily intervals for three consecutive weeks.

Each treatment consisted of five biological replicates per cultivar, resulting in a total of 45 experimental units (3 cultivars $\times$ 3 treatments $\times$ 5 replicates). Plant height was measured in millimeters at weekly intervals over a four week cultivation period. No additional fertilizers or nutrient supplements were applied during the experiment.

2.8. Data Analysis

All data are expressed as mean $\pm$ standard deviation. Dairy wastewater pretreatment and cultivation experiments were performed in three independent replicates per treatment ($n = 3$), while the agronomic experiment used five biological replicates per treatment $\times$ lettuce cultivar combination ($n = 5$; 45 experimental units in total).

Statistical analyses were performed using GraphPad Prism (version 11, GraphPad Software, San Diego, CA, USA). Prior to analysis, all datasets were tested for normality using the Shapiro–Wilk test.

One-way ANOVA with Tukey's HSD post hoc test was used to evaluate (I) differences in physicochemical properties of dairy wastewater among consecutive pretreatment steps and (II) differences in removal efficiencies of COD, TOC, NO₃-N and PO₄-P, as well as in chlorophyll-a concentration, among the three cultivation systems (*S. obliquus*, *L. minor* and the mixed system).

Two-way ANOVA was applied to two independent datasets. For the wastewater cultivation kinetics, cultivation system $\times$ cultivation time were used as factors to evaluate changes in COD, TOC, pH, NO₃-N and PO₄-P among the three cultivation systems over the 14-day period. For the agronomic experiment, treatment $\times$ lettuce cultivar were used as factors to evaluate final (week 4) plant height. Where a significant main effect or interaction was detected, Tukey's HSD post hoc test was applied for multiple comparisons. Statistical significance was accepted at $p < 0.05$.

3. Results and Discussion

3.1. Physicochemical Characteristics of Dairy Wastewater After Pretreatment

As shown in Table 1, the sequential pretreatment strategy significantly influenced the physicochemical composition of dairy wastewater (one-way ANOVA, $p < 0.05$), confirming its capacity to modify the medium prior to microalgal cultivation. Raw dairy wastewater was characterized by an acidic pH, moderate conductivity and elevated turbidity attributable to suspended solids, emulsified fats, proteins, and colloidal particles typical of dairy effluents [3]. Concurrently, substantial nitrogen and phosphorus concentrations, together with a high organic load expressed as COD and TOC, reflected its strong potential for nutrient recovery. However, the combination of high turbidity and organic matter burden poses a recognized limitation for photosynthetically driven treatment, as excessive light attenuation constrains microalgal productivity [44]. Comparable constraints have been documented for piggery wastewater, where photo-Fenton oxidation achieved >90% removal of most parameters and generated conditions most favorable for *Coelastrella* sp. cultivation [45], underscoring the critical role of targeted pretreatment in highly loaded agro-industrial effluents.

Heat treatment was applied to inactivate contaminating microorganisms and stabilize cultures, following approaches used in studies employing autoclaved swine wastewater as microalgal cultivation medium [27,46]. The increase in pH and conductivity reflects the release of dissolved ionic species [28], while reduced turbidity indicates coagulation

and settling of suspended solids during heating [29]. Concurrent increases in TN, $\text{NH}_4\text{-N}$ and $\text{PO}_4\text{-P}$ confirm conversion of bound nutrients into soluble, bioavailable forms [30]. The observed COD rise is attributed to solubilization of organic matter [31], whereas TOC reduction suggests thermal degradation of labile carbon compounds [32].

Table 1. Physicochemical properties of dairy wastewater after consecutive pretreatment steps. Values are presented as mean $\pm$ SD ($n = 3$). Different superscript letters within the same row indicate statistically significant differences among treatments according to one-way ANOVA followed by Tukey's HSD post hoc test ($p < 0.05$).

Parameter	Raw Wastewater	Wastewater After Heat Treatment	Wastewater After Ultrasound	Wastewater After Filtration
pH	5.84 ± 0.01^d	5.97 ± 0.01^c	6.03 ± 0.01^b	6.07 ± 0.01^a
Conductivity [ms cm^{-1}]	4.62 ± 0.01^c	5.03 ± 0.01^a	4.75 ± 0.02^b	4.75 ± 0.02^b
Turbidity [NTU]	793 ± 2^c	676 ± 2^d	1882 ± 4^a	1125 ± 3^b
TN [mg L^{-1}]	136.7 ± 2.1^c	169.7 ± 1.5^a	162.7 ± 2.5^b	134.0 ± 3.5^c
$\text{NO}_3\text{-N}$ [mg L^{-1}]	10.60 ± 0.03^a	9.85 ± 0.07^b	8.61 ± 0.02^c	8.23 ± 0.05^d
$\text{NH}_4\text{-N}$ [mg L^{-1}]	19.00 ± 0.53^c	25.60 ± 0.36^b	27.07 ± 0.32^a	28.03 ± 0.67^a
$\text{PO}_4\text{-P}$ [mg L^{-1}]	35.73 ± 0.23^b	41.10 ± 0.72^a	41.17 ± 0.67^a	19.83 ± 0.25^c
K [mg L^{-1}]	41.17 ± 0.76^a	38.00 ± 0.62^{ab}	34.33 ± 3.51^b	28.47 ± 0.78^c
COD [mg L^{-1}]	4246 ± 36^d	6231 ± 30^b	6574 ± 22^a	5635 ± 5^c
TOC [mg L^{-1}]	2080 ± 52^a	1548 ± 29^b	1354 ± 115^c	1369 ± 69^{bc}

