

Native microalgae for decentralized wastewater treatment for nitrogen and phosphorus removal

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ABSTRACT

This study presents the results of an exploratory experiment aimed at investigating the feasibility of microalgae-based tertiary wastewater treatment for the removal of phosphorus (P) and nitrogen (N) for decentralized systems. The effluent from a single-household on-site packaged activated sludge (AS) plant, not designed for targeted N and P removal, was used to feed an algal photo-sequencing batch reactor (Algae SBR; 4.5 L). The reactor was operated under laboratory conditions using natural sunlight (approximately 14 h light/10 h dark photoperiod) and a hydraulic retention time (HRT) of 20–21 h. The packaged AS plant exhibited pronounced effluent variability associated with changes in household occupancy (2–10 p.e.), with total phosphorus (TP) ranging from 5.1 to 13.1 mg/L and total nitrogen (TN) from 4.6 to 58.2 mg/L. These variations strongly influenced nutrient removal efficiency in the Algae SBR. Effluent nutrient concentrations were directly proportional to their influent concentrations, with R^2 values exceeding 0.99 for TN and 0.80 for TP. Phosphorus removal was significantly affected not only by influent phosphorus concentrations but also by influent nitrogen levels. Regarding compliance with legislative limits, nitrogen standards ($TN \leq 15$ mg/L) were met only when influent TN remained below 20 mg/L. Phosphorus standards ($TP \leq 2$ mg/L) were nearly achieved only when influent TN was below 8 mg/L and influent TP below 7 mg/L. These initial results indicate that algae-based tertiary treatment for on-site packaged plants is technically feasible. However, its performance appears to be highly sensitive to the effluent quality of upstream treatment units and therefore requires further in-depth investigation.

Keywords: algae, wastewater treatment, *Tetrademus obliquus*, decentralized sewerage systems, onsite, packaged plant.

INTRODUCTION

Decentralized wastewater treatment (WWT) is gaining momentum as a complementary alternative to centralized sewerage, particularly in the places where there are low housing density, seasonal occupancy, high sewerage connection costs, or geographical constraints that make conventional expansion inefficient or slow to deliver [Garrido-Baserba et al., 2024; Sha et al., 2024; Brooks et al., 2025]. In this context, compact packaged plants and other on-site systems are increasingly discussed as scalable, modular infrastructure that can be deployed rapidly, tailored to local loads, and upgraded over time [Sha et al., 2024; Ribarova et al., 2024; Subramanian

et al., 2024]. However, a recurring limitation of very small systems is that influent and hydraulic shocks propagate directly into process instability and irregular effluent nutrient quality, because buffering capacity and operational control are inherently constrained [Ribarova et al., 2024; Vidal et al., 2023; Micek et al., 2021]. This problem is particularly relevant for package activated sludge (AS) units serving single households or small clusters, where documented performance scatter and reliability issues for nitrogen and phosphorus are common even when organic removal is adequate [Vidal et al., 2023; Micek et al., 2021; Engstler et al., 2022].

At the same time, nutrient control expectations are tightening, driven by persistent eutrophication

pressures and by the need to protect receiving-water ecological status [Preisner et al., 2020; UN-Water, 2024; EEA, 2025]. Regulatory evolution is therefore shifting from basic compliance toward more systematic nutrient management, which increases the relevance of robust tertiary (polishing) options, especially where upstream processes do not include biological N and P removal [Preisner et al., 2020; Liao et al., 2024; Karczmarczyk et al., 2025]. In Europe, the recast Urban Wastewater Treatment Directive (UWWTD) materially strengthens this trajectory by broadening the scope to smaller agglomerations and expanding the ambition for nutrient control across the sector [Directive (EU) 2024/3019]. In particular, the recast sets tertiary-treatment parametric values of TP = 0.7 mg/L ($\geq 10,000$ p.e.) and 0.5 mg/L ($\geq 150,000$ p.e.), and TN = 10 mg/L ($\geq 10,000$ p.e.) and 8 mg/L ($\geq 150,000$ p.e.), indicating a clear downward shift in allowable nutrient concentrations for large dischargers relative to many legacy regimes [Directive (EU) 2024/3019]. Even where the smallest on-site systems are not directly regulated by the same numerical limits, these policy shifts shape receiving-water expectations, permitting practice, and the “direction of travel” for decentralized designs, especially in sensitive catchment areas [Council of the EU, 2024; Karczmarczyk et al., 2025; Preisner et al., 2020].

