

Article

Comparative Study of the Microalgae-Based Wastewater Treatment, in an Oil Refining Industry Cogeneration Concept

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Abstract

Microalgae are broadly recognized as promising agents for sustainable wastewater treatment and biomass generation. However, industrial effluents such as petroleum refinery wastewater (WW) present challenges due to toxic growth inhibiting substances. Three marine microalgae species: *Pseudochloris wilhelmii*, *Nannochloropsis gaditana* and *Synechococcus* sp. MK568070 were examined for cultivation potential in oil refinery WW. Their performance was evaluated in terms of growth dynamics, lipid productivity, and toxicity reduction, with a focus on their suitability for largescale industrial use. *N. gaditana* demonstrated the highest growth rate and lipid content (37% d.w.) as well as lipid productivity (29.45 mg/(Lday)) with the N-uptake rate of 0.698 mmol/(gday). The highest specific DIN uptake rate was observed in *P. wilhelmii* (0.895 mmol/(gday)) along with the highest volumetric productivity (93.9 mg/L/day) and WW toxicity removal (76.5%), while *Synechococcus* sp. MK568070 demonstrated lower performance metrics. A simple numerical model was applied to calculate continuous operation based on empirical results of batch experiments. Sustainability of the microalgae-based WW remediation under the conditions of optimized lipid biomass production was estimated, regarding 2019–2022–2025 cost dynamics. Parameters for optimum open raceway pond cultivation were calculated, and the biomass production accumulation was estimated, with the highest biomass production noted in *P. wilhelmii* (171.38 t/year). Comparison of treatment costs, production costs and revenue showed that the best candidate for WW remediation is *N. gaditana*.

Keywords: *Nannochloropsis gaditana*; *Pseudochloris wilhelmii*; *Synechococcus* sp. MK568070; lipid productivity; wastewater remediation; continuous culture



Academic Editor: Xiaohu Li

Received: 30 May 2025

Revised: 9 July 2025

Accepted: 11 July 2025

Published: 24 July 2025

Citation: Pritišanac, E.; Fafandel, M.; Haberle, I.; Geček, S.; Markić, M.; Bolf, N.; Vukadin, J.; Crnković, G.; Klanjšček, T.; Žilić, L.; et al. Comparative Study of the Microalgae-Based Wastewater Treatment, in an Oil Refining Industry Cogeneration Concept. *Water* **2025**, *17*, 2217. <https://doi.org/10.3390/w17152217>

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1. Introduction

Microalgal biofuel production and utilization are feasible on pre-existing technology platforms. However, high manufacturing costs have been hampering progress towards the commercial scale. Recently, cogeneration of microalgal biofuel using wastewater (WW) as a potential source of nitrogen, and flue gas as a source of CO₂, is an attractive option to reduce

the costs by 50% and at the same time remediate polluted water [1]. Such an approach is particularly interesting for heavily polluted industrial waters rich in ammonium, nitrate, and/or urea as sources of nitrogen, which are otherwise difficult and expensive to treat. While many studies explore the growth of microalgal biomass on municipal WW [2,3] or animal farm effluents [4,5], treatment of highly toxic industrial effluents such as petroleum refining industry WW remains a difficult challenge [6,7].

However, one of the greatest challenges is the treatment of petrochemical WW, a type of industrial effluent known to pose mutagenic risks, even to the purest water bodies [8]. To preserve environmental sustainability, it is essential to treat the oil refinery WW. However, only a small fraction of WW is treated worldwide [9]. Conventional WW treatments are not economically feasible and viable because they are expensive, require a large amount of energy, do not recover nutrients from the WW, and generate greenhouse gas emissions [10]. The quality of the oil refinery WW can vary depending on many factors, such as crude oil composition, processing technology, etc. Usually, they are rich in phenols, hydrocarbons, mercaptans, and ammonium levels, and contain substantial amounts of heavy metals, exhibiting high chemical oxygen demand (COD) [7,11]. All of these pose a major public health concern due to their toxicity at very low concentrations [12]. However, despite incorporating pretreatment methods (such as mechanical and chemical methods) for WW refinement, discrepancies may still arise, which can be mitigated by understanding the effluent nature and characteristics. Therefore, selecting an appropriate pretreatment method is crucial to ensure cost-effective secondary treatment of the effluent [13,14].

The bioremediation process involves the integration of selected microalgae strains into WW treatment. Biological treatment of petrochemical WW using natural, acclimated microbial consortia has been shown to be more effective than using commercial strains. Furthermore, halophilic strains demonstrate greater resilience to toxic stress and are more efficient in removing oil and COD. This is particularly important, as high COD levels are toxic to human health and can have detrimental effects on the environment [13,15]. There are several significant advantages to using microalgae strains in WW bioremediation [16]. These include a reduction in cultivation costs through the utilization of nutrients available in WW, thereby minimizing the need for external nutrient inputs; decreased reliance on external CO₂ supplies, enhancing the overall sustainability of the process [17]; lower energy consumption (0.2 kWh/m³ compared to 0.5 kWh/m³ for conventional treatment methods) while simultaneously enabling the production of valuable biomass [18]; the conversion of nutrients from WW into biomass, promoting the principles of a circular economy [10]; and the sequestration of CO₂, contributing to the advancement of a green economy [19].

There are a few autotrophic organisms capable of thriving in the presence of oil-derived toxic compounds, such as the previously mentioned mercaptans and hydrocarbons. Previous studies have found the picoeukaryotic algae *Pseudochloris wilhelmii* and *Nannochloropsis gaditana* to be promising candidates due to their fast growth and tolerance for high concentrations of NH₄⁺ and NO₃[−], which provide a favorable environment for growth and lipid accumulation [20,21]. In addition, these algae are rich in lipids with high proportions of mono- and polyunsaturated fatty acids, offering a wide variety of applications from biodiesel to feed production, or pharmacy and nutraceuticals (vitamins, essential fatty acids, and antioxidants). Other small autotrophic prokaryotes, such as *Synechococcus* sp. MK568070, are of increasing interest. Their rapid growth and high nutrient uptake efficiency lead to higher productivity and efficient toxicant removal [22,23].

It has been noted that microalgae cultivated in WW produce hydrocarbons in bulk, which can be converted into biofuels such as biogas, biodiesel, biohydrogen, bioethanol, and biobutanol through thermochemical and biological methods [7,24]. Despite high cultivation costs, biofuel production, along with integrated co-products, offers significant

commercial benefits from an industrial perspective [25]. Countries like China, Germany, Japan, and Taiwan collectively produce around 19,000 tons of dehydrated microalgal biomass annually, generating an estimated 5.7 billion USD in revenue from derived high-value products [26]. To our knowledge, there is a lack of studies comparing microalgae strains in WW remediation, lipid productivity, and toxicity removal, coupled with a robust economic assessment of the upscaled cogeneration concept.