Subsequent ultrasound treatment was introduced to further disrupt residual particles and microbial flocs via acoustic cavitation, enhancing nutrient accessibility. Consistent with previous reports showing ultrasound-driven increases in COD, protein and reducing sugar concentrations in organic waste streams [33], sonication caused a marked turbidity rise in the present study due to fragmentation and re-suspension of fine particles [34]. The accompanying COD increase confirms the release of organic matter, while changes in nitrogen forms suggest redistribution rather than loss: $\text{NH}_4\text{-N}$ increased due to mineralization, whereas $\text{NO}_3\text{-N}$ declined, likely due to inhibition of nitrifying microorganisms [35,36]. Elevated phosphate concentrations persisted, consistent with disruption of phosphorus complexes and prevention of re-precipitation [37].

The final filtration step was intended to remove residual particulate matter generated during the preceding thermal and ultrasonic treatments. Relative to the ultrasonicated wastewater, filtration reduced turbidity, COD, TN, $\text{NO}_3\text{-N}$, $\text{PO}_4\text{-P}$ and K. However, final turbidity remained considerably higher than in raw wastewater. This indicates that a substantial fraction of the fine colloidal particles generated by ultrasonic cavitation was not retained by the qualitative filter paper used. In contrast, $\text{NH}_4\text{-N}$ concentration did not decrease following filtration and instead remained slightly elevated relative to the ultrasonicated sample, consistent with ammonium being a small, freely dissolved ionic species that is not expected to be removed by a purely mechanical filtration step. The decreases observed in $\text{PO}_4\text{-P}$, TN and K following filtration likely reflect the removal of particulate-associated or precipitated fractions of these nutrients together with the retained solids. Similar filtration approaches have been widely applied as preparatory steps before microalgal wastewater cultivation, improving optical properties and operational stability of the cultivation medium [21,27]. Therefore, although some colloidal material and dissolved ammonium remained after filtration, the pretreated wastewater was considered suitable for subsequent use as a cultivation medium for microalgal growth. Overall, the applied pretreatment altered the distribution of nutrients between dissolved and particulate fractions, sonication increased the availability of some nutrient fractions by releasing intracellular and particle-bound compounds, while filtration subsequently removed part of the particulate-associated fraction. Consequently, the nutrient removal performance reported in this study reflects the behavior of pretreated dairy wastewater specifically and

may differ from that expected for untreated effluents. The applicability of these findings is limited to wastewater subjected to similar pretreatment conditions.

However, these improvements were achieved at a measurable energy cost, which should be considered when evaluating the overall feasibility of the process. Based on the rated power of the laboratory equipment used, the applied pretreatment (thermal treatment, ultrasonication and filtration) required an estimated 2.10 kWh L^{-1} of pretreated wastewater (2.10 kWh L^{-1} for heating, 0.002 kWh L^{-1} for ultrasonication, and negligible input for manual filtration) equivalent to approximately 2100 kWh m^{-3} . At an average electricity price of $\text{€}0.1761 \text{ kWh}^{-1}$ for household consumers in Slovakia [47], this corresponds to an estimated cost of $\sim\text{€}370$ per m^3 of treated wastewater, a value substantially higher than the typical energy demand of conventional aeration in activated sludge systems, reported at $0.3\text{--}0.6 \text{ kWh m}^{-3}$ for large-scale plants, including those in Slovakia [48]. This reflects the inefficiency of small-batch heating in a general-purpose laboratory oven rather than an inherent requirement of the pretreatment itself and could be considerably reduced through process intensification at larger scale.

3.2. Organic Matter Removal (COD and TOC)

COD and TOC declined progressively across all three biological systems over the 14-day cultivation period (Figure 1a,b and Table 2), confirming that *Scenedesmus obliquus*, *Lemna minor* and their co-culture each substantially reduced the organic load of pretreated dairy wastewater. Two-way ANOVA indicated that both cultivation system and time significantly affected COD dynamics ($p < 0.001$), with a significant interaction between these factors, demonstrating system-dependent removal patterns.

