Comparative study of the microalgae-based wastewater treatment, in an oil refining industry cogeneration concept

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ABSTRACT

Microalage are broadly recognized as promising agents for sustainable wastewater treatment, carbon sequestration, and biomass generation. However, industrial effluents such as petroleum refinery wastewater present challenges due to toxic growth inhibiting substances. Three marine microalgae species: *Pseudochloris wilhelmii, Nanochloropsis gaditana* and *Synechococcus* sp. were examined for cultivation potential in oil refinery wastewater. Their performance was evaluated in terms of carbon capture, nitrogen reduction, biomass generation and lipid productivity, with a focus on their suitability for largescale industrial use. A simple numerical model was applied to calculate continuous operation based on empirical results of batch experiments. Sustainability of the microalgae-based wastewater remediation (case study INA oil refinery Ltd) under the conditions of optimized lipid biomass production was estimated. *N. gaditana* demonstrated the highest growth rate (0.024 day^{-1}), lipid content (37% d.w.), and maximum productivity flow rate (0.758 L/day). *P. wilhelmii* achieved the greatest volumetric productivity (93.9 mg/L/day) and wastewater toxicity removal (76.5%), while *Synechococcus* sp. demonstrated lower performance metrics. By synergistically combining insights from laboratory research and an economic study, our findings established *N. gaditana as* a promising candidate for the bioremediation of oil refinery wastewater.

Key words: Nanochloropsis gaditana, Pseudochloris wilhelmii, Synechococcus sp., biodiesel production, wastewater remediation, continuous culture

1. INTRODUCTION

Microalgal biofuel production and utilization are feasible on the already 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 source of CO_2, is an attractive option to reduce the costs to 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].

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 [7]. To preserve environmental sustainability, it is essential to treat oil refinery WW. However, only a small fraction of WW is treated worldwide [8]. 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 [9]. 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) [10]. All of these pose a major public health concern due to their toxicity at very low concentrations [11]. 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 [12].

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 chemical oxygen demand (COD). This is particularly important, as high COD levels are toxic to human health and can have detrimental effects on the environment [12,13]. There are several significant advantages to using microalgae strains in WW bioremediation [14]. 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 CO_2 supplies, enhancing the overall sustainability of the process [15]; 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 [16]; the conversion of nutrients from WW into biomass, promoting the principles of a circular economy [9]; and the sequestration of CO_2, contributing to the advancement of a green economy [17] .

There are 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 NO^{3-}, which provide a favorable environment for growth and lipid accumulation [18,19]. 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* spp., are of increasing interest. Their rapid growth and high nutrient uptake efficiency leads to higher productivity and efficient toxicants removal [20,21].

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 [22]. Despite high cultivation costs, biofuel production, along with integrated co-products, offers significant commercial benefits from an industrial perspective [23]. 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 [24]. This study evaluates the feasibility of cultivating *P. wilhelmii*, *N. gaditana*, and *Synechococcus* sp. 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. These findings provide valuable insights into their potential application of selected microalgae in the industrial-scale implementation of sustainable cogeneration process.

2. MATERIALS & METHODS

2.1. Microalgae selection and maintenance

Marine picoeukaryotes *Pseudochloris wilhelmii* (SAG 55.87) and *Microchloropsis gaditana* (SAG 2.99) were obtained from the Culture Collection of Algae at Göttingen University (SAG). *Synechococcus* sp. was isolated at the Center for Marine Research and deposited into the NCBI GenBank database (https://www.ncbi.nlm.nih.gov/) under accession number MK568070. The cultures were activated in appropriate media: *P. wilhelmii* and *Synechococcus* sp. in saltwater BG 11 ($NaNO_3$, K_2HPO_4, $MgSO_4$ x $7H_2O$, $CaCl_2$ x $2H_2O$, citric acid, ammonium ferric citrate green, Na_2EDTA, Na_2CO_3 and trace elements: H_3BO_3, $MnCl_2$ x $4H_2O$, $ZnSO_4$ x $7H_2O$, Na_2MoO_4 x $2H_2O$, $CuSO_4$ x $5H_2O$, $Co(NO_3)_2$ x $6H_2O$), and *N. gaditana* in F/2 medium ($NaNO_3$, NaH_2PO_4 x $2H_2O$, trace elements: Na_2EDTA, $FeCl_3$ x $6H_2O$, $CuSO_4$x $5H_2O$, $ZnSO_4$ x $7H_2O$, $CoCl_2$ x $6H_2O$, $MnCl_2$ x $4H_2O$, Na_2MoO_4 x $2H_2O$ 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 µmol/m²/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. The growth conditions were as follows: light intensity (130 µm photon m−2s−1), light cycle (12:12 h day/night cycle), temperature (24°C) and pH (via CO_2 flux) were controlled by SCADA (Supervisory Control and Data Acquisition) system [10]. 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 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), and pH was controlled by pH-probe (Mettler Toledo).