Against this backdrop, algae-based treatment has become a prominent candidate for nutrient polishing because microalgae can assimilate nitrogen and phosphorus into biomass while simultaneously offering pathways for resource recovery [Díaz et al., 2022; Nguyen et al., 2022]. The appeal is not limited to removal. Coupling wastewater treatment with biomass valorization (e.g., biofertilizer products) is repeatedly framed as a circular-economy route that can improve sustainability and local acceptance, particularly in decentralized settings [Hussain et al., 2021; Santos et al., 2025]. Recent reviews instead emphasize that the decisive barriers for practical deployment are predominantly engineering and techno-economic - most notably reliable, low-cost biomass harvesting and overall system feasibility under real operating variability [Valchev and Ribarova, 2022; Abdelfattah et al., 2023; Nguyen et al., 2022]. These factors emphasize the potential for the implementation of the algae-based WWT technology on a smaller scale (on-site packaged application) and substantiate an investigation of its potential as a worthwhile endeavor.

Despite this progress, the published research linking household-scale packaged activated sludge effluents to algae-based polishing for N and P removal is still scarce compared to the extensive literature on municipal-scale streams. Accordingly, the goal of the present study is to evaluate microalgae-based tertiary polishing for N and P removal under realistic decentralized conditions by operating a laboratory scale photo-sequencing batch bioreactor (Algae SBR) fed with unsterilized secondary effluent from a household AS packaged plant, located in Bistritsa, Bulgaria. In order to assess the latter, the study i) quantifies the magnitude of P and N variability in the packaged plant effluent, and ii) determines how influent nutrient levels relate to their removal behavior and achievable effluent quality from the algae reactor.

MATERIALS AND METHODS

Experimental set-up

Algal strains

For cultivation of a suitable algae strain, a mix of naturally occurring microalgae in the wastewater of the municipal sewerage system of Sofia, Bulgaria was used for the experiment. The mixed culture was cultivated and propagated in the laboratories of the Department of Water Supply, Sewerage, and Water Treatment at the University of Architecture, Civil Engineering, and Geodesy (UACEG). The microalgae consortium used was clearly dominated by the strain *Tetradismus obliquus* (Turpin) Wynne (Figure 1). This strain acted as an invasive species in the reactors during the experiment, mainly due to its ability to reproduce rapidly. Its reproduction is mainly asexual through immobile spores, which are mini copies of the mother cell (autospores), with each cell having a well-defined pyrenoid [Komárek and Fott, 1983; Stoyneva-Gärtner and Uzunov, 2017; Valchev et al., 2023].

The strain itself is a freshwater alga that belongs to the green evolutionary line, specifically the Chlorophyta phylum [Guiry et al., 2022]. It is widespread and usually found in 4-celled coccidia, but during cultivation it can break down into single cells (Figure 1). Phylogenetically, *Tetradismus obliquus* is considered a relatively newly separated strain within the genus *Scenedesmus* [Wynne et al., 2016]. The genus is among the most preferred worldwide in microalgal wastewater treatment systems [Acevedo et al., 2017;

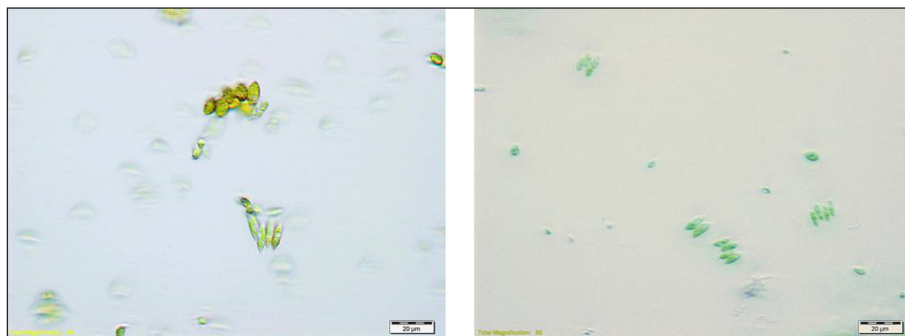


Figure 1. Light microscope images of the microalgae suspension used in the experiment (80x magnification)

Di Caprio et al., 2018]. Its main advantages are maintaining autotrophic and mixotrophic growth modes and retaining stable productivity in a variety of real and synthetic wastewater compositions [Ye et al., 2020; Sánchez-Zurano et al., 2021; Ferreira et al., 2022].

Wastewater source

The wastewater for the laboratory algae reactor was taken from the outlet of an onsite treatment facility for a single house - a packaged plant that uses activated sludge technology (AS packaged plant) without targeted phosphorus and nitrogen removal (Figure 2). The plant is located in a house in Bistritsa, Southwestern Bulgaria. The analysis of effluent quality indicates that BOD, COD, and TSS meet the requirements of Directive 91/271 and the national regulation (BOD < 25 mg/L, COD < 125 mg/L, TSS < 60 mg/L), whereas TN and TP exceed the prescribed limits [Ordinance 6; Directive 91/271]. This finding motivated the evaluation of microalgae-based treatment as a potential final stage of tertiary treatment for nitrogen and phosphorus removal in the present study.