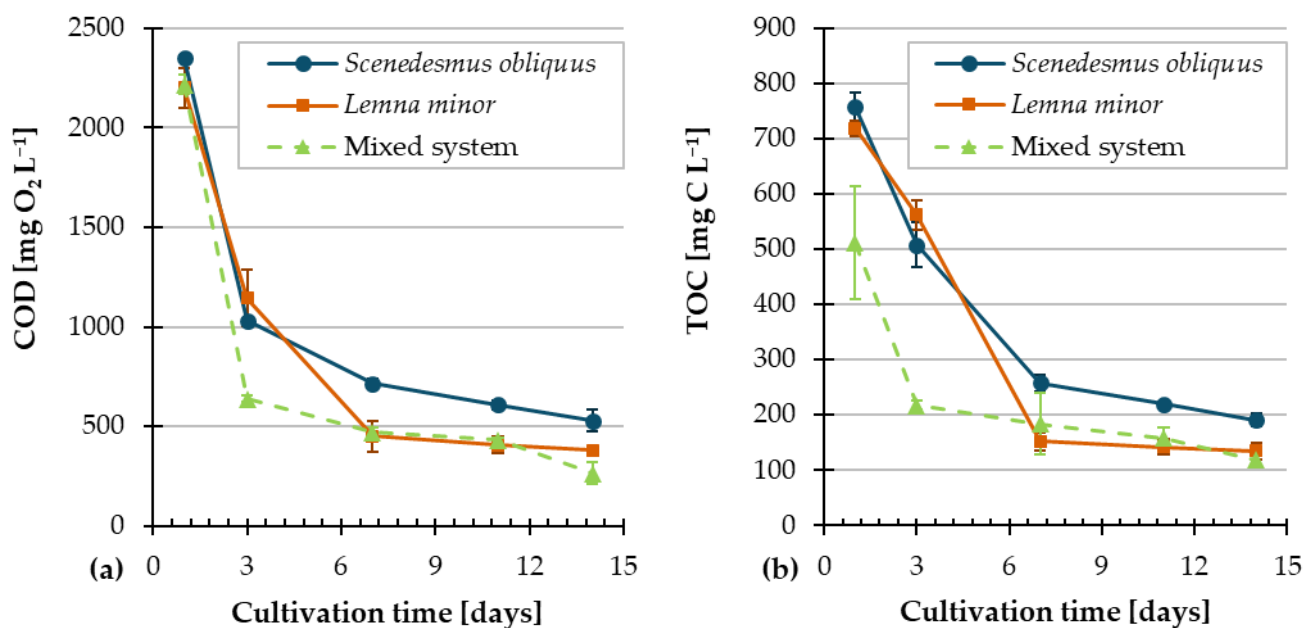


Figure 1. Changes in (a) chemical oxygen demand and (b) total organic carbon during a 14-day cultivation of pretreated dairy wastewater using *Scenedesmus obliquus*, *Lemna minor* and their mixed culture. Values are presented as mean $\pm$ SD ($n = 3$).

Final COD removal reached 90.6% for *S. obliquus*, 92.8% for *L. minor* and 95.3% for the mixed system, which attained the lowest residual concentration of 263.0 mg L^{-1} . These differences between systems were statistically significant, as confirmed using post hoc analysis ($p < 0.001$). The superior performance of the co-culture relative to individual mono-cultures reflects complementary degradation mechanisms: *S. obliquus* assimilates soluble organic carbon and inorganic nutrients via mixotrophic metabolism under illuminated

conditions [49], while *L. minor* takes up dissolved organic matter through its root system and stimulates microbial mineralization in the rhizosphere [50]. The advantage of the mixed system was evident from the early stage of cultivation. By day 3, it exhibited significantly lower COD concentrations compared to both monocultures ($p < 0.0001$), indicating accelerated initial removal kinetics and mirroring the early-phase advantages of integrated microalgae–duckweed systems reported before, where a combined approach achieved 95% COD reduction over 15 days in aquaculture wastewater [21].

Table 2. Removal efficiencies (%) of COD, TOC, NO₃-N, PO₄-P and turbidity achieved by different cultivation systems (*S. obliquus*, *Lemna minor* and mixed system). Values are presented as mean $\pm$ standard deviation ($n = 3$). Different letters within a column indicate statistically significant differences between systems according to one-way ANOVA followed by Tukey’s HSD test ($p < 0.05$).

System	COD [%]	TOC [%]	NO ₃ -N [%]	PO ₄ -P [%]	Turbidity [%]
<i>S. obliquus</i>	90.6 $\pm$ 1.0 ^c	86.1 $\pm$ 1.0 ^b	75.7 $\pm$ 4.0 ^b	92.0 $\pm$ 1.5 ^b	90.6 $\pm$ 1.8 ^b
<i>Lemna minor</i>	92.8 $\pm$ 0.2 ^b	90.2 $\pm$ 1.1 ^{ab}	83.4 $\pm$ 0.9 ^a	93.2 $\pm$ 0.4 ^{ab}	96.5 $\pm$ 0.2 ^a
Mixed system	95.3 $\pm$ 0.9 ^a	91.3 $\pm$ 0.9 ^a	97.1 $\pm$ 0.4 ^a	94.6 $\pm$ 0.5 ^a	92.2 $\pm$ 0.5 ^b

From day 7 onward, *L. minor* monoculture consistently outperformed *S. obliquus*, reaching a final COD concentration of 408.3 mg L^{−1} compared to 528.3 mg L^{−1} for the microalgal system. The comparatively lower removal efficiency of *S. obliquus* during the initial phase may be associated with light attenuation caused by high initial turbidity, which would constrain photosynthetic CO₂ fixation and associated organic carbon assimilation [51,52]. In contrast, *L. minor* grows at the water surface and accesses incident light independently of water clarity, conferring an inherent treatment advantage under turbid conditions. The high COD removal capacity of duckweed based systems is well supported by batch studies reporting up to 59.34% COD reduction [26], while the broader literature for *S. obliquus* cultivated in simulated dairy effluent documents removals of up to 95% [53], with a consortium of *Chlorella variabilis* and *S. obliquus* reaching only 54.72% under optimized conditions [54], suggesting that the macrophytic component of the present mixed system meaningfully augmented overall organic matter degradation.