The growth medium in the PBRs was untreated oil refinery wastewater (WW) mixed with artificial (ASW) seawater in 1:1 (vol:vol) (both prefiltered on 0.2 µm, Millipore) in order to obtain medium salinity of 19 psu for algae growth. The WW composition is presented in the Table 1. To obtain the fixed and uniform NH4+ 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 addition of KH_2PO_4 stock solution (100 µmol/L) to achieve 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 [25].

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
KPK	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. Biomass analyses

Daily biomass concentration was measured gravimetrically as dry weight (dw) according to the standardized 2540-D method [26]. For the determination of chemical composition, the biomass was harvested by centrifugation (Eppendorf centrifuge) of 5 mL aliquots of sample at 4200 rpm for 10 minutes. The pellets were washed twice with deionized water to remove salt and centrifuged again. Biomass was lyophilized in freeze-dryer (Labconco, FreeZone2.5), ground to powder and used for all further biomass composition analyses.

2.4. C:H: N elemental analysis

Elemental analysis of the algal pellets (% of dw) was performed using a "2400 CHN Elemental Analyzer" (Perkin Elmer, USA) and CHN 628 Analyzer (LECO, USA). For the determination of carbon, hydrogen, and nitrogen, the pre-weighed freeze-dryed 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.

2.5. Lipid content

Total lipids of the algal cultures during experimental growth were extracted according to [26]. 50 mL samples were filtered on pre-combusted GF/F filters (Whatman) and mechanically disrupted with a tissue homogenizer and total lipids were extracted according to the modified [27] method in a mixture of dichloromethane/methanol (DCM:MeOH, 2:1 v/v). Ultimately, samples were placed in an ultrasonic water bath (Aquasonic Model 750D). The extraction procedure was repeated for three 30 in cycles in an ultrasonic water bath at 35°C.

2.6. Inorganic N and P nutrients analysis

Aliquots (50mL) were sampled for determination of nitrate (NO_3^-), nitrite (NO_2^-), ammonium/ammonia (NH_4^+/NH_3), soluble reactive phosphorus (SRP) and dissolved organic phosphorus (DOP) in the PBRs. Samples are centrifuged (10 min, 5000 rpm) and supernatant was immediately analyzed. Inorganic N and P nutrients were analyzed following the procedures described by [28] and by [29]. When necessary, appropriate dilutions of the samples were used to fit the range of spectrophotometric determinations using Shimadzu UV 1800 at the path length of 1 cm for each method. Dissolved inorganic nitrogen (DIN) concentration was calculated as the sum of NO_3, NO_2^- and NH_4^+/NH_3 concentrations.

2.7. Toxicity assessment

The toxic potential of the wastewater-based growth medium was evaluated during experimental growth of *P. wilhelmi*, *N. gaditana*, and *Synechococcus* sp. in PBRs. This assessment employed the marine bioluminescent bacterium *Aliivibrio fischeri* using the Microtox® *in vitro* testing system. 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 [30] for media samples. In brief, 50 mL culture aliquots were centrifuged at 5000 rpm for 10 minutes. The resulting supernatants were extracted with 5 mL of DCM, evaporated to dryness, and dissolved in 50 µL of dimethyl sulfoxide (DMSO). EC_{50} values were obtained using Microtox Omni software and expressed as the percentage of wastewater equivalents in the growth medium, with 95% confidence intervals and used for assessment of medium detoxification.

2.8. 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 [31]. The growth of microalgal biomass in batch experiments was obtained using Verhulst logistic growth model [32], which accounts for self-limiting growth due to nutrient depletion of space limitations (Fig. 1).

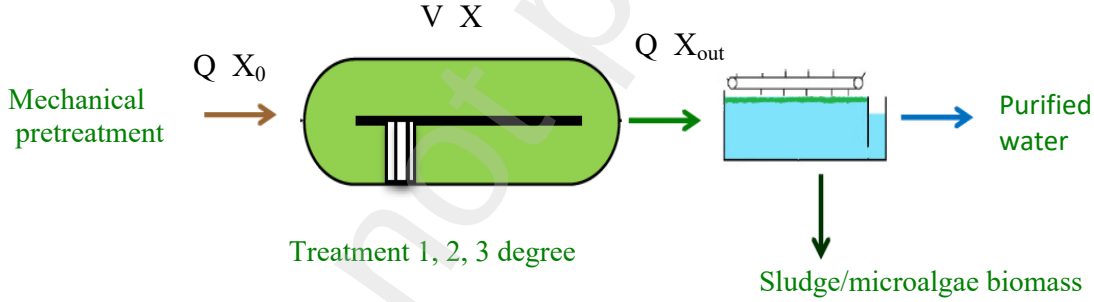

Figure 1. Approximation of the scaled up continuous operation. Q= flow rate, X_O=initial cell concentration, V=volume, X_{out}= concentration of biomass as output from the reactor.

