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Sewage Sludge Stabilization and Biomass Enhancement via Indigenous Microalgae: Pilot-Scale Validation, Nutrient Dynamics, EPS Evolution, and Impacts on Sludge Properties

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Abstract

This study evaluates the performance of an indigenous microalgae-assisted aerobic digestion process for sewage sludge stabilization in a pilot-scale photobioreactor (20 L) operated over 20 days without external aeration. The system achieved 53% volatile solids reduction, exceeding the 38–40% threshold required by European stabilization guidelines, alongside 34% total nitrogen and 75% total phosphorus removal. Indigenous microalgal biomass increased substantially from 1.6% to 17% of total biomass, with photosynthetically generated oxygen serving as the primary driver of aerobic bacterial activity and organic matter mineralization. Sludge settleability improved markedly (SVI from 154 to 54 mL/g), accompanied by significant floc size reduction (220 to 33 μm); however, this was accompanied by a clear deterioration in dewaterability, with CST increasing from 16 to 27 s and SRF rising approximately 4-fold, representing a practical trade-off that must be addressed for full-scale application. Extracellular polymeric substances (EPS) analysis showed a clear shift from tightly bound to soluble and loosely bound fractions during treatment, and Pearson correlations indicated that soluble EPS was linked to poorer settling and dewatering, while tightly bound EPS was associated with larger flocs and better sludge structure, confirming EPS restructuring as a key mechanism behind the observed changes in sludge properties. These findings highlight both the strong stabilization potential of this process and the need for targeted dewatering mitigation strategies to support its scale-up.

Keywords: sewage sludge; microalgae-bacteria symbiosis; nutrient removal; photobioreactor; EPS; stabilization



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1. Introduction

Sewage sludge (SS) is an unavoidable byproduct of wastewater treatment plants. It consists of a complex mixture of water, organic matter, microorganisms, inorganic solids, nutrients, and various contaminants [1]. In Europe, annual SS production is estimated at approximately 13 million tons of dry solids, posing a significant management challenge that requires technically robust, sustainable, and cost-effective treatment and disposal strategies [2,3].

Currently, several technologies are applied for SS stabilization. However, the choice of a specific option generally depends on country-specific conditions, plant size, local regulations, available infrastructure, energy and investment costs, and the possibilities for final reuse or disposal of the treated sludge [4,5]. For small- to medium-sized wastewater

treatment plants (typically <2000 Population Equivalent (PE) and up to about 10,000 PE), aerobic digestion is often the preferred stabilization option because it is simple to operate, requires relatively low capital investment, and does not need complex process control [6]. Its principle is to maintain the sludge under aerated and endogenous conditions for approximately 15–20 days, during which microorganisms oxidize their own biodegradable solids into CO₂, water, and nitrate. This process reduces volatile solids but requires a high oxygen input, on the order of 2.3 kg O₂ per kg of volatile solids destroyed, which leads to substantial energy consumption for aeration [7]. These energy constraints highlight the need for alternative sludge-stabilization strategies that lower aeration demand while maintaining treatment performance. In this context, integrating microalgae into sludge treatment systems is particularly attractive because photosynthetic microalgae generate oxygen in situ to sustain aerobic bacterial degradation of organic matter and simultaneously use the CO₂ released for their own growth [8]. This algal-bacterial symbiosis can reduce the need for external aeration and limit net CO₂ emissions, making sludge stabilization more energy-efficient and environmentally friendly [9,10]. Moreover, conventional aerobic digestion is known to solubilize and redistribute nutrients such as N and P from the solid matrix into the liquid phase, which can deteriorate effluent quality [11]. On the other hand, microalgae and cyanobacteria exhibit a high capacity to assimilate inorganic nitrogen and phosphorus from wastewaters and sludge, thereby converting these nutrients into valuable biomass and achieving high removal efficiencies [12,13].

In a previous laboratory-scale study conducted in 1 L photobioreactors, the feasibility, applicability, and performance of microalgae-assisted aerobic sludge digestion were successfully demonstrated [9]. Key results included a 47.55% reduction in volatile solids, efficient removal of fecal coliforms and *E. coli* (2.25 log and 6.72 log, respectively), complete *Salmonella* eradication after 15 days, and a 71.42% improvement in sludge settleability. Indigenous microalgae biomass production reached 16.56 mg/L/day, representing 10.35% of the total final biomass. These promising laboratory-scale results motivated the present pilot-scale validation study.

The objective of this study was to evaluate the performance of an indigenous microalgae-assisted aerobic digestion process for sewage sludge at pilot scale. Focus areas included sludge stabilization efficiency, biomass production, nutrient dynamics (N and P), extracellular polymeric substances (EPS) evolution, and correlations with sludge properties, and resulting changes in sludge characteristics such as settleability and dewaterability.

2. Materials and Methods

2.1. Photobioreactor Description

This study utilized a photobioreactor (Figure 1) constructed from a vertical plexiglass tube (14 cm diameter × 150 cm length). The system included an agitation motor, illumination, and mixing in the reactor core, as well as an accessible sampling valve. The internal mixing mechanism consisted of a rotary arm carrying four helices evenly distributed along the height at 20 cm intervals. Eight daylight fluorescent tubes (36 W/840, Philips, Pila, Poland) surrounded the reactor to create a uniform lighting band, positioned at an average distance of 10 cm from the wall. The lighting system was controlled by a timer to set the photoperiod. This pilot study was conducted using a single 20 L photobioreactor, consistent with its exploratory, proof-of-concept objective of testing process feasibility at a 20-fold larger scale than our previous laboratory work. Biological replicates and a parallel conventional aerobic digestion control were not included in this first pilot trial; the reported values and correlations should therefore be read as a single-run demonstration of feasibility, to be confirmed through replicated trials in future work.



Figure 1. Illustration of the photobioreactor.

2.2. Raw Sludge

Sludge samples were collected from the secondary settling tank of the Grosses Battes wastewater treatment plant in Liège, Belgium. Treated effluent was also collected at the plant's outflow, just prior to its discharge into the Meuse River, and used for dilution. The key characteristics of both samples are presented in Table 1.

Table 1. Characteristics of raw sludge and treated wastewater.

Parameter	Raw Sludge	Treated Wastewater
pH	7.44 ± 0.01	7.7 ± 0.70
TS (g/L)	6.6 ± 0.346	-
VS (g/L)	2.06 ± 0.23	-
TSS (g/L)	4.2 ± 0.67	0.006 ± 0.002
VSS (g/L)	2 ± 0.1	-
DCO (mg O ₂ /L)	3402.5 ± 168.0	19.55 ± 0.63
SVI (mL/g)	105.14 ± 12.39	-
TP (mg/L PO ₄ ³⁻)	64.4 ± 2.42	0.15 ± 0.06
TN (mg/L)	195.5 ± 4.65	15.7 ± 0.13

2.3. Mix Preparation

Based on preliminary trials, an initial total suspended solids (TSS) concentration of 4.5 g/L was targeted. Raw sludge was therefore mixed with treated wastewater and diluted to achieve 4.5 ± 0.2 g TSS/L in the photobioreactor. Experiments were conducted in a temperature-controlled room at 22 °C, with continuous agitation at 112 rpm. The

illumination regime consisted of continuous light for the first 5 days, followed by 12 h light/12 h dark cycles for the remaining 15 days (total 20 days). Continuous light at the start maximized microalgal oxygen production and prevented sludge septicity, as preliminary tests showed much higher dissolved oxygen levels compared to light-dark cycles. Photo-synthetic photon flux density (PPFD) provided by fluorescent tubes at the reactor surface was $79.29 \pm 11.37 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ (PAR), measured using a quantum sensor (PQS-1, Kipp & Zonen, Delft, The Netherlands). To promote gas exchange (O_2/CO_2) between microalgae and bacteria while preventing exchange with ambient air, the photobioreactor was tightly sealed.