This study evaluates the feasibility of cultivating *P. wilhelmii*, *N. gaditana*, and *Synechococcus* sp. MK568070 under these conditions, with a comparative assessment of their capacities for inorganic carbon sequestration, biomass accumulation, and nitrogen removal from toxic, heavily polluted wastewater. A series of batch culture experiments were conducted in photobioreactors using diluted oil refinery wastewater as the growth medium. The experimental conditions were designed to simulate industrial wastewater environments, enabling the assessment of microalgal performance under realistic operational constraints. Finally, the operational cost (OPEX) comparison within a specific time frame (2019–2022–2025) was performed. These findings provide valuable insights into the potential application of selected microalgae in the industrial-scale implementation of a sustainable cogeneration process.

2. Materials and Methods

2.1. Microalgae Selection and Maintenance

Marine picoeukaryotes *Pseudochloris wilhelmii* (SAG 55.87) and *Nannochloropsis gaditana* (SAG 2.99) were obtained from the Culture Collection of Algae at Göttingen University (SAG). *Synechococcus* sp. MK568070 was isolated at the Center for Marine Research and deposited into the NCBI GenBank database under accession number MK568070. The cultures were activated in appropriate media: *P. wilhelmii* and *Synechococcus* sp. MK568070 in saltwater BG 11 (NaNO_3 , K_2HPO_4 , $\text{MgSO}_4 \times 7\text{H}_2\text{O}$, $\text{CaCl}_2 \times 2\text{H}_2\text{O}$, citric acid, ammonium ferric citrate green, Na_2EDTA , Na_2CO_3 and trace elements: H_3BO_3 , $\text{MnCl}_2 \times 4\text{H}_2\text{O}$, $\text{ZnSO}_4 \times 7\text{H}_2\text{O}$, $\text{Na}_2\text{MoO}_4 \times 2\text{H}_2\text{O}$, $\text{CuSO}_4 \times 5\text{H}_2\text{O}$, $\text{Co}(\text{NO}_3)_2 \times 6\text{H}_2\text{O}$), and *N. gaditana* in F/2 medium (NaNO_3 , $\text{NaH}_2\text{PO}_4 \times 2\text{H}_2\text{O}$, trace elements: Na_2EDTA , $\text{FeCl}_3 \times 6\text{H}_2\text{O}$, $\text{CuSO}_4 \times 5\text{H}_2\text{O}$, $\text{ZnSO}_4 \times 7\text{H}_2\text{O}$, $\text{CoCl}_2 \times 6\text{H}_2\text{O}$, $\text{MnCl}_2 \times 4\text{H}_2\text{O}$, $\text{Na}_2\text{MoO}_4 \times 2\text{H}_2\text{O}$ and vitamin mix: vitamin B_{12} , vitamin B_1 , biotin) without silicate, respectively. The inoculum cultures were maintained in flasks at a temperature of 16 ± 1 °C, under a light intensity of $80 \mu\text{mol}/\text{m}^2/\text{s}$, with a 12:12 light–dark cycle.

2.2. Batch Growth in Photobioreactors (PBR)

Three independent growth experiments of the selected microalgae were conducted in batch mode using four double-walled borosilicate 2.6 L photobioreactors (PBRs), with each experiment dedicated to a single microalgae species: *Pseudochloris wilhelmii*, *Nannochloropsis gaditana*, *Synechococcus* sp. MK568070. The growth conditions were as follows: light intensity ($130 \mu\text{mol photon m}^{-2}\text{s}^{-1}$), light cycle (12:12 h day/night cycle), temperature (24 °C), and pH (via CO_2 flux) were controlled by a SCADA (Supervisory Control and Data Acquisition) system [11]. Air or air/ CO_2 mixture (97:3 v/v) was injected at the bottom of the reactor through a glass tube and served as a source of carbon and oxygen, as well as an agitator and pH controlling agent. The pH of the cultures was maintained at 8.2 during the experiments, which was regulated additionally by adding 1M NaHCO_3 when necessary. Salinity was monitored daily by conductometer (Mettler Toledo, Columbus, OH, USA), and pH was controlled by pH-probe (Mettler Toledo, Columbus, OH, USA).

The growth medium in the PBRs was untreated oil refinery wastewater (WW) mixed with artificial (ASW) seawater in a 1:1 (vol:vol) ratio (both prefiltered on $0.2 \mu\text{m}$, Millipore) in order to obtain a medium salinity of 19 psu for algae growth. The WW composition

is presented in Table 1. To obtain the fixed and uniform NH_4^+ concentration (1.1 mM) in the final medium in all experiments, the WW-stock was set to the desired value prior to the dilution with ASW. Medium was enriched by the addition of KH_2PO_4 stock solution (100 $\mu\text{mol/L}$) to achieve an initial nutrient ratio N:P = 8. After inoculation, the strains were grown in batch mode until the stationary phase was reached (16 days) to obtain a high biomass concentration and to determine batch growth kinetic parameters. The triplicates were checked for outliers using the modified z-score method [27].

Table 1. Approximate composition of the untreated oil refinery wastewater.

Parameter	Unit	Range
pH	-	7.48–10.62
Hydrocarbon	mg/L	5.4–152
Mineral oil	mg/L	3.3–81.3
COD	mg/L O_2	1–658
NH_4^+	mg/L	0.5–124
NO_3^-	mg/L	15–61
PO_4^{3-}	mg/L	0–1
S^{2-}	mg/L	0–122
Mercaptan	mg/kg	0–64

2.3. Analytical Procedures

2.3.1. Biomass Analyses

Daily biomass concentration was measured gravimetrically as dry weight (dw) according to the standardized 2540-D method [28]. For the determination of chemical composition, the biomass was harvested by centrifugation (Eppendorf centrifuge) of 5 mL aliquots of the sample at 4200 rpm for 10 min. The pellets were washed twice with deionized water to remove salt and centrifuged again. Biomass was lyophilized in a freeze-dryer (Labconco, FreeZone2.5, Kansas City, MO, USA), ground into powder, and used for all further biomass composition analyses.