Despite the substantial organic matter removal achieved across all systems, the final COD concentrations of 528.3 mg L^{−1} (*S. obliquus*), 408.3 mg L^{−1} (*L. minor*) and 263.0 mg L^{−1} (mixed system) remained relatively high in absolute terms. This outcome is consistent with observations from single-stage microalgal treatment of high-strength dairy wastewaters, where COD reductions of 70–92% have been reported but final effluent concentrations frequently exceed regulatory thresholds when applied to undiluted effluents [6,55]. For context, a recent study cultivating *Chlorella sorokiniana* in 100% undiluted moderate COD dairy wastewater achieved biomass productivity of 0.16 g L^{−1} d^{−1} but noted that COD compliance required a 50% dilution pretreatment [56]. Taken together, these findings indicate that the evaluated biological systems function more effectively as an on-site pretreatment stage at the dairy facility, prior to discharge into the municipal sewerage network and further treatment at a municipal wastewater treatment plant, rather than as standalone polishing units capable of achieving direct environmental discharge compliance.

TOC dynamics closely followed COD trends with final removal efficiencies of 86.1% for *S. obliquus*, 90.2% for *L. minor* and 91.3% for the mixed system and the lowest residual concentration (119.0 mg C L^{−1}) observed in the co-culture. Similar to COD, two-way ANOVA confirmed significant effects of system and time, as well as their interaction ($p < 0.001$), indicating system-dependent differences in removal dynamics. However, in contrast to COD, TOC removal exhibited an earlier stabilization phase. No statistically significant differences were observed between days 7 and 11 ($p = 0.581$) or between days

11 and 14 ($p = 0.556$), indicating that the majority of biodegradable organic carbon was removed by approximately day 7, after which further reduction was limited.

3.3. Nutrient Removal

Over the 14 day cultivation period (Figure 2a) nitrate nitrogen removal efficiencies reached 75.7% for *S. obliquus*, 83.4% for *L. minor* and 97.1% for the mixed system (Table 2). Two-way ANOVA indicated that both cultivation system ($p < 0.001$) and time ($p < 0.001$) significantly influenced $\text{NO}_3\text{-N}$ concentrations.

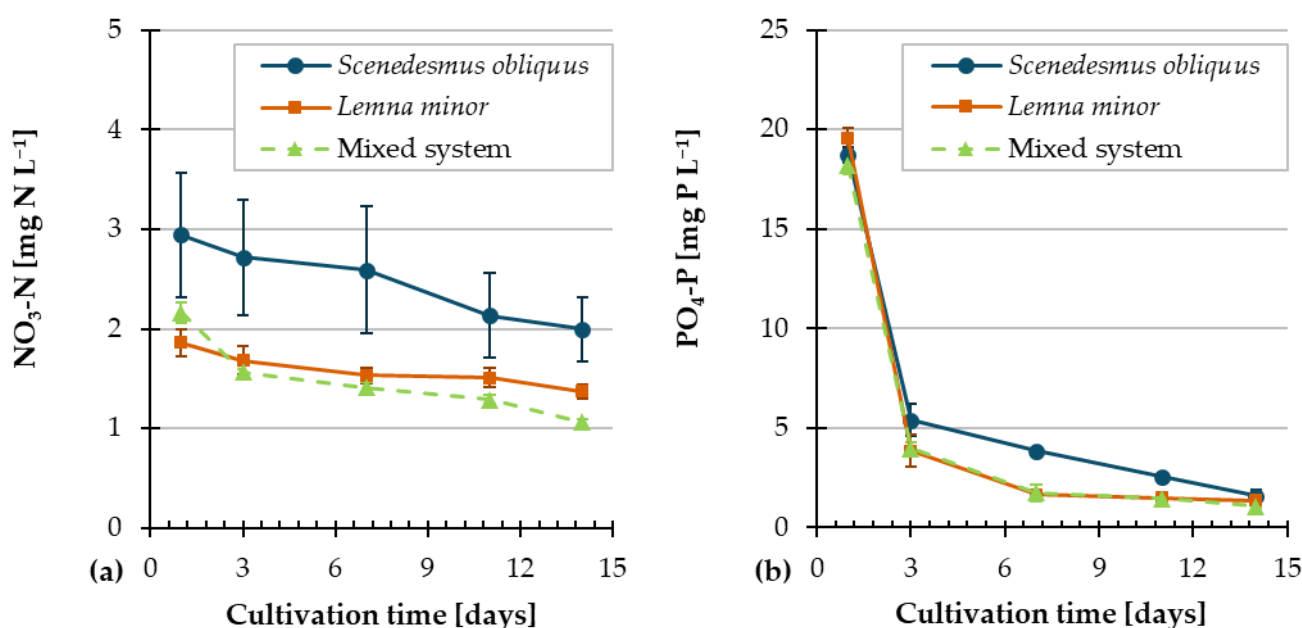


Figure 2. Changes in (a) nitrate nitrogen and (b) orthophosphate phosphorus concentrations during 14-day treatment of pretreated dairy wastewater using *Scenedesmus obliquus*, *Lemna minor* and their mixed culture. Values are presented as mean $\pm$ SD ($n = 3$).