2.4. Biomass Determination

Total biomass concentration was quantified using a standard gravimetric procedure. Briefly, 10 mL of homogenized culture was filtered through pre-weighed $0.45 \mu\text{m}$ membrane filters, and the retained solids were dried at 105°C to constant weight. In the co-culture system, total biomass (CTB, g/L) corresponded to the sum of microalgal biomass (Cm , g/L) and sludge biomass (Cs , g/L): $\text{CTB} = \text{Cm} + \text{Cs}$.

Microalgal biomass (Cm) was estimated from chlorophyll-a (Chl-a). Pigments were extracted with 90% acetone, and the absorbance of the extracts was measured at 663, 645, 630, and 750 nm with a spectrophotometer, using 90% acetone as the blank. Chl-a concentration ($\mu\text{g/L}$) was then calculated using the following equation:

$$\text{Chl_a} = \frac{[11.64(\text{OD}_{663} - \text{OD}_{750}) - 2.16(\text{OD}_{645} - \text{OD}_{750}) + 0.10(\text{OD}_{630} - \text{OD}_{750})] \cdot V_1}{V \cdot d}$$

where V is the sample volume (L), V_1 the volume of acetone extract (mL), OD the optical density at each wavelength, and d the optical path length of the cuvette (1 cm).

Finally, Chl-a concentration values were converted to microalgal dry weights using a calibration curve: ($\text{DW} = 9.74 \cdot \text{Chl-a} - 0.09$, $R^2 = 0.97$), which relates Chl-a content to microalgal biomass; the corresponding standard curve is shown in Figure A1 (Appendix A).

2.5. EPS Extraction and Analysis

According to the procedure described by [14], 50 mL of the mixture was first centrifuged at $4000 \times g$ for 5 min; the resulting supernatant was collected and referred to as soluble EPS (S-EPS). The pellet was then resuspended in 50 mL of 0.05% NaCl preheated to 70°C , vortexed for 1 min, and centrifuged again at $4000 \times g$ for 10 min. This second supernatant was designated as loosely bound EPS (LB-EPS). The remaining pellet was resuspended in 50 mL of 0.05% NaCl, incubated at 60°C for 30 min, and centrifuged at $4000 \times g$ for 15 min to obtain the tightly bound EPS (TB-EPS). The 0.05% NaCl solution was used throughout the extraction to match the conductivity of the original supernatant and thus avoid artifacts in EPS composition. Before analysis, all three EPS fractions were passed through a $0.45 \mu\text{m}$ membrane filter and stored at 4°C for no longer than 48 h. Proteins were measured with a modified Lowry assay kit (VWR, Leuven, Belgium) using bovine serum albumin as the standard, while polysaccharides were quantified by the phenol-sulfuric acid method with glucose as the standard [15].

2.6. Analytical Methods

pH and dissolved oxygen (DO) were measured 5–6 h after the light phase onset using a pH meter and DO probe (Hach, Loveland, CO, USA). Total solids (TS), volatile solids (VS), total suspended solids (TSS), and sludge volume index (SVI) were determined according to Standard Methods. Microscopic observations were performed using an optical microscope (Leica DM LS2 DFC 280, Diegem, Belgium). Floc sizes were measured with a particle size analyzer (DelsaNano, Beckman Coulter, Brea, CA, USA) based on dynamic

light scattering; samples were diluted tenfold prior to analysis. Capillary suction time (CST) and Specific Resistance to Filtration (SRF) were determined using a Triton Electronics type 319 multi-CST apparatus (Triton Electronics Ltd, Dunmow, Essex, UK). Total phosphorus and orthophosphate were analyzed using LCK 350 cuvette tests (Hach Lange), total nitrogen with LCK 138, and Chemical Oxygen Demand (COD) with HI93754B-0 (Hanna Instruments). All nutrient and COD analyses were conducted on both mixed liquor and supernatant to track nutrient partitioning dynamics between phases. All measurements were performed in triplicate.

3. Results and Discussions

3.1. pH and OD Changes

During the 20-day stabilization period, the pH of the mixture rose from 6.86 to 9.7, while dissolved oxygen increased from 2.1 to 13.5 mg/L before declining to 9.3 mg/L (Figure 2). The pH rise is characteristic of active microalgal growth: during photosynthesis, microalgae consume CO_2 and bicarbonate, releasing hydroxyl ions (OH^-) that progressively alkalinize the medium. Nitrogen uptake, particularly nitrate assimilation, further reinforces this effect by releasing additional OH^- during nitrate reduction [16,17]. The excretion of basic metabolites from organic matter biodegradation also contributes to the observed pH increase [18].

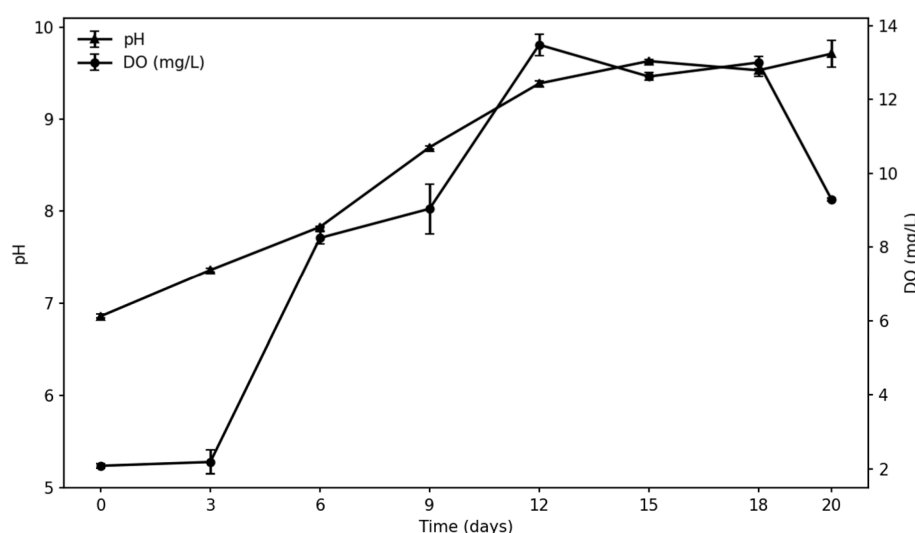


Figure 2. pH and dissolved oxygen changes during treatment.

The sharp increase in dissolved oxygen after day 6 (Figure 2) reflects a phase of intense microalgal photosynthesis, providing natural in situ aeration that activates aerobic bacteria involved in sludge stabilization. The subsequent decline suggests either reduced net photosynthesis or increased oxygen consumption by the microbial community as aerobic digestion intensifies. During this phase, heterotrophic bacteria oxidize organic matter according to $\text{C}_5\text{H}_7\text{NO}_2 + 7\text{O}_2 \rightarrow 5\text{CO}_2 + \text{NO}_3^- + 3\text{H}_2\text{O} + \text{H}^+$, with a stoichiometric oxygen demand of approximately 2.3 kg O_2 /kg of destroyed cells [7].

Overall, the combination of elevated dissolved oxygen and alkaline pH (9–10) creates favorable conditions for aerobic degradation of organic matter while inhibiting pathogenic and odor-producing microorganisms [19], thereby contributing to effective sludge stabilization.

3.2. Solids Changes

Figure 3 illustrates the evolution of total and volatile solids over the 20-day treatment. Total solids (TS) slightly increased from 0.41% to 0.45% at day 2, which can be attributed

to rapid growth of heterotrophic bacteria consuming the oxygen initially present in the mixed liquor. TS then decreased steadily to 0.235% by day 20, indicating a clear loss of solids and a corresponding reduction in sludge volume. Volatile solids (VS), representing the biodegradable organic fraction, decreased from 0.27% to 0.126%, corresponding to 53% removal over the treatment period.