2.3.2. C:H: N Elemental Analysis

Elemental analysis of the algal pellets (% of dw) was performed using a “2400 CHN Elemental Analyzer” (Perkin Elmer, Springfield, IL, USA) and a CHN 628 Analyzer (LECO, St. Joseph, MI, USA). For the determination of carbon, hydrogen, and nitrogen, the pre-weighed freeze-dried sample—sealed in a foil or capsule—is combusted in an oxidizing atmosphere within a vertical furnace. Elemental carbon, hydrogen, and nitrogen are converted into CO_2 , H_2O , N_2 , and NO_x . The resulting gases are directed through filters into a ballast cylinder, where the gas mixture is homogenized. Each element is detected using a dedicated detector: an infrared (IR) detector for carbon, an IR detector for hydrogen, and a thermal conductivity (TC) detector for nitrogen. The detection of the resulting gases occurs as the sample passes through a constant-volume dosing device into the infrared detection system. The content of carbon and hydrogen is determined by direct IR absorption, measuring the concentrations of CO_2 and H_2O . The mixture of combustion gases, carried by helium, is subsequently introduced into a catalytic furnace where NO_x is reduced to N_2 . Carbon dioxide and moisture are then removed via columns packed with appropriate chemical absorbents. The remaining mixture of helium and nitrogen is conveyed to the thermal conductivity detector, where the amount of N_2 is quantified.

2.3.3. Lipid Content

Total lipids of the algal cultures during experimental growth were extracted according to Bligh and Dyer [29]. First, 50 mL samples were filtered on pre-combusted GF/F filters

(Whatman, Little Chalfont, Buckinghamshire, UK) and mechanically disrupted with a tissue homogenizer, and total lipids were extracted according to the modified Bligh and Dyer method in a mixture of dichloromethane/methanol (DCM:MeOH, 2:1 *v/v*). Ultimately, samples were placed in an ultrasonic water bath. The extraction procedure was repeated for three 30 min cycles in an ultrasonic water bath (Aquasonic Model 750D, Aboyne, Scotland, UK) at 35 °C.

2.3.4. Inorganic N and P Nutrients Analysis

Aliquots (50 mL) were sampled for the determination of nitrate (NO_3^-), nitrite (NO_2^-), ammonium/ammonia ($\text{NH}_4^+/\text{NH}_3$), soluble reactive phosphorus (SRP), and dissolved organic phosphorus (DOP) in the PBRs. Samples were centrifuged (10 min, 5000 rpm), and supernatant was immediately analyzed. Inorganic N and P nutrients were analyzed following the procedures described by Parsons [30] and by Ivančić and Degobbi [31]. When necessary, appropriate dilutions of the samples were used to fit the linear range of spectrophotometric determinations for each method (Shimadzu UV 1800) using Lambert–Beer’s law at the path length of 1 cm.

$$c = A/(\varepsilon * d) \quad (1)$$

A = Absorbance

ε = Molar absorptivity [$\text{L mol}^{-1} \text{cm}^{-1}$]

d = Path length of the cuvette containing the sample [cm]

c = Concentration of the compound in solution [mol L^{-1}]

Dissolved inorganic nitrogen (DIN) concentration was calculated as the sum of NO_3^- , NO_2^- , and $\text{NH}_4^+/\text{NH}_3$ concentrations.

$$c(\text{DIN}) = c(\text{NO}_2) + c(\text{NO}_3) + c(\text{NH}_4) \quad (2)$$

2.4. Toxicity Assessment

The toxic potential of the wastewater-based growth medium was evaluated during experimental growth of *P. wilhelmi*, *N. gaditana*, and *Synechococcus* sp. MK568070 in PBRs. This assessment employed the marine bioluminescent bacterium *Aliivibrio fischeri* using the Microtox® in vitro testing system (Modern Water, York, England, UK). The reduction in bacterial luminescence was measured after exposure to serial dilutions of the organic extract from the growth medium. For this, commercially available freeze-dried *A. fischeri* was used in the AZUR 500 luminometer, following a protocol adapted from seawater testing [32] for media samples. In brief, 50 mL culture aliquots were centrifuged at 5000 rpm for 10 min. The resulting supernatants were extracted with 5 mL of dichloromethane (DCM), evaporated to dryness, and dissolved in 50 μL of dimethyl sulfoxide (DMSO). EC_{50} values were obtained using Microtox Omni software version 4.2 and expressed as the percentage of wastewater equivalents in the growth medium, with 95% confidence intervals, and used for assessment of medium detoxification.

2.5. Growth Analysis and Estimation of Volumetric Productivity in Continuous Operation

Kinetic modeling was performed using Primer-7 software (PRIMER-E Ltd., Devon, UK) for linear regression, with a convergence criterion of 10^{-4} [33]. The growth of microalgal biomass in batch experiments was obtained using the Verhulst logistic growth model [34], which accounts for self-limiting growth due to nutrient depletion or space limitations (Figure 1).

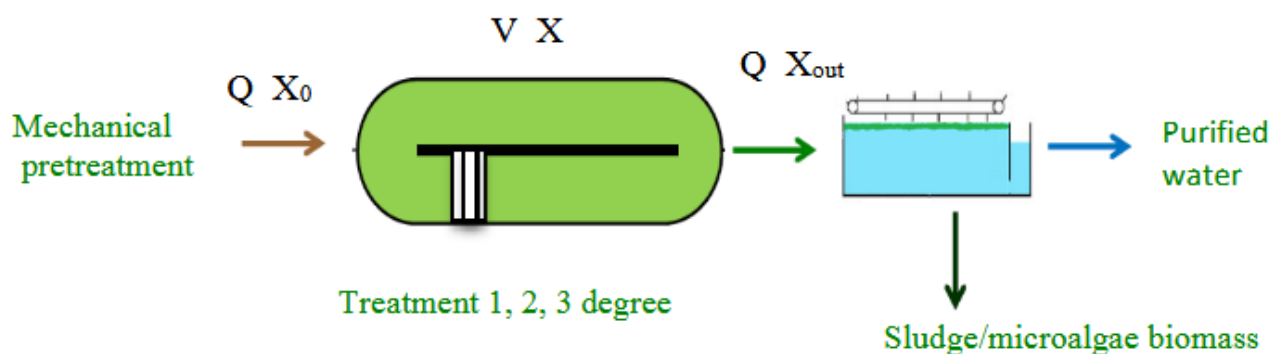


Figure 1. Approximation of the scaled-up continuous operation. Q = flow rate, X_0 = initial cell concentration, V = volume, X = biomass concentration in the reactor, X_{out} = concentration of biomass as output from the reactor.