During the period of the experiment, significant variations in the quality of the treated wastewater from the AS packaged plant were observed, likely caused by the number of people in the house. For this reason, each new batch of wastewater from the AS packaged plant outlet underwent additional filtration through paper filters with a pore size of 25 µm before being fed

into the algae reactor to remove residual coarse impurities (if any) and partially reduce the concentration of the TSS. Therefore, the inclusion of a filtration step in the final process flow diagram is recommended to ensure stable operation of the algal reactor.

Laboratory reactor set-up and working mode

The components of the laboratory reactor used to conduct the experiments in this study were:

- Glass cylinder with dimensions $D = 170$ mm and $H = 270$ mm ($V = 4.5$ L);
- Heidolph RZR 2021 electric mixer (mixer blade dimensions - $B = 120$ mm and $H = 50$ mm), set to 30–40 rpm;
- HACH SC1000 multi-parameter universal controller with luminescent dissolved oxygen (LDO) sensor, 1200-S pH sensor, and temperature sensor, recording the values of the three parameters hourly.

The microalgae system operated under laboratory conditions in a photo-sequencing batch bioreactor (Algae SBR) mode with natural sunlight, without an artificial light source (Figure 3). The approximate photoperiod was 14 hours of sunlight, followed by 10 hours of night darkness, with the installation located next to a North-facing window. The water temperature varied in the range of 19 °C to 33 °C with a mean temperature of 23.84 ± 2.95 °C. Since the algae were collected from an operating wastewater treatment plant, the

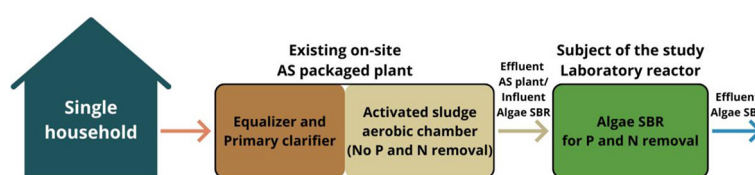


Figure 2. Place of the algae reactor in technological scheme

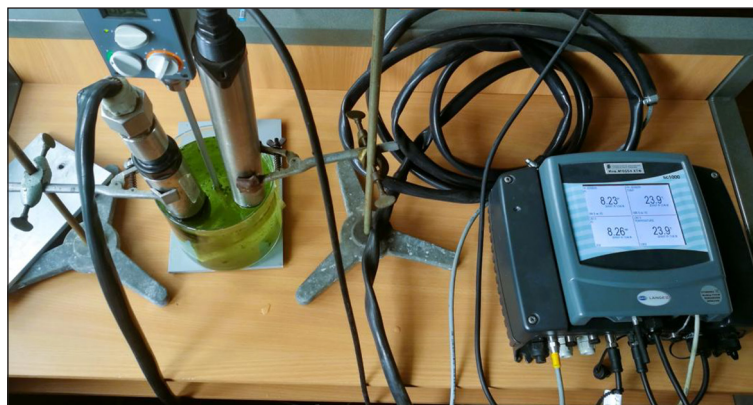


Figure 3. Image of the laboratory set-up

experimental temperature range corresponds to the conditions under which the species normally thrive.

The main phases of the operating mode of the Algae SBR were as follows: Phase 1: Filling – the pre-filtered wastewater from the AS packaged plant outlet was filled into the reactor, where the microalgae biomass is located. Phase 2: Mixing and reaction – the suspension (wastewater and microalgae) is homogenized with a vertical electric mixer. Phase 3: Sedimentation and decanting – at the end of the reaction phase, at $\text{pH} \approx 8\text{--}9$ (when autoflocculation occurs), the electric mixer was stopped, and the algal biomass was left to settle for 30 minutes. The decant was then separated and removed from the reactor, while the remaining settled biomass was left in the reactor for the next treatment cycle and waiting for Phase 1 to start again. The hydraulic retention time (HRT) of each of the photobioreactor cycles was 20 to 21 hours and the experiment was conducted over a period of approx. two months. In some stages of the experiment the HRT was extended to 45 and 70 hours to observe the influence of the parameter on treatment efficiency, but these results were not included in the analysis. The samples were taken at approximately the same time of the day in order to minimize the influence of the chemical precipitation mechanism of phosphorus removal.

Chemical and biological analyses

Each sample was filtered through a glass-fiber filter with an inorganic binding agent with a pore size of $0.45 \mu\text{m}$. The filtrate was then analyzed for the chemical parameters total phosphorus (TP) and total nitrogen (TN). To determine these parameters, a spectrophotometric method

of analysis with HACH Lange cuvette tests (ISO 15705) was used, with samples being thermally degraded in an LT200 thermostat and put into a DR2800 spectrophotometer. During the experiment, the water samples were regularly observed on non-permanent slides using a conventional Motic BA400 light microscope. During the work, microphotographs were taken on the same microscope with a Moticam2 camera provided by the Image Plus Program.