The comparatively modest removal by the *S. obliquus* monoculture contrasts with the substantially higher total nitrogen removals documented for this species in other matrices approximately 90% from piggery slurry under UV domestication [57] and up to 88.5% in brewery wastewater at extended hydraulic retention times [58] but is most plausibly explained by the low initial $\text{NO}_3\text{-N}$ concentrations in the present medium ($8.23 \pm 0.05 \text{ mg N L}^{-1}$). Its relatively low concentration reduces the driving force for nitrogen uptake and therefore limits removal efficiency, rather than indicating a fundamental limitation of the species itself. It is also relevant that many cited studies report total nitrogen removal, which integrates ammonium and organic nitrogen species that are assimilated more rapidly by microalgae [59], whereas the present study measured only the nitrate fraction. *Lemna minor* outperformed *S. obliquus* throughout the experiment, consistent with comparative studies in which duckweed consistently exceeded microalgae (*Chlorella vulgaris* and *Scenedesmus quadricauda*) in total nitrogen removal from citrus nursery wastewater under non-optimized conditions, attributed to the macrophyte's high intrinsic growth rate and efficient nutrient assimilation [60]. The capacity of duckweed to incorporate both nitrate and ammonium directly into plant biomass via high-affinity transport systems [61] likely underpins the greater nitrate depletion observed in *L. minor* and mixed treatments, particularly during the later cultivation phase when concentrations were lowest. The $83.4 \pm 0.9\%$ removal recorded for *L. minor* in the present study exceeds the values of 24.8% in undiluted and 65.4% in diluted real dairy wastewater reported elsewhere [62]. No significant differences were

observed between *L. minor* and the mixed system ($p = 0.715$), indicating duckweed-driven nitrate uptake. Comparable combined algae–duckweed systems show similarly variable nitrogen performance. Kotoula et al. [21] reported 88% total nitrogen removal using a sequential *C. sorokiniana*–*L. minor* system for municipal wastewater, whereas a combined *L. minor*–*Chlamydomonas incerta* system treating palm oil mill effluent achieved only 11.3 and 12.5% for $\text{NH}_3\text{-N}$ and NO_3^- [23]. This contrast highlights the strong matrix-dependence of nitrogen removal in such systems, limiting direct comparison across studies. Integrated microalgae–duckweed systems can achieve >90% $\text{NH}_4\text{-N}$ removal due to complementary physiology [63]. However, nitrate dominance and short cultivation time likely limited such synergy here.

Phosphorus removal was highly efficient across all systems, with PO_4P removal exceeding 90% in every treatment over 14 days: 92% for *S. obliquus*, 93.2% for *L. minor* and 94.6% for the mixed system (Table 2). Two-way ANOVA confirmed significant effects of both system and time ($p < 0.001$), as well as their interaction ($p < 0.001$), indicating system-dependent removal dynamics. The most dynamic phase of removal occurred during the first three days, when concentrations dropped from 19.83 mg P L^{−1} to 3.85–5.40 mg P L^{−1} across all treatments (Figure 2b), consistent with the well-documented luxury phosphorus uptake phenomenon in both microalgae and aquatic macrophytes, whereby cells accumulate polyphosphate reserves exceeding immediate metabolic requirements when phosphate is abundant [64]. Phosphorus starved microalgae exhibit 2–5 fold increases in alkaline phosphatase activity enabling rapid phosphate assimilation [65], a mechanism that likely contributed to the observed burst uptake across all systems during early cultivation. From day 7 onward, duckweed systems achieved significantly lower $\text{PO}_4\text{-P}$ concentrations than the *S. obliquus* monoculture ($p < 0.001$), indicating faster intermediate-phase removal kinetics. This difference persisted until day 11, after which all treatments converged to similarly low residual phosphate concentrations. The rapid phosphate assimilation characteristic of *Lemna* species is partly attributable to the large surface-area-to-volume ratio of frond tissue and direct contact with the surrounding medium, enabling efficient passive and active uptake [10]. Incorporation of *L. minor* into a sequential anaerobic–aerobic bioreactor treating dairy wastewater has previously been shown to increase total phosphorus remediation from 58.5% to 73% [16], reinforcing the particular contribution of duckweed to phosphorus removal in combined biological systems. By day 11–14, however, phosphorus removal stabilized, with no significant differences between these time points ($p = 0.096$). At the end of the cultivation period, all systems converged to similarly low residual concentrations (1.07–1.59 mg P L^{−1}), with no significant differences among treatments ($p > 0.05$), demonstrating that *S. obliquus* ultimately achieved comparable phosphorus removal to duckweed–based systems over the full cultivation period, albeit through slower kinetics. The phosphorus removal efficiencies obtained here are consistent with the upper range reported for combined microalgae–duckweed systems, such as the 91% total phosphorus removal achieved by a sequential *C. sorokiniana*–*L. minor* system treating municipal wastewater [21], and considerably exceed the 70.47% maximum $\text{PO}_4\text{-P}$ removal reported for a combined *L. minor*–*Chlamydomonas incerta* system in palm oil mill effluent [23], further supporting the efficiency of the present simultaneous co-cultivation approach.

It is also important to note that the *S. obliquus* inoculum was introduced together with its growth medium, corresponding to approximately 86 mg L^{−1} N and 5.5 mg L^{−1} P in the undiluted medium. Following dilution into the 1 L reactor volume, this represents an estimated additional contribution of ~39 mg L^{−1} N and ~2.5 mg L^{−1} P to the microalgal and hybrid systems, alongside the nutrient load supplied by the wastewater itself. This confound is particularly relevant to the modest nitrate removal reported above for the *S. obliquus* monoculture, as part of the observed decrease in N and P concentrations may reflect

uptake of nutrients originating from the growth medium rather than exclusively from the wastewater. In the duckweed-only system, dilution was performed using deionized water, which did not introduce additional nutrients, so nutrient removal in this system can be attributed directly to the wastewater matrix. Future studies could use washed/centrifuged microalgal biomass resuspended in an inert medium to eliminate this confounding factor.