The Verhulst model is given as:

$$\frac{dX}{dt} = \left[\mu \cdot X \cdot \left(1 - \frac{X}{X_m} \right) \right] \quad (3)$$

where X is the biomass concentration (mg/L), μ is the maximum specific growth rate (day^{-1}), and X_m is the maximum biomass concentration the system can support.

To extend the model to a continuous stirred-tank reactor, a mass balance is performed, presuming that the growth kinetics remain equal as in the batch phase. A constant WW flow rate of $5000 \text{ m}^3/\text{day}$ is considered in the calculation as representative of conditions at the INA oil refinery (personal communication), and an open raceway pond depth of 0.3 m was chosen as optimum based on previously published research by Sompech et al. [35]. The flow rate of the medium is expressed as Q (L/day), and the hydraulic retention time (HRT) is defined as:

$$\theta = \text{HRT} = \frac{V}{Q} \quad (4)$$

The mass balance on biomass is:

$$\frac{dX}{dt} = \mu \cdot X \cdot \left(1 - \frac{X}{X_m} \right) - D \cdot (X - X_{in}) \quad (5)$$

where $D = Q/V = 1/\theta$ is the dilution rate (1/day) and X_{in} is the biomass concentration in the inflow (typically zero). Assuming $X_{in} = 0$, the equation simplifies to:

$$\frac{dX}{dt} = \mu \cdot X \cdot \left(1 - \frac{X}{X_m} \right) - D \cdot X \quad (6)$$

At $(dX/dt = 0)$, the biomass concentration in the continuous reactor becomes:

$$X = X_m \cdot \left(1 - \frac{D}{\mu} \right) \quad (7)$$

The volumetric productivity in a continuous reactor can be calculated as:

$$P = D \cdot X = \frac{Q}{V} \cdot X = \frac{X}{\theta} \quad (8)$$

Combining Equations (5) and (6), the volumetric productivity under continuous operation becomes:

$$P = D \cdot X_m \cdot \left(1 - \frac{D}{\mu} \right) \quad (9)$$

This relationship shows that productivity is a quadratic function of the dilution rate D . In order to determine the optimal dilution rate that also maximizes productivity, the first derivative of P with respect to D is set to 0:

$$\frac{dP}{d\theta} = X_m \cdot \left(1 - \frac{2D}{\mu}\right) = 0 \quad (10)$$

Solving for D yields:

$$D_{opt} = \frac{\mu}{2} \Rightarrow \theta_{opt} = \frac{2}{\mu} \quad (11)$$

Substituting D_{opt} into the productivity equation gives the maximum volumetric productivity:

$$P_{max} = \frac{\mu \cdot X_m}{4} \quad (12)$$

This model demonstrates that the optimal biomass productivity in a continuous reactor is achieved when the dilution rate equals half of the maximum specific growth rate, corresponding to an HRT of $\theta = 2/\mu$. These connections are essential for designing and operating continuous systems for large-scale microalgal cultivation.

2.6. Sustainability Assessment of the Cogeneration Production of Microalgal Biodiesel in Oil Refinery Concept

The preliminary analysis of the operation costs required for industrial-scale microalgal growth provides critical insights into investment risk and long-term profitability of cogeneration systems utilizing microalgae biomass. The use of flue gases, originating from the arbitrary industrial process in the studied case scenario of the INA oil refinery, is planned, and for the purposes of the study, the associated CO₂ cost is considered negligible. Therefore, CO₂ consumption expenditure was not included in the study.

In the cost assessment, alongside key parameters such as biomass analyses, lipid content, nutrient analysis, toxicity assessment, and growth analysis, we incorporated the variability and dynamics of EU market prices for electricity and water between 2019, 2022, and 2025 [36]. This time frame includes three distinct economic conditions: 2019 as a pre-COVID baseline, 2022 as a representative of post-pandemic market recovery and volatility, and 2025 as a representation of current economic conditions. Oil markets were consistently affected by the pandemic, which further justifies the inclusion of a specific time frame to account for its effect on the cogeneration production economic justification [37,38].

A preliminary operational sustainability assessment was performed to evaluate the economic viability of microalgal biodiesel production within the oil refinery framework, considering only operational expenditures (OPEX) [39]. A scenario of the continuous growth of *P. wilhelmii* (SAG 55.87), *N. gaditana* (SAG 2.99), and *Synechococcus* sp. (MK568070) was predicted. The flotation process costs were calculated based on a specific electricity consumption of 0.005 kWh per cubic meter. Specific OPEX cost categories include recurring costs related exclusively to the raceway pond operation, separation processes, and lipid extraction operation, such as energy consumption, flotation coagulant consumption [40], water and nutrient inputs, and other consumables [41,42].

3. Results and Discussion

The application of microalgae for wastewater treatment has gained increased attention due to its dual ability to remediate pollutants and produce biomass for biofuel generation [40]. Our findings are consistent with Chisti [39], who highlighted the dual benefit of microalgal systems for wastewater remediation and biomass production. Experiments con-

ducted in batch mode in a photobioreactor provided species-specific patterns on nutrient uptake, biomass growth, and biomass composition.

3.1. Nutrient Uptake and Intracellular Carbon and Nitrogen Composition

CHN analysis results track nitrogen incorporation into the microalgae, cellular carbon synthesis, and the C/N ratio of the cell. Tested strains exhibit distinct physiological responses and different carbon and nitrogen composition, carbon and nitrogen ratio, and dissolved inorganic nitrogen uptake (Figure 2). Although the accumulation dynamics of cellular nitrogen and carbon differ, increases in both are observed, accompanied by the elimination of dissolved inorganic nitrogen from the medium. During the lag and early exponential phase (between days 1 and 5), growth rates, nitrogen, and carbon incorporation were similar in all three tested strains (Figure 2a,b). After day 6, a visible difference in cellular C and cellular N content was observed among the tested strains. *P. willhelmii* exhibited the highest intracellular C content (Figure 2a). *Synechococcus* sp. MK568070 showed the lowest intracellular C and highest cellular N content by the end of the experiment (Figure 2a,b). *N. gaditana* exhibited 40% intracellular C content and 2% intracellular N content (Figure 2a,b). All strains show a decline in cellular N at the beginning of the stationary phase, consistent with nitrogen limitation.