Table 2. List of parameters.

Parameters	Unit	Definition
X_o	mg/L	Initial cell concentration
X_m	mg/L	Maximum concentration that the system can achieve in batch
µ	h-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
Θp		HRT at maximum productivity
Q	L/day	Flow rate
Qp	L/day	Flow rate at maximum productivity
Xp	mg/L	Concentration at maximum productivity

The Verhulsts model is given as:

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

Where X is the biomass concentration (mg/L), μ is the maximum specific growth rate (1/day), 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 batch phase. In this configuration, fresh medium continuously enters the reactor and equal volume exits, maintaining a constant working volume V (L). For all calculations, a constant WW flow rate of 5000 m^3/ day and an open raceway pond depth of 0.3 m were used. The flow rate of the medium is expressed as Q (L/day), and the hydraulic retention time (HRT) is defined as:

$$\theta = HRT = \frac{V}{Q} \quad (2)$$

The mass balance on biomass is:

$$\frac{dX}{dt} = \mu \cdot X \cdot \left(1 - \frac{X}{Xm}\right) - D \cdot (X - Xin) \quad (3)$$

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

$$\frac{dX}{dt} = \mu \cdot X \cdot \left(1 - \frac{X}{Xm}\right) - D \cdot X \quad (4)$$

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

$$X = Xm \cdot \left(1 - \frac{D}{\mu}\right) \quad (5)$$

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

$$P = D \cdot X = \frac{Q}{V} \cdot X = \frac{X}{\theta} \quad (6)$$

Combining Eq. 5 and 6, the volumetric productivity under continuous operation becomes:

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

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 derivate of P with respect to D is set to 0:

$$\frac{dP}{d\theta} = Xm \cdot \left(1 - \frac{2D}{\mu}\right) = 0 \quad (8)$$

Solving for D yields:

$$Dopt = \frac{\mu}{2} \rightarrow \theta opt = \frac{2}{\mu} \quad (9)$$

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

$$Pmax = \frac{\mu \cdot Xm}{4} \quad (10)$$

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.

2.9. Sustainability assessment of the cogeneration production of microalgal biodiesel in oil refinery concept

By aligning our methodology with established feasibility study frameworks, the economic analysis provides critical insights into investment risk and long-term profitability of cogeneration systems utilizing microalgae biomass. The use of flue gases, originating from an arbitrary industrial process or refinery, is planned, and for the purposes of the study, the associated CO_2 cost is considered negligible. The portion of CO_2 that needs to be purchased is estimated at 0.5%, with potential savings anticipated through CO_2 quota trading on the international carbon market, at a project price of 20 EUR/ton.
The flotation process costs were calculated based on an influx rate of 0.005 kWh per cubic

meter. The consumption of coagulant agents is estimated at 4 EUR/kg [33]. A scenario of the continuous growth of *Pseudochloris wilhelmii* (SAG 55.87), *Nannochloropsis gaditana* (SAG 2.99) and *Synechococcus* sp. (MK568070) was predicted. 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, water, and land between 2019, 2022, and projected trends for 2025. A detailed feasibility study approach was applied to evaluate the economic viability of microalgal biodiesel production within the oil refinery framework, considering both operational expenditures (OPEX) and capital expenditures (CAPEX) [34]. Recent studies emphasize the importance of integrating fluctuating resource prices into techno-economic assessments to ensure realistic financial projections and risk mitigation strategies [1]. Furthermore, the inclusion of sensitivity analysis based on market trends allows for a more resilient sustainability assessment model [35].

3. RESULTS AND DISCUSSION

The application of microalgae for wastewater treatment has gained increased attention due to their dual ability to remediate pollutants and produce biomass for biofuel generation [34]. Our findings are consistent with [34], who highlighted the dual benefit of microalgal systems for wastewater remediation and biomass valorization. Experiments conducted in batch mode in a photobioreactor provided species-specific patterns on nutrient uptake, biomass growth and qualitative 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 exhibited distinct physiological responses and different carbon and nitrogen composition, carbon and nitrogen ratio, and dissolved inorganic nitrogen uptake (Fig. 2). Although the accumulation dynamics of cellular nitrogen and carbon differ, increases in both are observed, accompanied by elimination of dissolved inorganic nitrogen. During the lag and early exponential phase (between days 1 to 5), growth rates, nitrogen, and carbon incorporation were similar in all three tested strains (Fig. 2a and 2b). After day 10, a visible difference in cellular C and cellular N content was observed among tested strains. *P. willhelmii* exhibited the highest intracellular C content (Fig. 2a), whereas *Synechococcus* sp. showed the lowest intracellular C and highest intracellular N content by the end of the experiment (Fig. 2a and 2b). *N. gaditana* exhibited 40% intracellular C content and 2% intracellular N content (Fig. 2a and 2b). All strains show a decline in cellular N at the beginning of stationary phase, consistent with nitrogen limitation.