3.4. Changes pH During Cultivation

A progressive alkalization was observed across all treatments, with pH increasing from 6.07 to 9.75–10.08 by day 14 (Figure 3a). Two-way ANOVA showed significant effects of time ($p < 0.001$) and a weaker but significant effect of system ($p = 0.049$), with no significant interaction ($p = 0.073$), indicating that the temporal trend of pH change followed a broadly similar pattern across systems, despite differing overall mean pH levels between them. This pattern likely reflects an initial phase of active biomass growth during the first 7 days, followed by reduced growth activity, consistently across all systems. Early pH increases were uniform across systems, with values of approximately 8.9 in all treatments by days 1–3 ($p > 0.05$), reflecting similarly vigorous photosynthetic CO₂ assimilation during the acclimation phase. This photosynthetically driven alkalization arises from cellular proton uptake during water photolysis and the plastoquinone redox cycle, with the rate of increase proportional to light intensity and cell concentration [66]. Significant divergence emerged at day 11, when the mixed system reached pH 10.56, *L. minor* pH 10.45 and *S. obliquus* a significantly lower pH of 9.56 (*S. obliquus* vs. *L. minor*: $p = 0.0309$; *S. obliquus* vs. mixed: $p = 0.0085$). The higher pH in duckweed systems reflects the additive photosynthetic activity of both organisms in the mixed treatment and the more rapid metabolic turnover of *L. minor* documented throughout the experiment. By day 14, pH stabilized at 9.75–10.08 across all systems with no significant intertreatment differences ($p > 0.05$), consistent with progressive nutrient depletion attenuating photosynthetic activity. The alkaline conditions sustained in the later cultivation phase represent an operational co-benefit of these biological systems, as elevated pH has been associated with substantial reductions in fecal bacteria and pathogenic coliforms, extending treatment performance beyond nutrient removal.

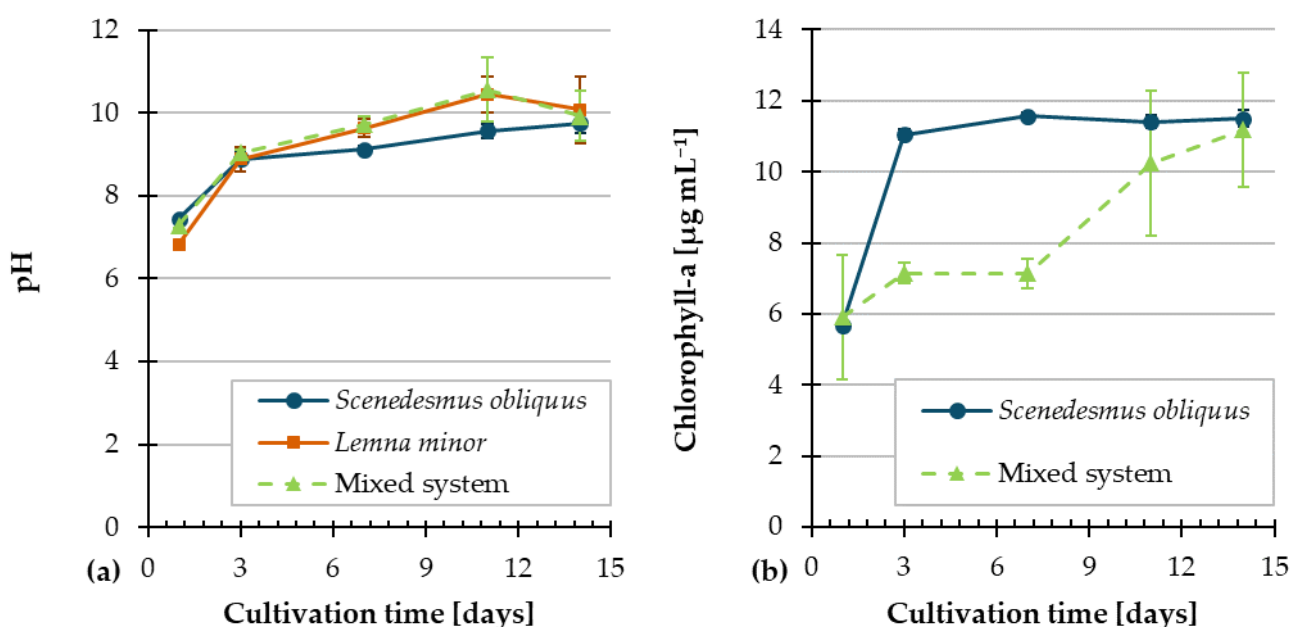


Figure 3. Changes in (a) pH and (b) chlorophyll-a concentration during 14-day treatment of pretreated dairy wastewater using *Scenedesmus obliquus*, *Lemna minor* and their mixed culture. Values are presented as mean $\pm$ SD ($n = 3$).

3.5. Biomass Development and Chlorophyll-a Dynamics

3.5.1. *Lemna minor* Biomass Development

L. minor exhibited clear biomass accumulation over the cultivation period, with greater final fresh weight in monoculture compared to the mixed system, most likely due to interspecific competition with *S. obliquus* for nutrients and light. Despite this, the maintenance of full surface coverage indicates stable growth and high adaptability under the applied wastewater conditions. The observed biomass production is in line with previously reported growth rates for *L. minor* in nutrient-rich wastewaters ($0.14\text{--}0.35\text{ d}^{-1}$), where performance is primarily driven by nutrient availability [67,68].