The initial rapid increase in intracellular N reflects the uptake of available nitrogen and fuels synthesis of nitrogen-rich cellular components during the exponential growth phase. The subsequent decline in intracellular nitrogen indicates that the cells are becoming nitrogen-limited relative to carbon availability [21]. Particularly, *Synechococcus* sp. reaches the highest peak cellular N in the exponential growth phase (day 5), suggesting it either takes up nitrogen faster or accumulates nitrogen-rich compounds more effectively than picoeukaryotes during the early phase (Figure 2b).

The intracellular C/N ratio follows the expected pattern: it remained steady around the Redfield ratio until inorganic nitrogen was removed. At this point, the ratio increased because photosynthesis continued to add carbon, while nitrogen levels remained constant [43]. *P. willhelmii* and *N. gaditana* showed high intracellular carbon accumulation relative to nitrogen, reaching C/N ratios of 18 and 22 mol/mol, respectively. *Synechococcus* sp. MK568070 had a significantly lower (40.9%) final C/N ratio of 13 mol/mol (Figure 2c). The observed increase in C/N ratio is an indicator of nitrogen limitation in the tested strains. All strains show a decline in cellular N in the stationary phase, consistent with nitrogen limitation. As a result of nitrogen limitation during the stationary phase, an increase in the C/N ratio was observed, indicating the accumulation of carbon storage compounds, such as lipids [21].

The fastest DIN removal was observed by *N. gaditana* by day 5, i.e., during the exponential growth phase (Figure 2d). Other strains exhibited prolonged DIN removal while remaining in the exponential growth phase, with *P. willhelmii* completing DIN removal by day 6 and *Synechococcus* sp. MK568070 by day 7 (Figure 2d). Interestingly, although carbon accumulation through photosynthesis is expected to continue for more than 10 days after nitrogen depletion [44,45] *P. willhelmii* exhibited an unexpected continuous increase in cellular nitrogen following the removal of dissolved nitrogen (Figure 2b,d), which suggests that oil refinery WW may have had some nitrogen of organic and inorganic origin taken up by the cell [21].

Since WW samples contained inorganic and organic nitrogen, the observed nitrogen uptake implies that *P. willhelmii* could employ a mixotrophic strategy. While previous studies indicated that marine *Nannochloropsis* species, including *N. gaditana*, are obligate photoautotrophs [46], a recent study by [47] reported evidence of mixotrophy in novel *Pseudochloris* sp. (Trebouxiophyceae). Our results also suggest that *P. willhelmii* may exhibit

mixotrophy. The continued growth and accumulation of carbon with increasing C/N ratio during the stationary phase observed here suggests the deployment of mixotrophic metabolism in both picoeucaryote tested strains in the presence of organic compounds in the oil refinery WW. The apparent ability of *P.wilhelmii* and *N. gaditana* to remove organic C- and N-containing contaminants from WW, in addition to the proven ability to remove inorganic nutrients, is a significant feature for the use of these algae in the bioremediation of WW. These results are aligned with recent findings of Goswami et al. [48], who explored the cultivation of *Pseudochloris* sp. under mixotrophic conditions and found that mixotrophic growth, with organic carbon supplementation, yielded the highest biomass productivity. Pang et al. [47] also confirmed mixotrophy in the *Pseudochloris* strain, even showing phagomixotrophy while exposed to different light and nutrient conditions. Mixotrophy easily explains *P. wilhelmii*'s superior efficiency of contaminant removal in a complex nutrient environment, such as oil refinery WW.