Data processing

Data processing was performed in Microsoft Excel. To visualize the temporal dynamics of the measured parameters, a two-point moving average was applied to generate smoothed trendlines. Linear relationships between the measured parameters were evaluated using a linear trendline described by the equation $y = ax + b$, where „a“ is the slope and „b“ is the intercept. The coefficient of determination (R^2), calculated automatically in Excel, was used to assess the goodness of fit, with values closer to 1 indicating a better fit.

RESULTS

Activated sludge packaged plant effluent quality

A variable with substantial effect on the studied nutrient removal efficiency is the quality of the AS packaged plant's effluent since it is used for Algae SBR inlet feed (see Figure 2). Hence, TP and TN concentration dynamics at the outlet of the AS packaged plant were measured and monitored throughout the experiment. Their variation

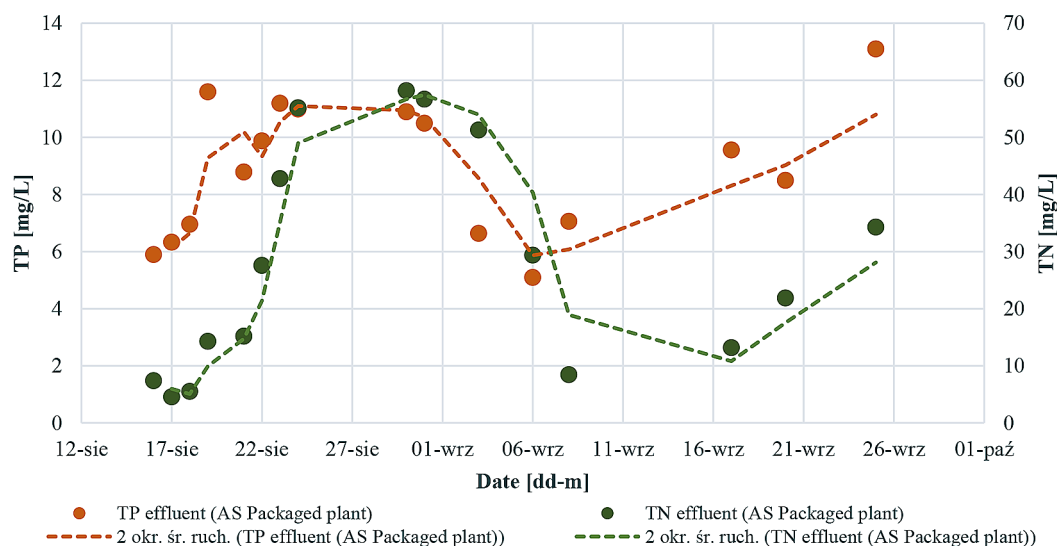


Figure 4. TP and TN concentration dynamics of the AS packaged plant effluent (Algae SBR influent)

visualised with moving average trendline is presented in Figure 4.

The graph shows that the effluent quality is irregular in terms of the TP and TN concentrations. The TP varied between 5.1 and 13.1 mg/L, while the TN concentration was between 4.6 and 58.2 mg/L. The moving average trendline on the graph confirms that the peaks and the lows in the TP are at approximately the same time as the ones in the TN. Furthermore, the peaks and the lows coincide with the variation in the amount of residents that were occupying the household in the period of the experiment. Their number changed back and forth in the range of 2 to 10 p.e., leading to periods with largely fluctuating influent loading of the AS packaged plant and major deterioration of its effluent quality. This shows that even though the on-site AS packaged plant has an equalization tank at the inlet, the amount of residents of the household is still a key factor in the plant's proper function, and the inlet tank cannot negate the negative effects of the major load and quantity fluctuations.

Total phosphorus and total nitrogen concentrations in the Algae SBR

Given the results from the AS packaged plant effluent quality, the effects of the fluctuations of the TP and TN concentrations on their removal were examined. A graph with their dynamics and moving average trendline is presented in Figure 5.

The results show that the TP removal increases with an increase of the initial TP and TN concentration until a threshold inlet TN concentration

above approx. 20 mg/L, and the inlet TP concentration – above approx. 8 mg/L. Once these threshold concentrations occur, the phosphorus removal declines. On the other hand, the TN removal curve remains relatively flat. These results indicate the sensitivity of the studied technology toward the influent concentration of the nutrient. Thus, these relationships were analyzed in more detail in the following subsections.

The effect of the influent TN on the effluent TN concentration

The analysis of the effects of the influent TN concentration on the effluent TN concentration in the Algae SBR are presented in Figure 6. Additionally, the graph on Figure 6 includes the effluent normative concentration of 15 mg/L for municipal wastewater treatment plants (WWTPs) with a capacity of 10 000 to 100 000 p.e., according to the current Bulgarian legislation and the Directive 91/271 which is still active on a national level [Ordinance 6; Directive 91/271]. This limit is depicted in a red horizontal line. Furthermore, the established threshold of 20 mg/L from the previous subsection for the initial TN concentration is also added to the graph in a vertical green line.