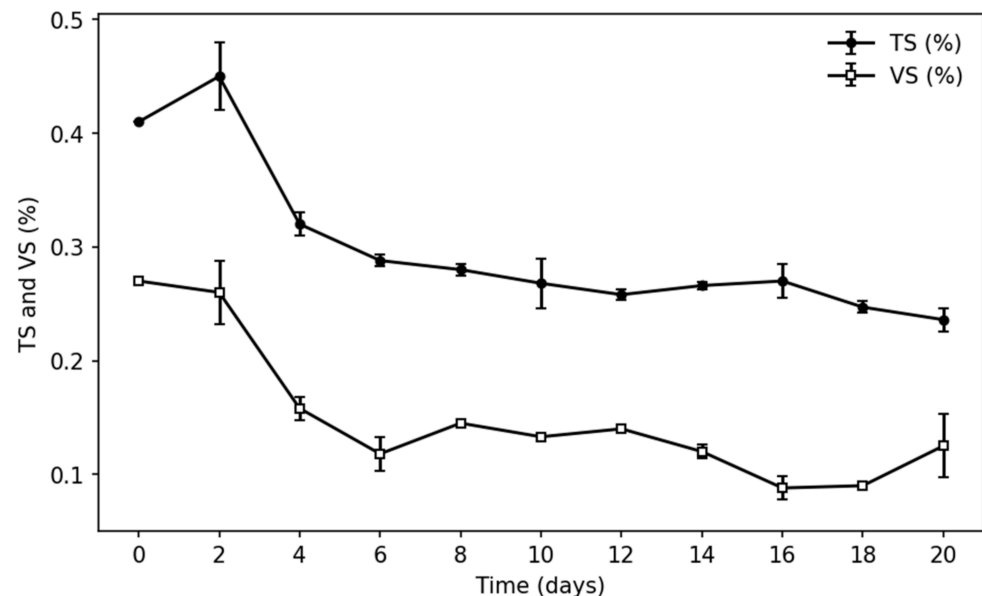


Figure 3. TS and VS reduction during treatment.

According to European guidelines, sludge is considered stabilized when VS reduction exceeds 38–40% [20]. The 53% VS removal achieved in this study therefore confirms that full stabilization was reached without external aeration or chemical pretreatment, well above the 17% [21] and 23% [22] reductions typically reported for conventional aerobic digestion. Although a higher VS reduction of 66% was reported by [23], this was achieved only after 90 days of treatment, which is more than four times the duration applied in the present study, further highlighting the efficiency of the microalgae-assisted process. This result derives from a single pilot-scale run, consistent with the exploratory design described in Section 2.1. Its agreement with the trends previously observed at laboratory scale [9] supports the robustness of this finding, though confirmation through replicated pilot trials, alongside a parallel conventional digestion control, remains a priority for future work.

3.3. Biomass Growth and Autotrophic Index

Figure 4 illustrates the evolution of biomass composition and autotrophic index over the 20-day treatment. Total biomass decreased gradually from 4.10 to 2.35 g/L, indicating net mineralization of the initial sludge solids, consistent with the TS and VS trends shown in Figure 2. In parallel, microalgal biomass increased from 0.06 g/L at day 0 to 0.41 g/L on day 20, with its share rising from 1.6% to nearly 17% of total biomass, while the sludge fraction declined from 4.03 to 1.94 g/L, dropping from a share of 98.4% to approximately 83%. This shift in composition demonstrates that, as sludge was degraded, microalgae successfully proliferated using the sludge as their sole source of nutrients and organic carbon, without any external feeding.

This dynamic is consistent with previous findings showing that microalgae assimilate organic carbon sources such as sugars and organic acids more rapidly than heterotrophic bacteria, potentially restricting bacterial growth under illuminated conditions [24]. Furthermore, when microalgae were cultivated in liquid digestate, the bacterial community

became less abundant and less diverse, as competition for available substrates increasingly favored microalgae over bacteria [25,26].

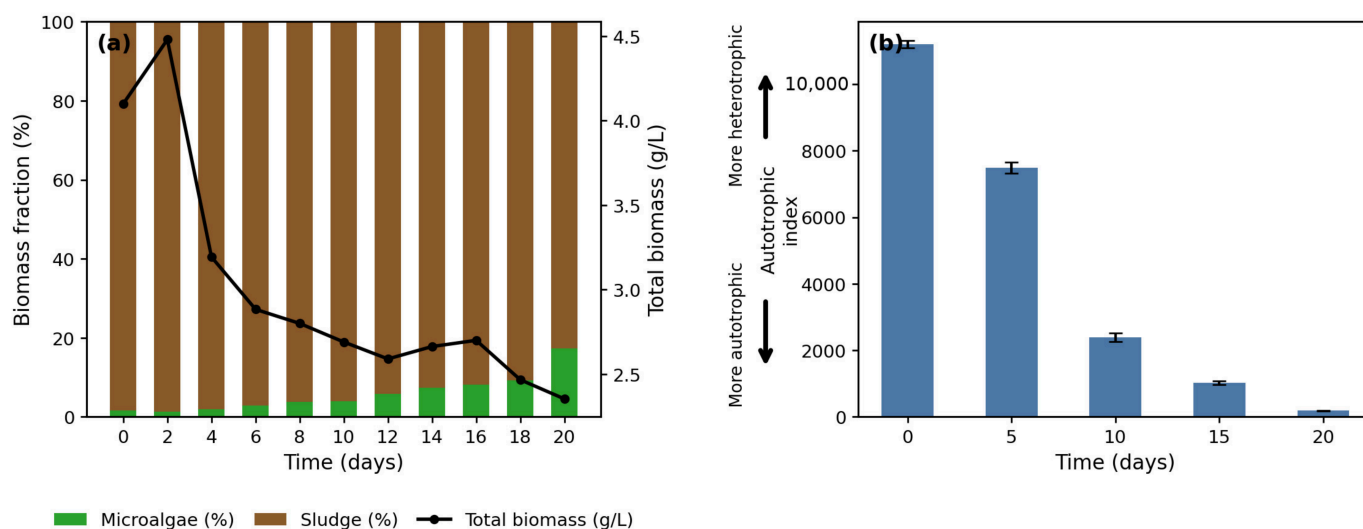


Figure 4. Changes in biomass (a) and autotrophic index (b) during the 20-day treatment.

The evolution of the autotrophic index (AI) further confirms this community shift (Figure 4b). The AI, defined as the ratio of chlorophyll-a concentration to volatile suspended solids (Chl-a/VSS) [27], reflects the balance between autotrophic and heterotrophic biomass: low values indicate a microalgae-dominated community, while high values reflect a heterotrophic, sludge-dominated system [28]. At the start of treatment, the AI was very high at approximately 11,203, reflecting a strongly heterotrophic sludge with negligible microalgal contribution. By day 20, the AI dropped sharply to around 188, confirming the establishment of a microalgae-rich, autotrophic consortium in agreement with the observed increase in microalgal biomass fraction.

Microscopic observations (Figure 5) further support these changes in biomass composition. At the beginning of treatment, flocs were dominated by bacterial aggregates with only a few dispersed microalgal cells. Microalgae were identified by their green pigmentation (chlorophyll content), larger cell size compared to bacteria, and distinct morphology, appearing either as round to oval single cells or as filamentous green structures under light microscopy (Figure 5) [29]. By day 20, well-defined microalgae–bacteria flocs were clearly visible, with microalgal cells and filaments tightly embedded within the sludge matrix.