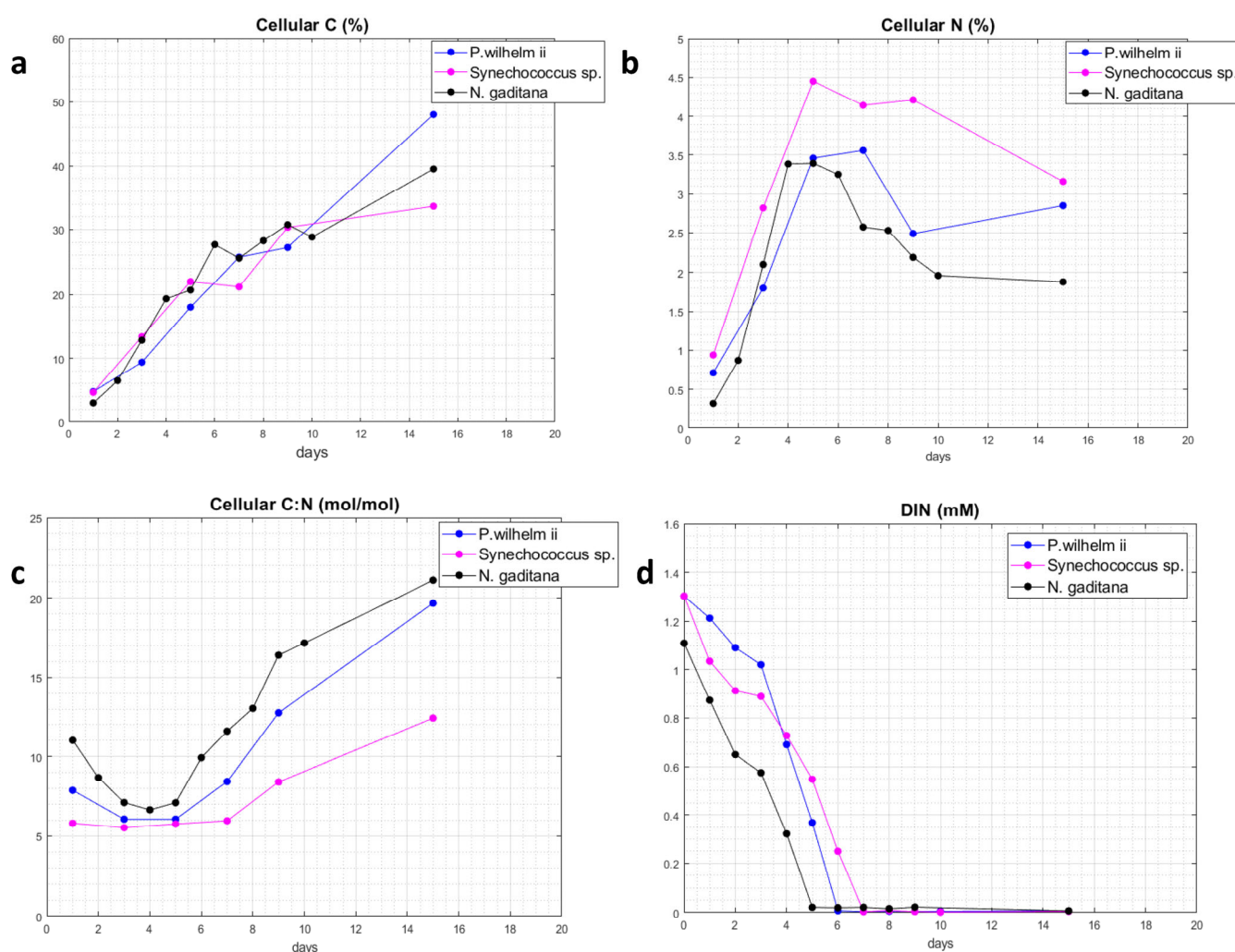


Figure 2. Nitrogen and carbon dynamics in batch cultures by *P. wilhelmii*, *Synechococcus* sp., and *N. gaditana* grown in a 1:1 (v/v) medium of ASW and oil refinery wastewater (1:1): (a) cellular C (%) content in *P. wilhelmii*, *Synechococcus* sp., and *N. gaditana*; (b) cellular N (%) content in *P. wilhelmii*, *Synechococcus* sp., and *N. gaditana*; (c) cellular C/N (mol/mol) ratio *P. wilhelmii*, *Synechococcus* sp., and *N. gaditana*; (d) DIN (mM) *P. wilhelmii*, *Synechococcus* sp., and *N. gaditana*.

3.2. Algae Growth Dynamics and Lipid Production

The three tested species are compared in terms of growth dynamics, dissolved inorganic nitrogen (DIN) uptake, hydraulic retention time (HRT), volumetric productivity, lipid

productivity, lipid content, and toxicity reduction. In general, the most marked differences were found between prokaryotes and eukaryotes, regarding nutrient uptake rate and toxicity reduction. Results of calculated parameters are presented in Table 2 and for the detailed presentation of the data please see the Supplementary Materials (Table S1).

Table 2. Summary of the observed growth indices in experimental cultures.

	μ day ^{−1}	Maximum Specific DIN Uptake Rate mmol/(gday)	Maximum Volumetric Productivity mg/(Lday)	HRT (day)	Final Lipid Content % d.w.	Lipid Productivity mg/(Lday)	Toxicity Reduction %
<i>P. wilhelmii</i>	0.432	0.895	93.9	2.28	28	26.30	76.5
<i>Synechococcus</i> sp.	0.336	0.531	71.0	3.02	21	14.91	12.4
<i>N. gaditana</i>	0.576	0.698	79.6	1.72	37	29.45	51.0

P. wilhelmii demonstrated the highest DIN uptake rate (0.895 mmol/(gday)), maximum volumetric productivity (93.9 mg/L day), and the highest toxicity reduction (79%) (Table 2). Removal of inorganic nitrogen corresponds with the increase in cellular N observed in Figure 2b; however, the dynamics of C and N accumulation differ significantly between species. High accumulation of carbon results in high C/N ratios, high final lipid content (28% d.w.), and lipid productivity (26.30 mg/Lday).

N. gaditana exhibited the highest specific growth rate (0.576 day^{−1}) and lipid productivity (29.45 mg/Lday), as well as final lipid content (37%) (Table 2). It also showed the shortest hydraulic retention time (1.72) required for nitrogen removal (Table 3 and Figure 2d). *N. gaditana* exhibited both high N- as well as high C-demand, resulting in the early N-limitation (fifth day) and substantially dynamic C:N ratio, indicating a high accumulation of storage compounds such as lipids, compared to the N-rich organic compounds such as proteins. These characteristics make *N. gaditana* one of the most promising species for both valuable biomass production and wastewater remediation.

Table 3. Calculated open raceway pond (ORP) parameters for *Synechococcus*, *N. gaditana*, and *P. wilhelmii*.

		<i>Synechococcus</i> sp.	<i>Nannochloropsis gaditana</i>	<i>Pseudochloris wilhelmii</i>
Volume of ORP	m ³	30.241	17.161	22.785
Surface of ORP	Ha	10.08	5.72	7.59
Biomass production	t/year	129.62	145.23	171.38
Θ_p	Day	6.05	3.43	4.56

The highest nitrogen demand, and consequently the lowest C:N ratio, were observed in *Synechococcus* sp. (Figure 2c), accompanied by the lowest specific DIN uptake rate of 0.531 mmol/ (gday) and the lowest lipid content (21%) (Table 2). *Synechococcus* sp., exhibited the lowest maximum specific growth rate and, consequently, the longest HRT among the three tested strains (Table 2). Based on these results, *Synechococcus* sp. was qualified as the least suitable for bioremediation applications within the context of this study. In addition, the low capacity for toxicity reduction (12.4%) compared to the tested picoeukaryotes indicates insufficient removal of toxic compounds from the oil refinery WW. There are several studies on diverse strains of *Synechococcus* showing promising results for WW bioremediation, demonstrating successful removal of nitrogen > 98% [49,50]; however, no record was found about the toxicity removal from various WWs. Considering the ability of cyanobacteria to produce toxins themselves under unfavorable environmental

conditions, it is very likely that *Synechococcus* sp. exhibits such a metabolic response as well [51].

Synechococcus sp. displayed a much slower growth rate than both *P. willhelmii* (22% slower) and *N. gaditana* (41.67% slower). It can be generally stated that none of the previous studies took into consideration the toxicity decrease in the WW as a measure of remediation efficiency. In the case of cyanobacteria, this seems to be very important, having a negative impact on their suitability for remediation purposes. On the other hand, among the studied species, *P. willhelmii* demonstrated the highest efficiency in nitrogen removal and the highest efficiency in WW toxicity reduction (Table 2). Although it had a slower N uptake, *N. gaditana* showed the highest potential for biofuel production based on high lipid proportions and lipid productivity.

3.3. Sustainability of Upscaled Cogeneration Concept

Observed values of biomass production, nutrient uptake, lipid productivity, and toxicity removal provide a general idea of the behavior of different cultures, but due to methodological limitations, it is challenging to assess the behavior of microalgae exposed to WW in an open continuous system based on empirical data alone. To address this, ideal specific growth rates (μ_{MAX} , day^{-1}) were calculated from accumulated biomass in batch mode using the logistic Verhulst model [33,52]. Growth kinetics was used to find the optimal dilution rate, i.e., wastewater flow in the continuous operation of the bioreactor (see the data in Supplementary Materials, Tables S1 and S2).

The resulting spatial requirements for the open raceway pond (ORP) in terms of volume and surface, as well as the hydraulic retention time (Θ_p) are presented in Table 3. Calculated parameters for optimum open raceway pond (ORP) cultivation and biomass production (t/year) are compared between three tested species [33,52].