3.5.2. Chlorophyll-a Dynamics

Chlorophyll-a dynamics confirmed active *S. obliquus* growth, with significant effects of system ($p = 0.003$) and time ($p < 0.001$), but no interaction ($p = 0.055$), indicating similar temporal patterns across treatments. Comparable initial concentrations on day 1 reflect consistent inoculation and early acclimation to the wastewater matrix (Figure 3b). The rapid increase in chlorophyll-a in monoculture from $\sim 5.6\text{ }\mu\text{g mL}^{-1}$ to $\sim 11.0\text{ }\mu\text{g mL}^{-1}$ by day 3, followed by a plateau at $\sim 11.6\text{ }\mu\text{g mL}^{-1}$ by day 7, is consistent with typical growth kinetics of *S. obliquus* in nutrient-rich wastewaters. This pattern corresponds to a short lag phase (days 2–7) and subsequent pigment accumulation reaching maximum cell density around day 7 [69]. The stabilization observed here suggests the onset of growth limitation, likely due to light attenuation or self-shading in denser cultures. Reported maximum chlorophyll-a levels of $18.46\text{ }\mu\text{g mL}^{-1}$ in diluted swine wastewater [11] indicate that the values obtained in this study fall within the expected physiological range, although below peak levels, likely due to system-specific constraints.

Chlorophyll-a in the mixed system was significantly lower than in monoculture during the early phase (7.15 ± 0.28 vs. $11.05 \pm 1.74\text{ }\mu\text{g mL}^{-1}$ at day 3, $p = 0.0303$; 7.14 ± 0.40 vs. $11.57 \pm 1.87\text{ }\mu\text{g mL}^{-1}$ at day 7, $p = 0.0118$), likely due to shading by *L. minor* (Figure 3b). Previous studies show duckweed reduces light availability and suppresses microalgal growth in co-cultures [66,70], with effects dependent on frond density and irradiance [71]. In this study, rapid *L. minor* growth likely intensified light limitation. Faster phosphorus uptake by duckweed may have further constrained *S. obliquus*, as phosphorus is essential for photosynthesis and chlorophyll synthesis and its depletion can inhibit growth and pigment production [72,73].

From day 7, chlorophyll-a in the mixed system increased and reached levels comparable to the monoculture by days 11–14 ($p > 0.05$), indicating recovery (Figure 3b). This reflects the ability of *Scenedesmus obliquus* to acclimate to suboptimal light by adjusting its photosynthetic machinery [74]. Reduced shading due to stabilization or partial senescence of *L. minor* likely further improved light availability. The convergence of chlorophyll-a confirms that co-cultivation did not cause long-term inhibition, but only a transient response. Similar resilience has been reported in integrated microalgae–duckweed systems, where stable microalgal growth is maintained alongside enhanced nutrient removal [63].

3.6. Valorisation of Harvested Biomass

Elemental analysis of the harvested biomass (Table 3) confirmed the accumulation of substantial macro- and micronutrient concentrations, supporting its potential use as a biofertilizer within a circular bioeconomy framework. The dominant macronutrient was calcium, followed by sulfur, iron, magnesium, potassium and phosphorus. The lead concentration ($2 \pm 0.7\text{ mg kg}^{-1}\text{ DW}$) was markedly below the threshold of $100\text{ mg kg}^{-1}\text{ DW}$ stipulated for organic fertilizers under EU Regulation 2019/1009 [75], indicating negligible contamination risk from this potentially toxic element. The overall elemental profile

is consistent with values reported for microalgal biomass cultivated on agro-industrial wastewaters [56] and confirms that the recovered material constitutes a nutrient-rich substrate suitable for agricultural reuse.

Table 3. Elemental composition of harvested biomass (mean $\pm$ SD, $n = 3$).

Parameter	Concentration [mg kg^{-1}]
Mg	8369 ± 1100
Si	2524 ± 48
P	4328 ± 29
S	$14,008 \pm 40$
K	8061 ± 24
Ca	$207,543 \pm 38$
Ti	137 ± 37
Mn	452 ± 9
Fe	$11,815 \pm 30$
Cu	23 ± 2
Zn	52 ± 1
Pb	2 ± 0.7

Final plant height after four weeks of cultivation is presented in Figure 4.