The graph on Figure 6 demonstrates a very strong positive linear correlation with an R^2 of above 0.99. That suggests that for the Algae SBR, the increase in the initial TN resulted in an increase in the effluent TN. Hence, even though there is a rise in the removed TN, according to Figure 5 from the previous subsection, this incremental gain does not correspond proportionally to the increase in the

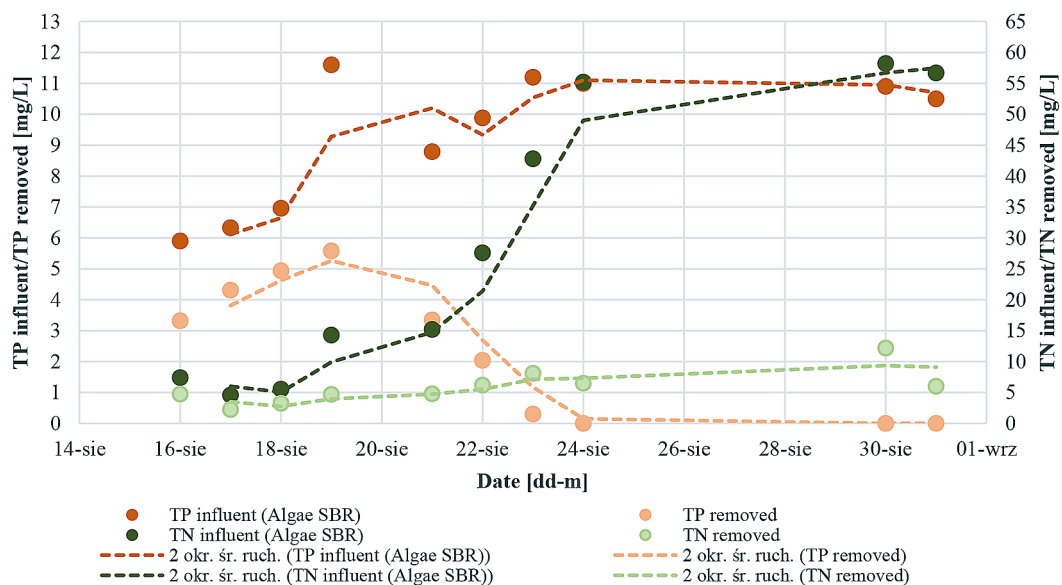


Figure 5. TP and TN reduction in relation to the inlet TP and TN in the Algae SBR

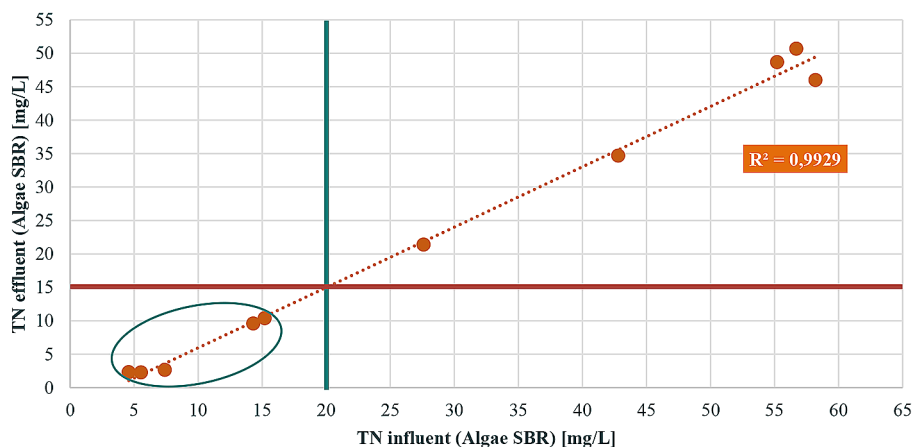


Figure 6. TN outlet concentration in relation to the inlet TN in the Algae SBR

initial TN concentration and cannot compensate for it. Thus, the final concentration remains under the limiting value of 15 mg/L, that is required legally, only for the cases when the inlet TN values remain below the noticed threshold of 20 mg/L. This signifies that every increase in the inlet TN concentration above the threshold in an Algae SBR of the kind that our study examines for an on-site (decentralized) secondary treatment, results in a high probability of non-compliance with the effluent norms for TN and deterioration of the water quality of the receiving water body.