3.4. Removal of Nitrogen and Phosphorus During Digestion

3.4.1. Nitrogen Removal

Total nitrogen (TN) distribution between mixed liquor and supernatant during the digestion period is presented in Figure 6.

Total nitrogen in the mixed liquor decreased from 147.25 mg/L to 97.65 mg/L, corresponding to an overall reduction of approximately 34%. At the start of digestion, TN was predominantly associated with the liquid phase (142.66 mg/L), with only a minor fraction detected in the supernatant (4.59 mg/L). During the initial stage (days 0–9), mixed liquor TN decreased substantially while supernatant TN rose to approximately 74 mg/L, indicating active mineralization of organic nitrogen and its release into the liquid phase. A sharp decline in supernatant TN was then observed on day 12 (9.41 mg/L), suggesting intense microalgal nitrogen uptake and incorporation into biomass [30]. In the later stage (days 15–20), TN concentrations in both fractions stabilized, reflecting a dynamic equilibrium between bacterial mineralization and microalgal assimilation [31].

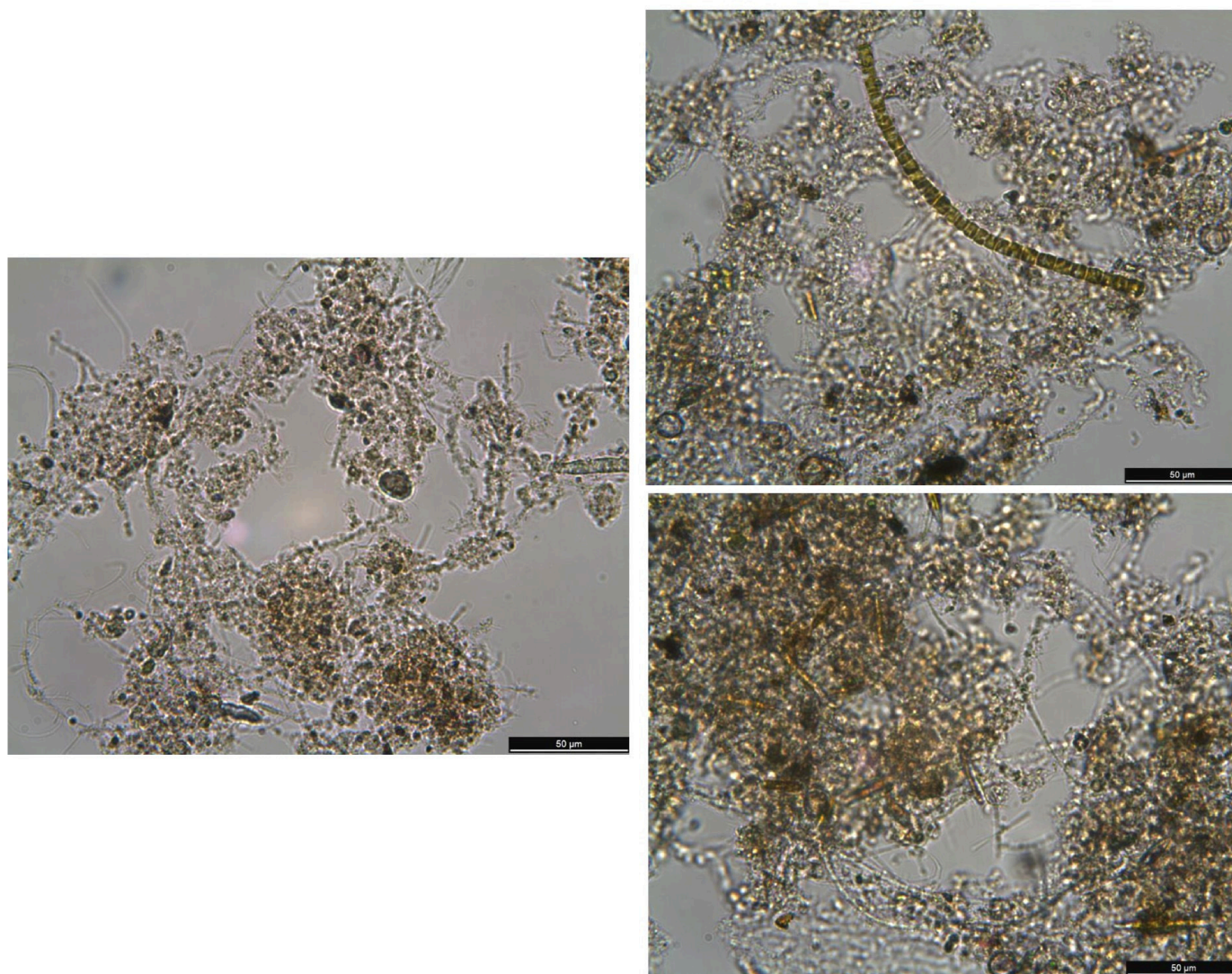


Figure 5. Microscopy images of the mixture (40×): on the left, from the initial day of treatment; on the right, from the end of treatment (20 days). Depicted scale bars measure 50 μm in length.

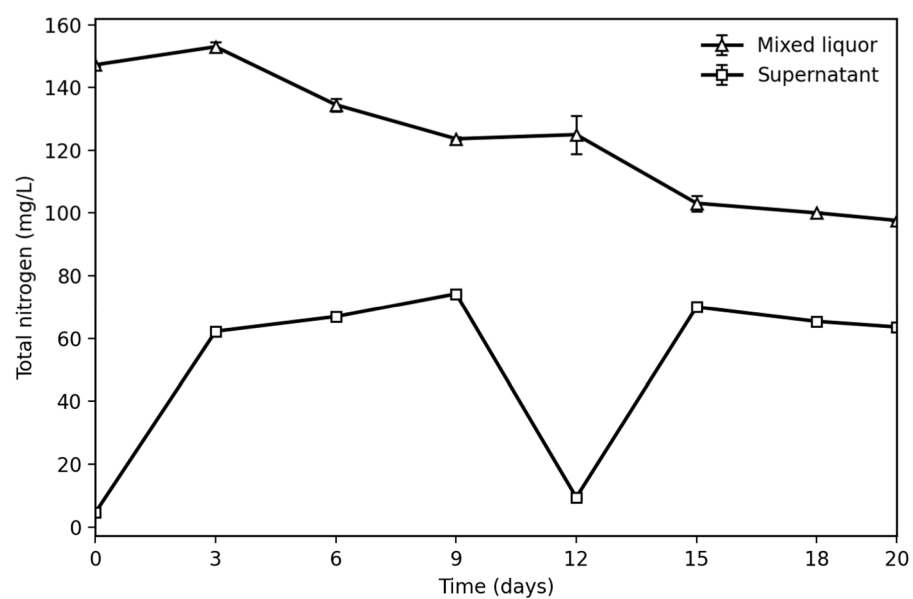


Figure 6. Total Nitrogen concentration in mixed liquor and supernatant over time.

Microalgae primarily remove nitrogen through direct assimilation into their biomass [31–34]. They can utilize various nitrogen forms, including ammonium, nitrate, nitrite, and urea, with ammonium being preferentially assimilated due to its lower energy requirement [32–35]. While bacteria contribute to nitrogen removal through assimilation and denitrification. Unlike conventional aerobic digestion, where nitrogen is mostly conserved and redistributed between solid and liquid phases with limited overall removal [36,37], the microalgae-assisted system achieved a clear net decrease in total nitrogen. In a classical aerobic digester without microalgae, nitrogen is released from biomass as organic nitrogen, rapidly converted to ammonium, and only partly nitrified. Hence, a substantial fraction accumulates in the liquid phase unless anoxic periods enable denitrification [36,37]. In contrast, the associated microalgae–sludge process couples aerobic mineralization with photosynthetic oxygen production and microalgal nitrogen uptake, so part of the released nitrogen is re-assimilated into biomass or removed via nitrification–denitrification, limiting nitrogen accumulation in the supernatant and making the process more sustainable.