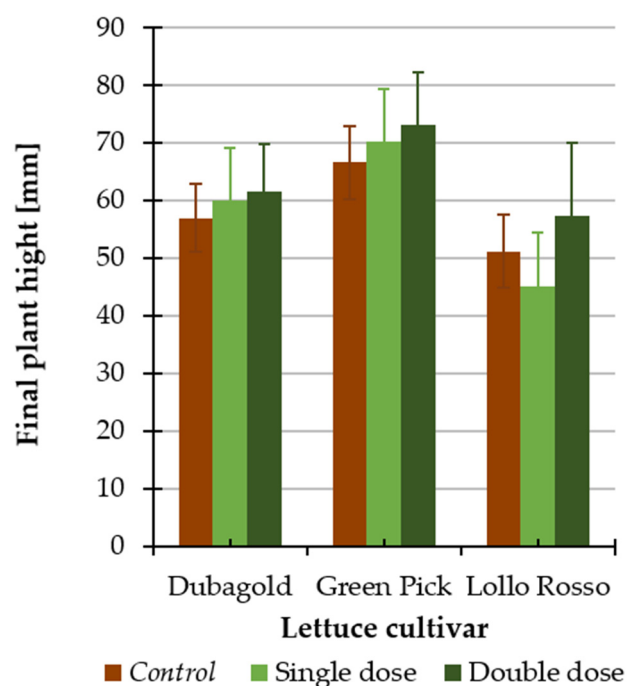


Figure 4. Final height of lettuce (*Lactuca sativa* L.) cultivars after application of liquid microalgal biomass. Values are presented as mean $\pm$ SD ($n = 5$). The same lowercase letters above bars indicate no statistically significant differences among treatments within each cultivar according to Tukey's HSD test ($p < 0.05$).

Application of the microalgal biomass did not result in statistically significant differences in final plant height within any cultivar after four weeks of cultivation (two-way ANOVA, Tukey's HSD, $p > 0.05$). In contrast, cultivar had a statistically significant effect on plant height ($p < 0.001$), whereas no significant interaction between cultivar and dose was observed ($p = 0.534$). Nevertheless, consistent positive trends were observed in two of the three cultivars. In Dubagold, mean final height increased from 54.4 ± 9.0 mm in the control to 60.0 ± 10.2 mm and 61.6 ± 8.0 mm in the single- and double-dose treat-

ments, respectively. In Green Pick, final height similarly increased from 66.6 ± 6.4 mm to 70.2 ± 10.1 mm and 73.2 ± 9.6 mm with increasing dose. These trends are consistent with the growth-promoting properties documented for wastewater-grown *S. obliquus* biomass, which has been reported to increase seed germination index by 40% and enhance cytokinin-like bioactivity by up to 187.5% relative to controls [12], effects attributable to the presence of phytohormone precursors and readily available macro- and micronutrients in the algal matrix. Similar observations were reported by Álvarez-Montero et al. [76], who demonstrated that *Scenedesmus* sp. cultivated in dairy wastewater significantly improved germination, plant height, and leaf development in *Phaseolus vulgaris*, confirming the agronomic potential of wastewater-derived microalgal biomass as a biofertilizer. In addition, Renganathan et al. [77] emphasized that microalgal biomass contains phytohormones, amino acids, polysaccharides and essential nutrients that can enhance plant growth, nutrient uptake and stress tolerance, with the magnitude of the response depending on the crop species and application dose.

Overall, the results demonstrate that biomass recovered from pretreated dairy wastewater treatment by *S. obliquus* and *L. minor* represents a nutrient-rich material with preliminary potential for biofertilizer applications and a positive effect on plant growth. Future research should include broader agronomic parameters, larger sample sizes and evaluation of higher application doses to confirm and enhance the observed effects. Assessment of nutrient content, heavy metal accumulation and scalable harvesting remains essential for practical implementation.

Several limitations of this study should be acknowledged. The system was evaluated under batch conditions only and the observed decline in removal efficiency after the initial treatment phase suggests that performance under semi-continuous or continuous operation requires further investigation. In addition, microalgal biomass was assessed indirectly using chlorophyll-a, which may introduce uncertainty due to physiological variability. The absence of ammonium monitoring limits interpretation of total nitrogen transformation pathways during cultivation. Furthermore, microbial community dynamics were not analyzed, restricting detailed insight into the underlying biological mechanisms governing pollutant removal. Finally, although the produced biomass showed potential for reuse as a biofertilizer, its safety and potential accumulation of contaminants were not evaluated. These aspects should be addressed in future studies to support scale-up and practical implementation of the proposed system.

4. Conclusions

This study demonstrated the feasibility of a simultaneous *Scenedesmus obliquus*–*Lemna minor* co-cultivation system as an integrated approach for dairy wastewater treatment, using wastewater pretreated by heat, ultrasound and filtration. The mixed system achieved 95.3% COD removal and 91.3% TOC removal, confirming its superior effectiveness in organic matter degradation. High nutrient removal was also obtained, reaching 87.1% for $\text{NO}_3\text{-N}$ and 94.6% for $\text{PO}_4\text{-P}$. These results indicate that the simultaneous presence of microalgae and duckweed promoted complementary removal mechanisms, combining biological assimilation, nutrient uptake, and physical clarification processes. Temporal analysis further demonstrated that the mixed system operated most efficiently during the initial cultivation phase, with the highest removal rates observed within the first 3–6 days; thereafter, the process gradually stabilized, with TOC removal reaching a plateau around day 7 and phosphorus removal around day 11. This behavior reflects the limitations of batch operation and suggests that process efficiency could be further improved through strategies such as periodic wastewater supplementation or semi-continuous operation. Despite these high removal efficiencies, final COD concentrations remained relatively high

in absolute terms, indicating that the mixed system is best suited as an on-site pretreatment step prior to discharge into the municipal sewerage network, rather than as a standalone solution. The sieved, microalgal-dominated suspension recovered from the mixed system did not result in statistically significant improvements in plant growth parameters; however, consistent positive trends observed in two of the three tested cultivars suggest preliminary potential for its reuse as a biofertilizer resource, warranting further validation. Overall, the integrated *S. obliquus*–*L. minor* system represents a promising, low-impact approach for dairy wastewater treatment, combining efficient pollutant removal with biomass valorization and supporting circular resource utilization.

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