For the depth of ORP set at 0.3 m (optimum photosynthesis efficiency), the calculated volume of ORP was 30.241 m^3 for *Synechococcus* sp. MK568070, 17.161 m^3 for *N. gaditana*, and 22.785 m^3 for *P. willhelmii*. The surface area needed for each strain was also determined. *N. gaditana* exhibited the smallest spatial requirement, only 5.72 ha (Table 3). These results are aligned with [53], who demonstrated that microalgae production costs are significantly influenced by cultivation scale. In small-scale aquaculture hatcheries, the cost may exceed EUR 500 per kg of dry biomass, whereas larger systems dramatically reduce production costs. The maximum productivity retention time was highest in *Synechococcus* sp. MK568070 (6.05). Considering all calculated parameters, biomass production was estimated. The lowest biomass production (129.62 t/year) was recorded for *Synechococcus* sp. MK568070, while *N. gaditana* and *P. willhelmii* demonstrated higher biomass production rates.

The period from 2019 to 2025 represents a critical time frame for the global economy, marked by significant disruptions and shifts. The COVID-19 pandemic, geopolitical tensions, and inflationary pressures have created a volatile environment with far-reaching consequences. A rise in global energy demand, particularly in the power sector, and a growing investment in clean energy sources have a significant impact on the electricity costs and consequently the fuel prices. To obtain a better insight into the techno-economic trends within this time frame, three milestone years are analyzed. The changes in operational cost (OPEX) of the microalgae-based cogeneration production, and classical wastewater treatment, depending on the global market, are estimated and presented in Figure 3.

Based on the data from this study (see Supplementary Materials, Table S2), the microalgae-based wastewater remediation process is less energy-demanding and consequently more economically sustainable than the conventional bacterial wastewater treatment. Due to the record electricity prices in 2021 driven by the COVID-19 pandemic, and

followed by further supply-side issues caused by the Russian invasion of Ukraine, the year 2022 demonstrated the highest financial discrepancy between the contrasting WW remediation processes. WW treatment costs in 2019 amounted to EUR 530,726. By 2022, a substantial increase was recorded, with costs rising to EUR 1,112,178, effectively doubling. In 2025, WW treatment costs decreased significantly and stabilized at EUR 821,452 (Figure 3). The revenue for each strain was calculated by subtracting the market value of the produced biodiesel from the overall cost of production and adding the savings from CO₂ quotas. The total biodiesel production cost based on microalgae wastewater remediation holds a similar year-to-year trend on all three tested species. However, along with the markedly lower operational expenditures in all the tested scenarios, there is a well-expressed financial resilience of the microalgae-based process against the fluctuations of the energy prices incurred by the global crisis situations.

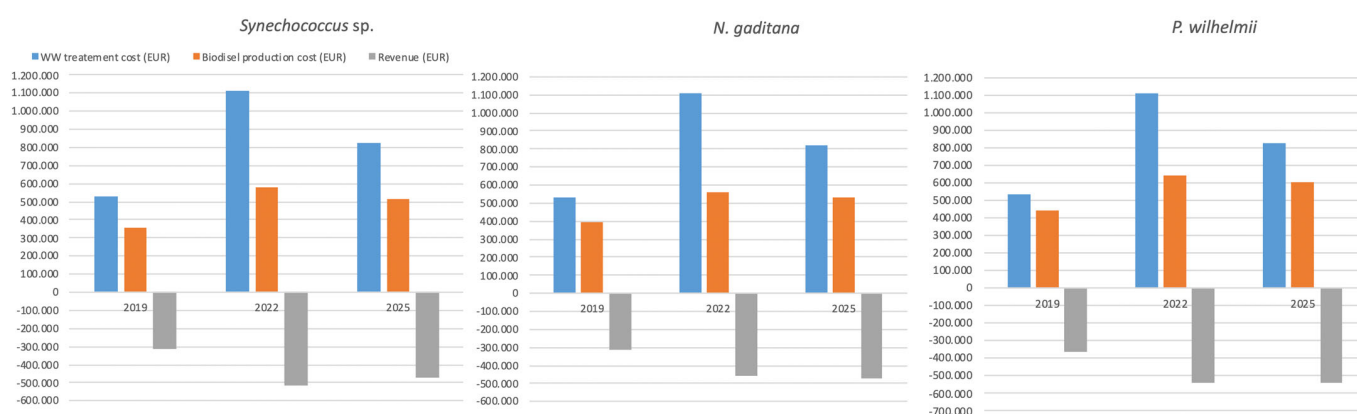


Figure 3. Comparison of WW treatment costs, biodiesel production costs, and revenue for *Synechococcus*, *N. gaditana*, and *P. wilhelmii*.

The biodiesel production cost (i.e., phycoremediation process) was in the period 2019–2025 in the range EUR 357,784 – 653,928, showing the highest value for *P. wilhelmii* (2022). The lowest expenditures were recorded for *Synechococcus* sp. In terms of financial efficiency in the bioremediation process, *Synechococcus* sp. MK568070 can be considered the most economically sustainable tested strain, in particular when considering the non-demanding wastewaters requiring only N and P removal. However, there are several drawbacks mentioned before regarding cyanobacteria metabolism that hamper future considerations of this species for the cogeneration remediation purposes. Although showing higher operational costs required for the growth, picoeukaryotes *N. gaditana* and *P. wilhelmii*, these costs are related to the processing of the high proportions of valuable lipids for further use in the oil refinery. In the framework of the set working capacities of 5000 m³ WW/year, the maximum lipid production capacity of *N. gaditana* and *P. wilhelmii* is calculated to be 54.40 t/year and 48.16 t/year, respectively, contributing substantially to the sustainability of the concept. The microalgae-based bioremediation concept under the ideal conditions seems to incur lower costs than conventional biological WW treatment; however, the sustainability of the cogeneration is still under many doubts due to the low and very variable productivity, process sensitivity, and still negative revenue from the end-product marketing. In this study, the most promising revenue trends were recorded for *N. gaditana* with low negative values, and the least year-to-year variability (from EUR −311,913.14 to EUR −498,533.77; Figure 3).