To further clarify the relative contribution of nitrogen removal pathways, a semi-quantitative mass-balance estimate was performed using microalgal biomass production and literature-reported nitrogen content of microalgae (1–14% of dry weight, depending on species and conditions) [38]. Applying a conservative mid-range value (~8%) to the net microalgal biomass produced (0.35 g/L), assimilation is estimated to account for ~25–30 mg/L of the 49.6 mg/L total nitrogen removed (~50–60%), with the remainder attributable to nitrification–denitrification. This indicative estimate suggests microalgal assimilation is a major, though not exclusive, nitrogen removal pathway.

3.4.2. Phosphorus Removal

Figure 7 illustrates the evolution of total phosphorus (TP) and orthophosphate ($\text{PO}_4\text{-P}$) in the mixed liquor and supernatant during the 20-day microalgae–sludge treatment. Mixed liquor TP declined markedly from 102.75 to 25.75 mg/L, representing an overall reduction of approximately 75%. Throughout the treatment, supernatant TP remained consistently low, ranging between 2.52 and 10.55 mg/L, confirming that phosphorus did not accumulate in the liquid phase but was effectively retained within the biomass.

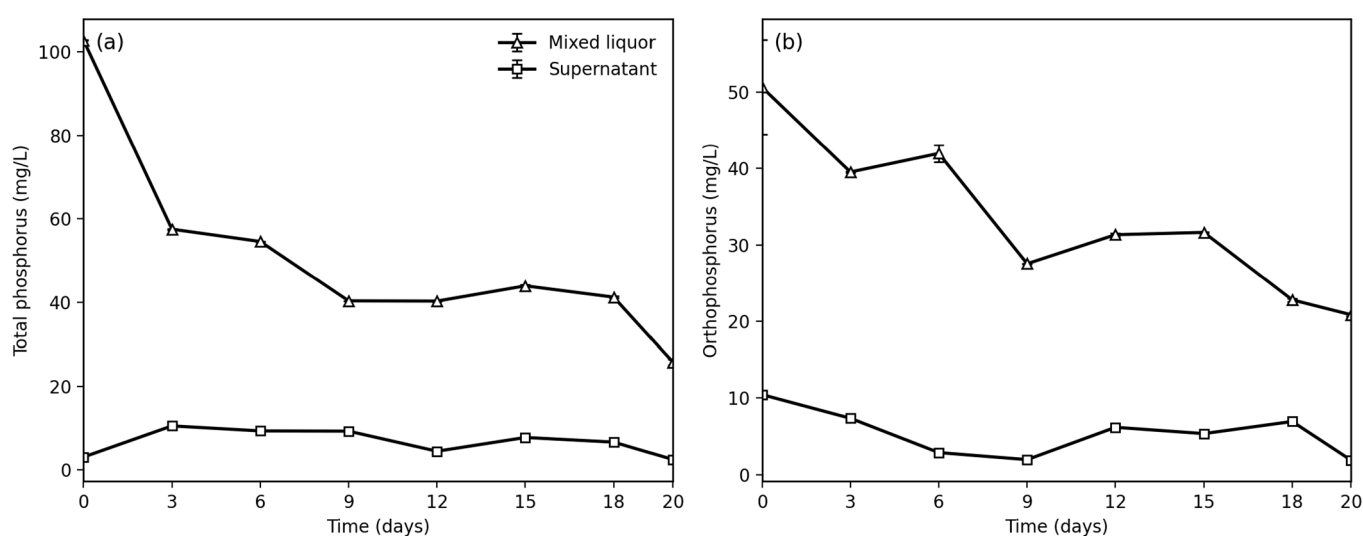


Figure 7. Changes in phosphorus during microalgae-sludge treatment. (a) Total phosphorus; (b) Orthophosphorus, in mixed liquor and supernatant over time.

Orthophosphate in the mixed liquor followed a similar declining trend, decreasing from 50.65 to 20.85 mg/L, corresponding to approximately 59% removal. A temporary increase was observed around day 6, likely reflecting the mineralization of organic phosphorus during the early biological activity phase, followed by subsequent microalgal uptake as algal growth intensified. Supernatant $\text{PO}_4\text{-P}$ remained consistently low throughout the treatment, ranging between 1.875 and 10.45 mg/L, confirming effective phosphorus retention within the biomass rather than release into the liquid phase.

Previous studies have shown that microalgae–bacteria systems can efficiently remove phosphorus, with TP removal efficiencies ranging from 53.4% to 98% and orthophosphate removal reaching up to 73% [26,39–43]. In this study, the removal efficiencies were approximately 75% for TP and 59% for orthophosphate, which are within the range reported in the literature. The observed removal can be attributed to several complementary mechanisms, including microalgal assimilation, bacterial transformation, and precipitation processes favored by the photosynthetically induced increase in pH [43]. In contrast to conventional activated sludge systems, which typically require strict anaerobic–aerobic cycling for biological phosphorus removal [44,45], the microalgae–sludge system ensured effective phosphorus removal while maintaining relatively low TP concentrations in the supernatant throughout the treatment period. This suggests that phosphorus was predominantly retained within the biomass rather than released back into the liquid phase.

To clarify phosphorus removal pathways, a similar mass-balance estimate was performed using literature-reported P content of microalgae (~1% of dry weight, ranging up to ~4% under luxury uptake) [46]. Applying ~1% to the microalgal biomass produced gives only ~3.5 mg/L of the 77 mg/L TP removed (~4–5%), indicating that most phosphorus removal is driven by chemical precipitation from the pH rise from 6.86 to 9.7 [47].

3.5. COD Removal

Figure 8 illustrates the evolution of chemical oxygen demand (COD) in the mixed liquor and supernatant during the 20-day microalgae–sludge treatment. Mixed liquor COD decreased substantially from 3447.5 mg/L on day 0 to 1420.5 mg/L on day 20, indicating progressive organic matter removal and sludge stabilization. At the start of treatment, supernatant COD was high at 2013 mg/L, reflecting the dominance of soluble organic matter in the liquid phase. During the initial phase (days 0–3), both mixed liquor and supernatant COD declined from 3447.5 to 2139.7 mg/L and from 2013 to 1212.5 mg/L respectively, suggesting active hydrolysis and consumption of soluble organics by heterotrophic bacteria. From day 3 to day 9, supernatant COD dropped sharply from 1212.5 to 63 mg/L, consistent with intense bacterial degradation of dissolved organic matter, while mixed liquor COD continued to decrease steadily to 1599 mg/L. In the later stage (days 13–20), both fractions stabilized, with mixed liquor COD fluctuating between 1533 and 1420.5 mg/L and supernatant COD remaining low between 30 and 231 mg/L, reflecting a near-steady state where biomass growth, endogenous respiration, and mineralization were approximately balanced. In a co-culture, bacteria are mainly responsible for COD removal while microalgae contribute indirectly via O_2 supply and uptake of soluble metabolites [48]. Several studies on algal–bacterial systems report high COD removal, mainly driven by bacterial degradation. For example, mixed algal–bacterial cultures achieved around 80% COD removal in wastewater in the work of [49], and similar removal efficiencies (~80%) were observed by [50].

In this study, microalgae sustained aerobic bacterial degradation through continuous photosynthetic oxygen supply while simultaneously assimilating organic carbon under mixotrophic conditions [50,51]. Bacteria, in turn, converted refractory organics into bioavailable forms for algal uptake and supplied CO_2 to stimulate algal photosynthe-

sis [39,52], creating a synergistic COD removal mechanism superior to isolated algal or bacterial systems.