Influent TN affecting the effluent TP concentration in the Algae SBR

Analogically to the previous subsection, the influence of the influent TN concentration on the

effluent TP concentration in the Algae SBR is depicted in Figure 7. The currently active regulatory permissible value of 2 mg/L for the effluent TP concentration for municipal WWTPs is also included with a red line. The R^2 of above 0.93 on the chart exhibits a very strong positive linear correlation between the two examined parameters. It is visible that regardless of the initial TN concentration, the normative outlet TP concentration can barely be reached. Only two measurements were on the borderline of 2 mg/L, and one was slightly above it with a value of 2.6 mg/L. Furthermore, in terms of reaching the regulatory outlet TP, the initial TN concentration above which the effluent TP becomes significantly greater was much lower than the established threshold of 20 mg/L from Figure 5 and Figure 6. In this case, all inlet samples above $TN=8$ mg/L (the vertical green line on

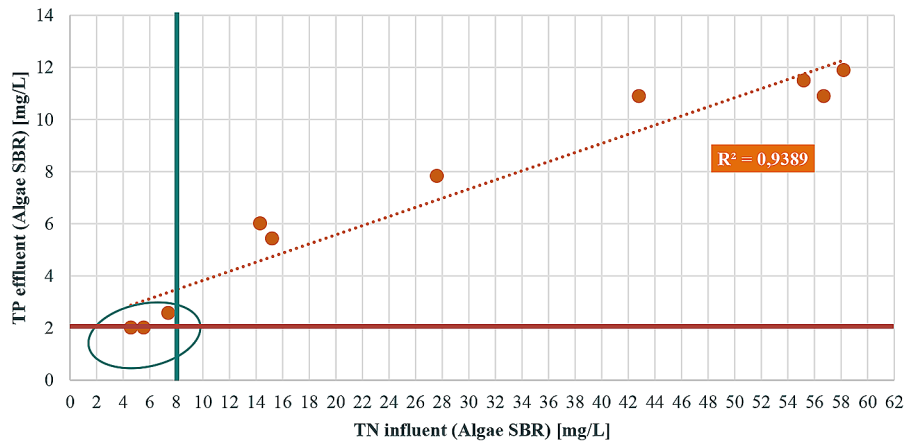


Figure 7. TP outlet concentration in relation to the inlet TN in the Algae SBR

Figure 7) lead to inefficient TP removal and outlet concentrations substantially beyond TP=2 mg/L. Thus, the inhibition due to the high inlet TN was much more pronounced with regards to the TP removal in the Algae SBR. This indicates that in the cases of TN>8mg/L, the effluent TP could also remain high and, depending on the receiving water body, it could still potentially lead to an environmental area that is prone to eutrophication in it.

Total phosphorus concentration from the Algae SBR

In order to further examine the influences on the influent TP concentration and to determine in which cases the regulatory limit can be reached, an additional analysis of the influence of the influent TP concentration on the effluent TP was analyzed. The graph with the data and the permissible value (horizontal red line) is presented in Figure 8 [Ordinance 6; Directive 91/271]. Additionally, in order to consider the combined influence of the

influent TP and the TN, the data on the graph was divided into two series – samples with influent TN below the threshold established in the previous subsection of 8 mg/L (green dots and trend-line) and samples with influent TN concentrations above it (orange dots and trendline).

Both trendlines represent a very strong positive linear correlation with R² above 0.8, suggesting that generally not only the higher influent TN concentration affects negatively the outlet TP, but also the inlet TP. Even though the graph on Figure 5 exhibits an initial rise in the removed TP with a corresponding increase in the influent TP concentration, according to the data from Figure 8, algae are not able to compensate for the initial TP increase beyond a certain threshold. This TP threshold is marked with a green vertical line on Figure 8 and its value is 7 mg/L. Furthermore, it is visible from the graph that in order to achieve the effluent regulatory value of TP≤2 mg/L, the

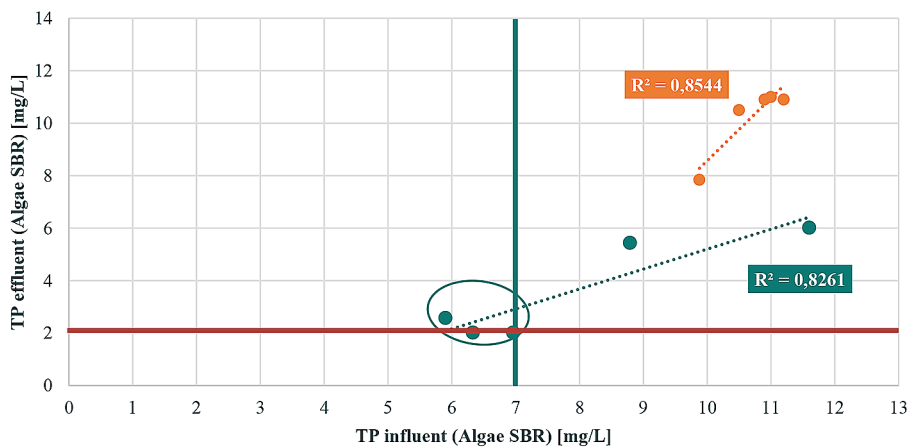


Figure 8. TP outlet concentration in relation to the inlet TP in the Algae SBR

most unfavorable inlet concentrations are TP>7 mg/L and TN>8 mg/L.

Technological parameters at an average hydraulic retention time of 21 h

In order to further assess the applicability of the algae-based technology for decentralized application for TP and TN removal from secondary effluent from AS packaged plants, the technological parameters for the Algae SBR are summarized in Table 1. The table includes three separate rows with relation to the thresholds for the initial TP and TN concentrations at the inlet of the Algae SBR discussed in the previous section.