The electricity consumption required for mixing the ponds was calculated based on the pond volume, using a factor of 0.065 kWh/m³/day. At the time the study was conducted, the electricity price was 0.4 EUR/kWh (February 2025), compared to the earlier value of

0.2 EUR/kWh recorded in 2019, and a value of 0.6 EUR/kWh in 2022 [54]. It is important to note that the trend of electricity price changes on the market affects the profitability of the process. However, when compared to conventional treatment, a significant cost advantage is observed in favor of the algal treatment, due to fewer processing steps and the potential revenue generated from biomass utilization [16,53,55]. Recent economic assessments also highlight that integrated systems combining wastewater treatment with biomass valorization can achieve improved cost-efficiency, especially when price fluctuations in utilities are considered [1].

In addition, according to our calculations, which considered the CO₂ quota (approximately 72.9 EUR/t in 2025) [36] and the estimated CO₂ uptake incorporated into microalgae biomass, using flue gas as a source of CO₂ provides some annual savings, which may vary depending on the microalgae strain. Despite the fact that these savings do not represent a significant financial contribution to the overall sustainability of the production process, they are a positive factor in terms of reducing atmospheric CO₂ emissions.

The cost of the land required for the installation of the ponds was not included in the calculation, but the data show that a large area is needed, varying depending on the species. In this case, it ranges from 5.72 to 10.08 hectares (Table 3). Land requirements vary and are directly linked to the strain-specific biomass productivity. The strains with lower biomass yield per unit area, such as *Synechococcus* sp. MK568070, requires larger pond surfaces to achieve the same output. Oostlander et al. [54] highlighted how spatial constraints can significantly affect the feasibility of algal systems. Our results show that land requirements depend on the strain, underscoring the importance of optimizing species selection based on both biological performance and land availability. *N. gaditana*, which requires the smallest land area while yielding the highest biomass and lowest biodiesel production cost, emerges as the most sustainable option for long-term industrial application. Based on its biomass production, biodiesel production economics, and compact spatial requirement, *N. gaditana* is the most promising candidate for upscaling in a cogeneration framework and for WW remediation (Table 3; Figure 3).

Our findings highlight the complex and dynamic nature of integrating microalgal systems into existing industrial frameworks. There is a lot of potential for future research. Future studies should explore cultivation performance under variable conditions (temperature, light, and WW quality) [56]. Additionally, a more detailed life cycle analysis would improve the long-term feasibility assessment. There is also an opportunity for genetic or metabolic strain engineering to enhance traits like lipid yield [57]. Finally, developing predictive economic models that incorporate the utility market volatility and land use constraints, building on the bioprocess frameworks above, would support better policy alignment and industrial application.

4. Conclusions

Three selected strains: *P. wilhelmii*, *Synechococcus* sp. MK568070, and *N. gaditana* were tested for cultivation potential in oil refinery wastewater. Among the tested strains, *Synechococcus* sp. MK568070 was quantifiably the least suitable.

P. wilhelmii demonstrated the highest efficiency in toxicity reduction, maximal volumetric productivity, and rapid nitrogen removal, qualifying to be a promising candidate in the bioremediation of oil refinery WW. However, biochemical analysis and growth kinetics revealed that *N. gaditana* exhibited very good biomass yield and nitrogen removal, and the highest lipid productivity. In terms of treatment costs, production expenses, and revenue comparison in the framework of a cogeneration concept, *N. gaditana* generated the highest savings (revenue) over the period 2019–2022–2025.

The use of microalgae in bioremediation is a promising and innovative approach due to its low cost, high efficiency, and environmental friendliness. However, concerns remain regarding the limited reduction of toxicity and the persistence of petroleum-derived toxic substances in the treated wastewater. Future research should focus on pilot-scale validation of microalgal wastewater treatment systems, including the assessment of system robustness across varying industrial effluent types. To gain a more accurate estimate of the overall sustainability of the cogeneration remediation process, CAPital EXpenditures categories should be included, in particular, equipment procurement (raceway ponds and pumps), installation costs, infrastructure development, pretreatments, and initial system integration. These costs fall under the broader framework of Life Cycle Analysis, which includes temporal factors, maintenance cycles, and depreciation, providing a more comprehensive financial risk assessment. As a promising result of this study, it is important to note that further investigation should be undertaken to fully understand the long-term resilience and stability of the tested strains under real industrial-scale conditions.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/w17152217/s1>, Table S1: Calculation of biomass production for conditions of maximum specific growth rate for *Synechococcus* sp. *N. gaditana* and *P. wilhelmii* based on experimental growth data; Table S2: Calculation of the operational expenditure (OPEX) during *Synechococcus* sp. *N. gaditana* and *P. wilhelmii* biomass production and processing in the industrial-scale raceway pond. Comparison of the year 2019, 2022 and 2025.

Author Contributions: Conceptualization, methodology, data analysis, writing—original draft—E.P.; Formal analysis, Writing—review and editing—M.F.; experimental design, data acquisition, data curation, literature collection and overview—I.H.; Validation, formal analysis, writing—review and editing—S.G.; Formal analysis—M.M.; Conceptualization, writing—review and editing—N.B.; Formal analysis, writing—review and editing—J.V.; Investigation, Review and editing—G.C.; Conceptualization, writing—review and editing—T.K.; Methodology, data acquisition, data curation—L.Ž.; Supervision, funding acquisition, project administration, writing—review and editing—M.B. All authors have read and agreed to the published version of the manuscript.

Funding: The research was funded by the: Croatian Science Foundation under the project number IP-2024-05-8858 (A3-PHYCO-TOX) and by the HAMAG-BICRO project NPOO.C3.2.R3-11.05.0263.

Data Availability Statement: All relevant data are within the manuscript and in the Supplementary Materials.

Acknowledgments: The authors are thankful to Lana Husinec, Andrea Budiša and Enis Hrustić for the technical assistance during the experimental part of the study. The support of INA-INDUSTRIJA NAFTE d.d. for data provisions and expert advises regarding wastewater treatment process is highly acknowledged.

Conflicts of Interest: The authors declare no conflict of interest.

List of Abbreviations and Symbols

Parameters	Unit	Definition
X_o	mg/L	Initial cell concentration
X_m	mg/L	Maximum concentration that the system can achieve in batch
μ	day ⁻¹	Maximum specific growth rate
X_{in}	mg/L	Concentration of inflow in the reactor
X_{out}	mg/L	Concentration of biomass as output from the reactor
dX/dt	mg/(Lh)	Change in biomass

V		Reactor volume
D		Dilution rate
P		Volumetric productivity
θ	Day	Hydraulic retention time (HRT) in the reactor
Θ_p		HRT at maximum productivity
Q	L/day	Flow rate
Q _p	L/day	Flow rate at maximum productivity
X _p	mg/L	Concentration at maximum productivity

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