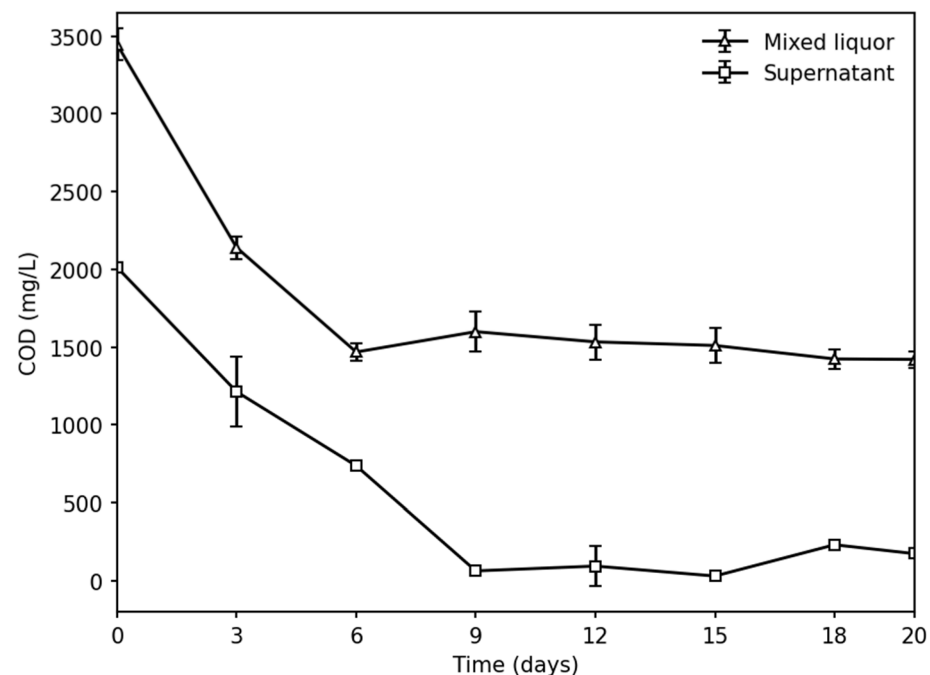


Figure 8. COD removal during treatment.

3.6. Sludge Settleability, Dewaterability, and Floc Size

During the 20-day treatment, sludge settleability improved substantially, as evidenced by the SVI decreasing steadily from 154.4 to 54.4 mL/g (Figure 9a). This marked reduction indicates excellent floc compaction, eliminating any risk of sludge bulking and ensuring effective solid–liquid separation. Concurrently, mean floc size dropped steadily from 220.2 to 32.8 μm (Figure 9d), confirming the progressive formation of smaller, denser flocs that settle rapidly under gravity [53]. This apparent contradiction is explained by floc density rather than size alone, since settling depends on both. Two results support increased density despite the smaller size: (i) the 53% VS removal (Figure 3) preferentially degraded light organic matter, leaving flocs relatively enriched in denser mineral content; and (ii) the shift from tightly bound to soluble/loosely bound EPS (Section 3.7) implies a loss of the water-retaining gel matrix normally formed by TB-EPS, allowing flocs to compact and expel water. The parallel decline of SVI and floc size (Figure 9a,d) supports this compaction mechanism, though direct density or SEM measurements would be needed for confirmation.

In contrast, dewaterability deteriorated progressively over the same period. CST increased from 15.9 to 26.9 s (Figure 9b), reflecting slower water release from the sludge matrix during filtration [54]. Similarly, SRF rose sharply from 5.59×10^{13} to 2.31×10^{14} m/kg (Figure 9c), indicating the formation of an increasingly resistant filter cake that hinders mechanical dewatering [54]. Notably, the most significant deterioration occurred between days 9 and 14, where CST increased from 15.2 to 22.3 s, and SRF nearly doubled from 8.27×10^{13} to 1.90×10^{14} m/kg, coinciding with the period of most intense microalgal growth.

The synchronized evolution of SVI, floc size, CST, and SRF reflects the structural transition described above: the sludge shifted from large, loosely aggregated flocs to compact, water-retentive particles that excel at settling but pose challenges for downstream dewatering, consistent with EPS-mediated changes in floc structure and water-binding capacity [9,55]. This divergence presents a real management challenge. The low SVI and small floc size favor rapid gravity thickening and reduce clarifier footprint an advantage for

space-constrained small-to-medium WWTPs. However, the rise in CST and SRF shows the sludge holds water more strongly, likely due to increased soluble EPS and finer particles, implying higher energy and chemical demand for downstream dewatering. Possible mitigation strategies include cationic polyelectrolyte dosing to bridge soluble EPS and restore floc permeability, pre-oxidation (Fenton, ozonation, peracetic acid) to degrade hydrophilic biopolymers, or thermal hydrolysis/electro-dewatering to disrupt the water-binding EPS matrix. These strategies should be tested in future pilot trials to balance settleability gains with acceptable dewatering costs.

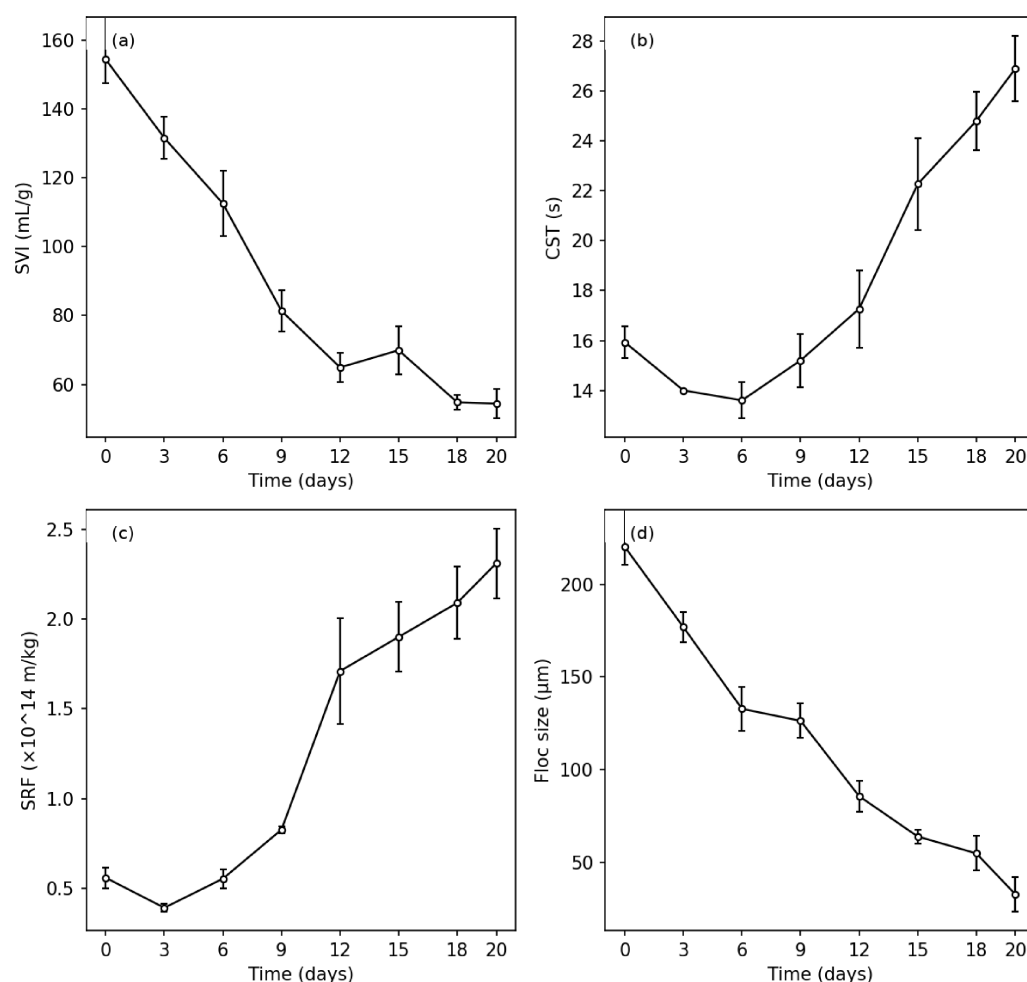


Figure 9. Evolution of sludge settleability, dewaterability, and floc structure during the 20-day treatment. (a) Sludge volume index (SVI, mL/g); (b) Capillary suction time (CST, s); (c) Specific resistance to filtration (SRF, m/kg); (d) Mean floc size (μm).