The table shows that the performance of the on-site Algae SBR depends strongly on the influent nutrient levels, and that TP and TN do not respond in a similar manner. When the influent has high TN (>20 mg/L), the system produces poor effluent quality. This is consistent with very low TP removal (0.47 mg/L; 0.02 mg/L·h; 4.67%) and only modest TN removal (7.80 mg/L; 0.38 mg/L·h; 16.94%). In contrast, when TN is below 20 mg/L, treatment improves. The TN<20 mg/L and TP<7 mg/L condition gives the lowest effluent concentrations (TP = 2.21 mg/L; TN = 2.43 mg/L) and the highest removal efficiencies (TP = 65.11%; TN = 57.28%), with stable removal rates (TP = 0.21 mg/L·h; TN = 0.17 mg/L·h). When TN<20 mg/L but TP>7 mg/L, TP removal remains substantial in absolute terms (4.47 mg/L) and proceeds at a similar rate (0.21 mg/L·h), but the higher influent TP leads to a higher effluent TP (5.73 mg/L; 43.11%), while TN removal is moderate in this case (TN = 10.01 mg/L; 32.19%; 0.23 mg/L·h).

Using the currently active discharge limits (TN ≤ 15 mg/L; TP ≤ 2 mg/L), the results indicate that the Algae SBR can meet the TN target under both TN<20 mg/L regimes (effluent TN of 2.43 and 10.01 mg/L), but it could not constantly meet the TP target in any regime tested. The

best-case scenario in the table is TN<20 mg/L and TP<7 mg/L (row marked in grey), because it combines clear TN compliance with the lowest TP residual (2.21 mg/L, which is very close to the 2 mg/L limit). This suggests that the technology is most suitable for moderate-strength wastewater with relatively low influent TP, where a small operational improvement or a simple polishing step could likely bring TP consistently below the discharge requirement.

DISCUSSION

In light of the irregular AS packaged plant effluent quality, often reported, similar results have been observed in the literature. For example, Vidal et al., 2023 examined five AS packaged plants that use an SBR process for the treatment of wastewater from 1 to 5 p.e. and the effluent TP and TN concentrations varied dramatically [Vidal et al., 2023]. For TP the range was from 0.1 to 21 mg/L, and for TN – from 4.8 to 220 mg/L [Vidal et al., 2023]. Another study by Micek et al., 2021 that investigated two households with AS packaged plants reported effluent TP and TN ranges of 9.5 to 19.0 mg/L and 35.0 to 159 mg/L, respectively [Micek et al., 2021]. Furthermore, the maximum effluent concentrations exceeded the maximum influent concentrations for both parameters in some cases in their study, suggesting an extremely irregular effluent TP and TN quality that could be utterly difficult to predict [Micek et al., 2021].

Regarding the performance of the Algae SBR, since, to our knowledge, there are no studies on treating secondary effluent from an AS packaged plant with algae for P and N removal, the results in this study were compared with papers researching algae-based secondary treatment of municipal and synthetic wastewater. The literature suggests that in algae-based polishing of secondary effluents, high influent TN and TP

Table 1. Technological parameters for onsite Algae SBR used as final stage for TP and TN removal

Parameter	Avg. Algae SBR TP effl. conc.	Avg. Algae SBR TN effl. conc.	Avg. reduced TP	Avg. reduced TN	Avg. TP reduction rate	Avg. TN reduction rate	Avg. TP rem. eff.	Avg. TN rem. eff.
	mg/L	mg/L	mg/L	mg/L	mg/L·h	mg/L·h	%	%
At influent TN>20 mg/L	10.61	40.30	0.47	7.80	0.02	0.38	4.67%	16.94%
At influent TN<20 mg/L; TP<7 mg/L	2.21	2.43	4.19	3.41	0.21	0.17	65.11%	57.28%
At influent TN<20 mg/L; TP>7 mg/L	5.73	10.01	4.47	4.75	0.21	0.23	43.11%	32.19%

Note: *Abbreviations: avg. – average; effl. – effluent; conc. – concentration; rem. eff. – removal efficiency.

often translate into nutrient loading rates that exceed the reactor's effective removal capacity (set by light availability, biomass inventory and retention, the time available for uptake and associated pathways, etc.). For example, Ding et al. 2018 report that in pilot-scale high-rate algae ponds treating synthetic secondary effluent with influent TN of 23.5 mg/L and TP of 4.65 mg/L, the high initial algae still yielded only 34.2% TN removal (effluent TN 15.8 mg/L) and 67.0% TP removal (effluent TP 1.52 mg/L) during autumn trials with 72 h HRT, since the nutrient overload outstripped algal metabolic rates, causing incomplete uptake and biomass washout [Ding et al. 2018]. Another example is the Aparicio et al., 2024 study that uses microalgae in a consortium with nitrifiers [Aparicio et al., 2024]. They report that when a membrane high-rate algal pond was fed with effluent wastewater, the reduction of the HRT lead to performance decrease to removal levels below 10% for TN and below 5% for TP. A process driven by a “loading outpaces removal” response that raised effluent TN and TP [Aparicio et al., 2024]. The same study links poor TN polishing in low-carbon feeds to limited denitrification potential (insufficient organics content), which tends to leave more NO_x (nitrate/nitrite) in the effluent even when nitrification proceeds. Importantly, the same study reached much higher removals (91% for TN and 71% for TP) when operated under conditions that better supported the dominant mechanisms for nitrification–denitrification for N, highlighting that high influent concentrations are not inherently fatal, but they become detrimental when they bring the system beyond its light, carbon or retention time constraints [Aparicio et al., 2024]. These studies indicate that algae reactors in the literature achieve relatively similar treatment efficiencies and tendencies for TN and TP removal as the current study with respect to the increase in the initial concentration exceeding the algae's uptake potential.

Similar to the observations in our study, the most practically important negative effect at high TN is often NO_x stress, especially nitrite, because it can move the system from capacity-limited into inhibition, sharply reducing algal activity and thus TN and/or TP uptake. For example, González-Camejo et al., 2020 show that sustained nitrite exposure in microalgae–nitrifier cultures caused large performance losses: 5-day assays reduced nitrogen recovery efficiency by 32%, 42%, and 80% at 5, 10, and 20 mg NO₂-N/L, respectively

(even though short 30 minute exposures showed little immediate effect) [González-Camejo et al., 2020]. Mechanistically, Aparicio et al., 2022 demonstrate that nitrite stress can directly suppress photosynthetic function as the electron transport chain between photosystems II (PSII) and photosystems I (PSI) was hindered in their study at NO₂-N > 25 mg/L, and the half-inhibition constant for net oxygen production was $K_i \approx 23.7$ mg NO₂-N/L, implying a relatively sharp threshold range where additional NO₂-N disproportionately degrades algal-driven treatment [Aparicio et al., 2022]. Another study by Rani and Maróti, 2021 reports that nitrate and nitrite inhibition directly impairs algal performance at these levels via physiological stress. In their study the *Chlamydomonas* sp. MACC-216 removed only 53.6% of 15 mM nitrate (with residual ~7 mM, relevant to secondary TN of 20–40 mg/L) over 6 days, with chlorophyll dropping by 25–35%, reactive oxygen species (ROS) rising, and PS II efficiency falling, as nitrate overload competes with CO₂ fixation and raises pH. Nitrite accumulation (1–5 mg/L from endogenous partial oxidation during high-TN spikes) worsens toxicity in pure cultures, cutting nitrate reductase activity and growth by 20–40% through membrane peroxidation. This results in effluent TN above 10–15 mg/L and TP over 1 mg/L [Rani and Maróti, 2021].

These algal limitations for decentralized (on-site) application of the technology for P and N removal that have been registered in our study, and the similar effects, described in the literature for other algae-based WWT applications emphasize the needs for influent moderation or reactor design tweaks to boost standalone polishing. Such measures for avoidance of these bottlenecks could include implementation of operational modifications to dampen hydraulic and nutrient shocks and to keep the algal process within a stable operating window. Effluent equalization (mixed buffer tank) followed by flow-paced feeding can reduce short-term variability from occupancy and upstream AS performance, while internal recirculation/bypass can further dilute transient peaks before they reach the algal reactor. Stability can also be improved by maintaining a constant design HRT (or surface loading rate) independent of daily flow swings, and by pH/inorganic carbon management (CO₂ or alkalinity dosing) to prevent high-pH excursions and sustain consistent uptake kinetics. Finally,

since nutrient removal is strongly linked to biomass retention, robust solids capture/harvesting (settling with coagulation, membrane separation, or attached-growth configurations) together with simple operational monitoring and control triggers (e.g., pH, turbidity/optical density, and periodic NO_x/PO₄ checks) can reduce effluent variability by enabling timely feed reduction, recirculation adjustment, or harvesting during periods with unfavorable conditions.

CONCLUSIONS

The exploratory experiment on the application of microalgae-based technology for nitrogen and phosphorus removal from secondary treated wastewater for decentralized systems revealed both strengths and limitations under the specific tested conditions. Treatment efficiency and overall performance were strongly influenced by household occupancy and variability in influent TN and TP concentrations. Despite this variability, a notable strength of the technology is its ability to achieve stable nutrient removal at influent TN < 20 mg/L and TP < 7 mg/L. However, at higher influent TN and TP concentrations, the required discharge standards for sensitive areas could not be consistently achieved. The results also suggest that phosphorus removal efficiency depends not only on its influent concentration but also on the influent nitrogen concentration. These preliminary findings require further confirmation through more detailed and comprehensive investigations under varying operational conditions.

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