The following section examines EPS composition evolution and its quantitative correlations with these sludge separation properties.

3.7. EPS Changes

Figure 10 illustrates the evolution of extracellular polymeric substances (EPS) fractions during the 20-day treatment. Total EPS remained relatively stable, decreasing slightly from 60.2 to 47.0 mg/g, but its composition changed markedly. Soluble proteins (PN-S) increased progressively from 2.1 to 9.2 mg/g, while tightly bound proteins (PN-TB) declined substantially from 40.5 to 14.8 mg/g. Similarly, soluble polysaccharides (Poly-S) rose from 1.3 to 7.6 mg/g and loosely bound polysaccharides (Poly-LB) increased from 1.4 to 5.2 mg/g, while tightly bound polysaccharides (Poly-TB) decreased from 7.9 to 4.5 mg/g.

Consequently, total soluble EPS (EPS-S) increased from 3.4 to 16.8 mg/g, loosely bound EPS (EPS-LB) rose from 8.4 to 10.9 mg/g, and tightly bound EPS (EPS-TB) declined sharply from 48.4 to 19.3 mg/g. These trends collectively indicate a progressive conversion of tightly bound EPS into soluble and loosely bound fractions throughout the treatment period.

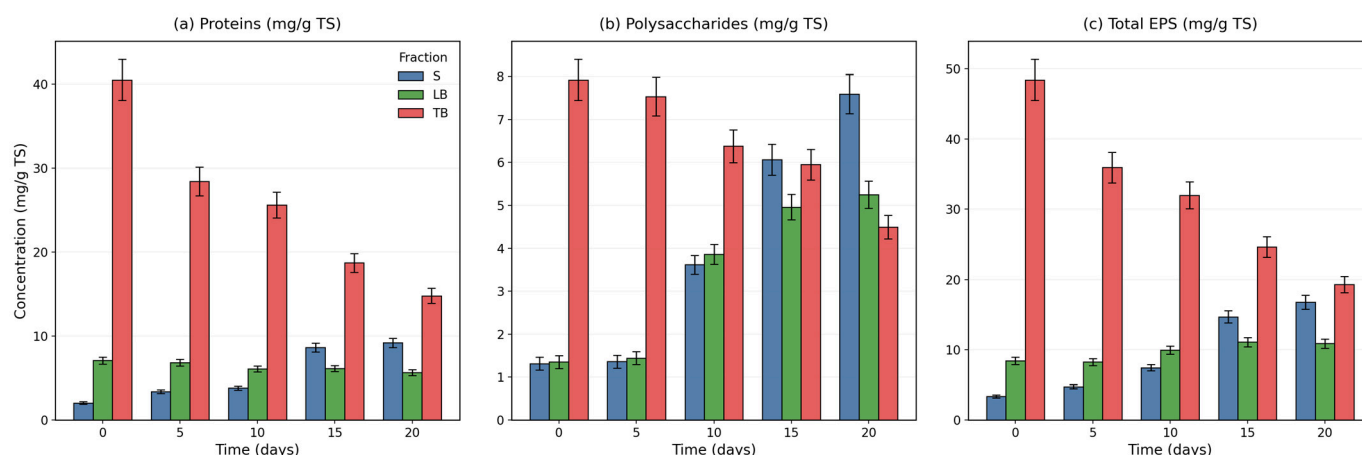


Figure 10. Effects of the process on EPS dynamics at different times. (a) The changes in protein content, (b) polysaccharide content, and (c) total EPS content in different fractions.

While the biochemical data establish this EPS redistribution, confirming its precise drivers would require microbial community and enzymatic analysis. Two non-exclusive mechanisms are proposed: (i) as biodegradable organic matter declined (Figure 3), bacteria likely entered endogenous decay, known to trigger hydrolytic self-degradation of the TB-EPS matrix; and (ii) the shift toward a microalgae-dominated consortium (autotrophic index dropping from ~11,203 to ~188, Figure 4b) suggests declining bacterial populations responsible for the original TB-EPS, while proliferating microalgae may not produce EPS with the same tightly bound character. We propose microbial sequencing and enzymatic EPS characterization as priorities for future work to confirm these mechanisms.

3.8. Correlation Between EPS Fractions and Sludge Physicochemical Properties

The relationship between EPS distribution and sludge physicochemical properties remains complex and sometimes contradictory in the literature. As discussed in our previous review [55]. Therefore, Pearson correlation analysis was performed to explore semi-quantitative relationships between EPS fractions, their biochemical composition, and key sludge properties, including SVI, CST, SRF, and floc size. Exact r and p -values for all tested variable pairs are reported in the extended correlation matrix provided in Table A1 (Appendix A).

As shown in Figure 11, clear differences in how EPS fractions and their components are associated with sludge physicochemical properties. SVI was negatively correlated with the soluble fractions, particularly PN-S (-0.87), PS-S (-0.87), and EPS-S (-0.88 , $p < 0.05$), whereas positive correlations were observed with tightly bound fractions, including PN-TB (0.60), PS-TB (0.61), and EPS-TB (0.60). This pattern suggests that an increase in soluble EPS is linked to poorer settling characteristics, while tightly bound EPS is associated with improved sludge structure and settleability [55,56]. A similar trend was found for CST and SRF, which were strongly and positively correlated with PN-S and EPS-S, but negatively correlated with PN-TB and EPS-TB. These relationships indicate that soluble EPS fractions were associated with poorer dewatering behavior, whereas tightly bound fractions were more closely related to improved sludge dewaterability [57].

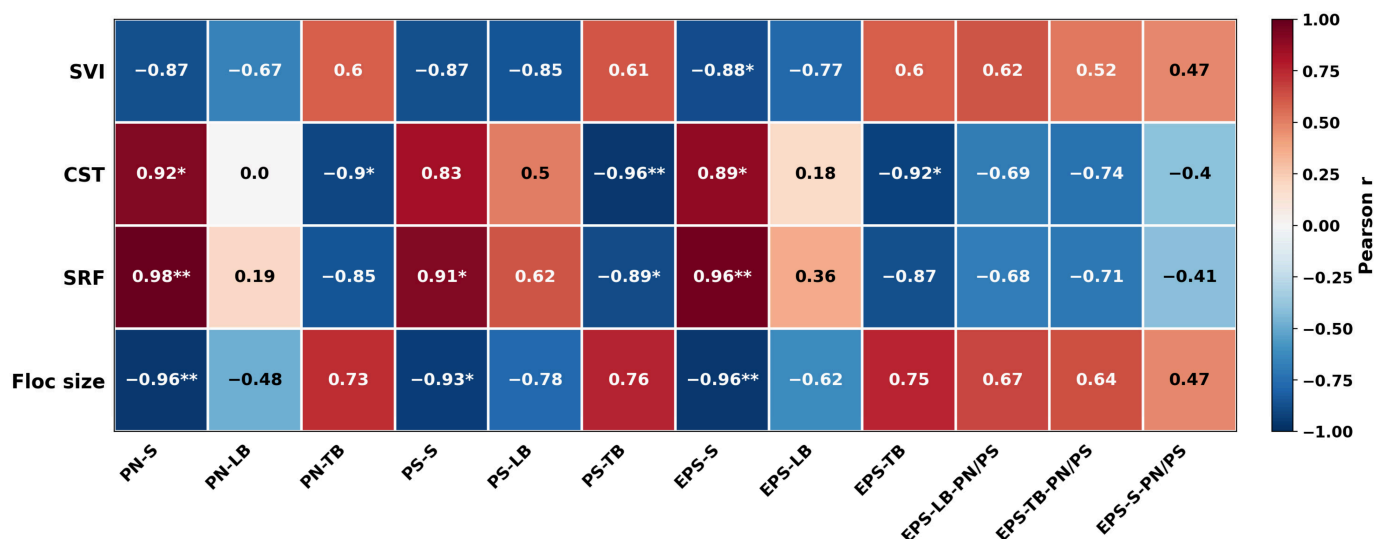


Figure 11. Pearson correlation between EPS fractions, their components, and sludge physicochemical properties. * $p < 0.05$, ** $p < 0.01$.

Floc size showed the opposite tendency, with strong negative correlations with soluble fractions such as PN-S (-0.96 , $p < 0.01$) and EPS-S (-0.96 , $p < 0.01$), and positive correlations with tightly bound components, including PN-TB (0.73) and EPS-TB (0.75). This suggests that soluble EPS fractions were related to smaller and less compact flocs, while tightly bound EPS favored the formation of larger and denser aggregates. In addition, the PN/PS ratios of LB-EPS and TB-EPS were positively associated with floc size and negatively associated with SVI, further indicating that a relative enrichment in proteinaceous components may contribute to better sludge structure [56,58,59]. Overall, the correlation patterns support the view that tightly bound EPS, particularly its protein-rich components, is closely linked to improved sludge aggregation, larger floc size, and better settling and dewatering properties, whereas soluble EPS fractions appear to have a detrimental effect on sludge physicochemical performance.

4. Conclusions

This 20 L pilot study confirms that indigenous microalgae-assisted aerobic digestion eliminates the need for external aeration while achieving 53% volatile solids reduction and substantial nutrient removal (34% TN, 75% TP), validating the process beyond laboratory scale. Scaling to small-to-medium WWTPs will require addressing three engineering constraints: mixing (adequate here at pilot scale, but larger units may need air-lift configurations), light penetration (self-shading likely at higher biomass density, requiring flat-panel/tubular designs), and biomass harvesting, since the improved settleability (SVI 154→54 mL/g) contrasts with deteriorated dewaterability (4-fold SRF increase), likely requiring polymer conditioning and adding operational cost.

This trade-off has direct economic implications: energy savings from eliminated aeration could be partly offset by higher dewatering costs. Mitigation strategies (polymer conditioning, oxidative pre-treatment, thermal hydrolysis) should be tested in future pilot trials.

Further research should include replicated reactors with a conventional digestion control, molecular identification of the microalgal consortium, direct N/P speciation, deeper EPS characterization, outdoor cultivation trials, and a full techno-economic assessment to move this technology toward full-scale application.

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Abbreviations

The following abbreviations are used in this manuscript:

SS	Sewage sludge
TS	Total solids
VS	Volatile solids
TSS	Total suspended solids
VSS	Volatile suspended solids
SVI	Sludge volume index
COD	Chemical oxygen demand
TN	Total nitrogen
TP	Total phosphorus
DO	Dissolved oxygen
EPS	Extracellular polymeric substances
S-EPS	Soluble extracellular polymeric substances
LB-EPS	Loosely bound extracellular polymeric substances
TB-EPS	Tightly bound extracellular polymeric substances
CST	Capillary suction time
SRF	Specific Resistance to filtration
Chl-a	Chlorophyll a

Appendix A

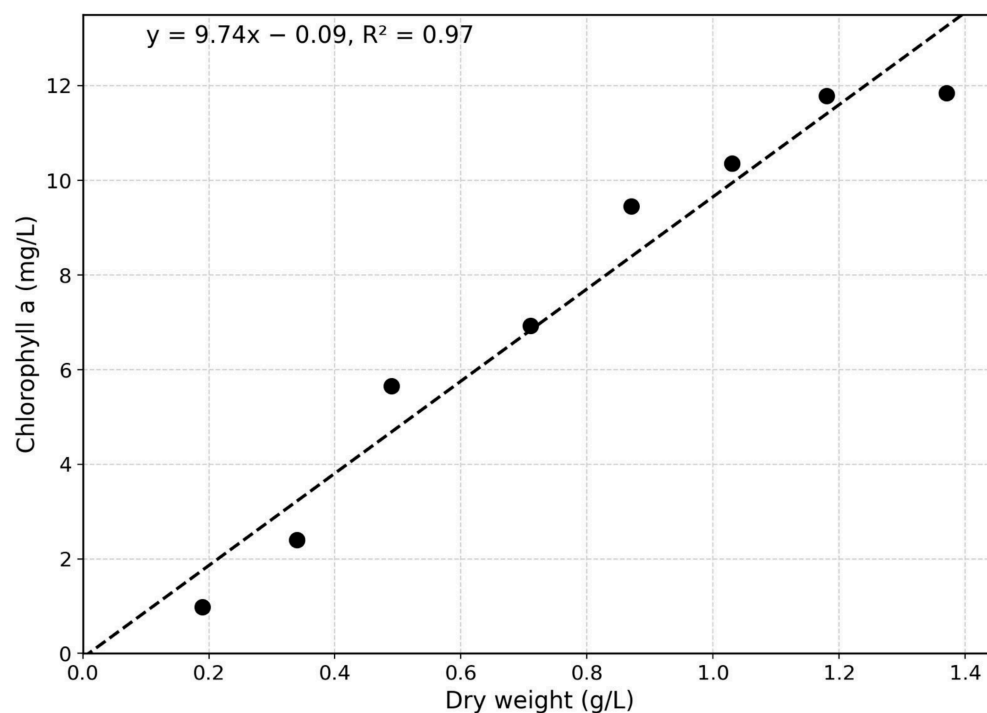


Figure A1. The standard curve of chlorophyll-a content and dry weight of microalgae.

Table A1. Extended Pearson correlation matrix between EPS fractions, their biochemical components, and additional sludge physicochemical parameters, sorted by p -value (* $p < 0.05$; ** $p < 0.01$; ns = not significant).

Variable 1	Variable 2	r	p-Value	Signification
SVI	Floc size	0.949	0.0039	**
EPS-S	EPS-TB	0.927	0.0079	**
PN-LB	Poly-LB	0.886	0.0189	*
TS	CST	−0.869	0.0247	*
Poly-LB	EPS-LB	0.862	0.0271	*
SVI	EPS-S	−0.854	0.0305	*
PN-TB	Poly-TB	0.841	0.0359	*
EPS-S	Total EPS	0.823	0.0443	*
SRF	Poly-TB	0.764	0.0770	ns
Poly-TB	EPS-S	−0.748	0.0870	ns
TS	SRF	−0.745	0.0889	ns
PN-LB	EPS-LB	0.738	0.0939	ns
SVI	EPS-TB	−0.737	0.0944	ns
TS	Total EPS	0.695	0.1251	ns
Floc size	EPS-S	−0.691	0.1283	ns
SVI	PN-TB	0.671	0.1443	ns
Poly-TB	EPS-TB	−0.670	0.1454	ns
Poly-LB	Poly-TB	−0.657	0.1563	ns
EPS-TB	Total EPS	0.657	0.1564	ns
PN-TB	EPS-S	−0.652	0.1603	ns

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