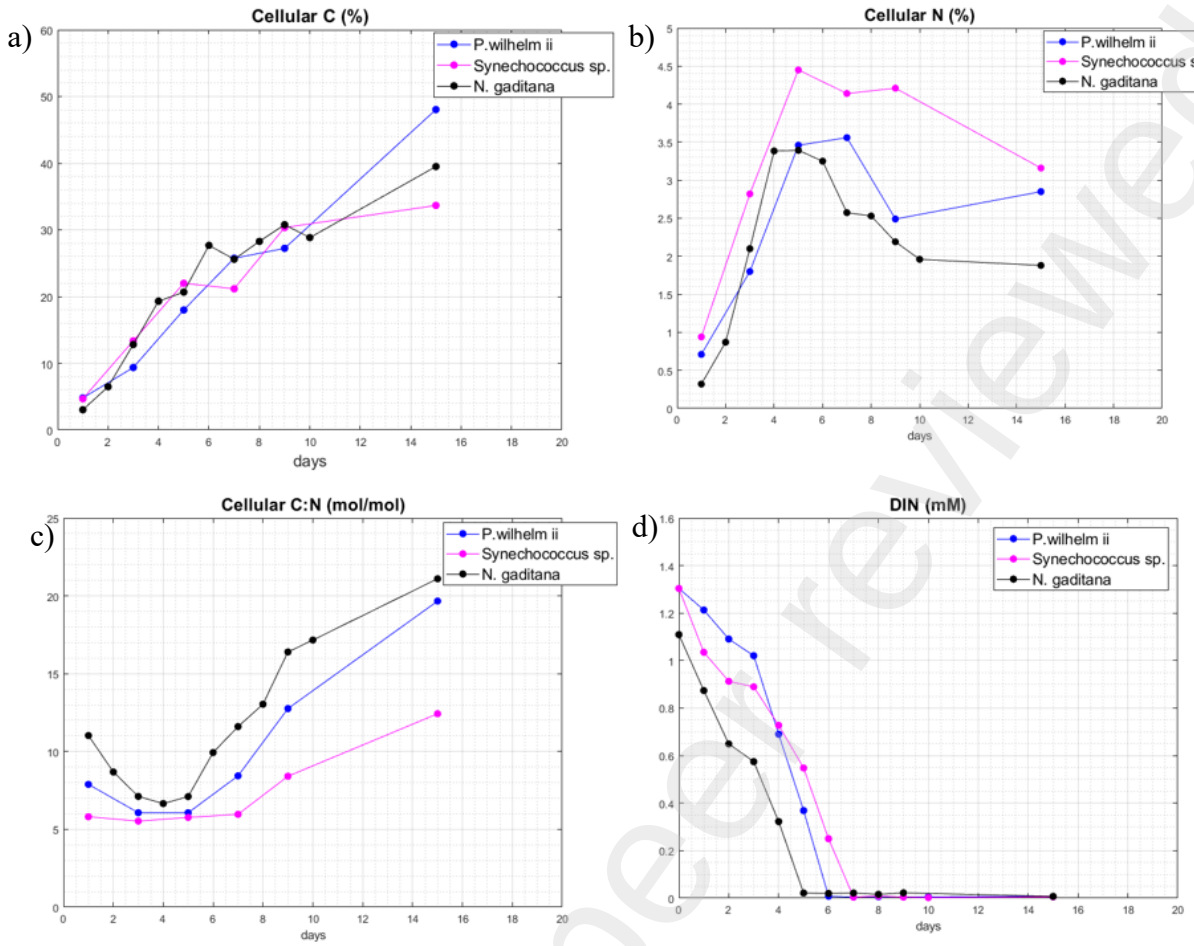

Figure 2. Nitrogen and carbon dynamics in batch cultures by *P. wilhelmii, N. gaditana* and *Synechococcus* sp. grown in a 1:1 (v/v) medium of ASW and oil refinery wastewater (1:1).

The initial rapid increase in intracellular N reflects the uptake of available nitrogen and rapid 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 [19]. 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 during the early phase (Fig 2b). All strains show a decline in cellular N in stationary phase, consistent with nitrogen limitation.

Intracellular C/N ratio follows the expected pattern: it remained steady around the Redfield ratio until inorganic nitrogen was removed during the late exponential and stationary phase. At this point, the ratio increased because photosynthesis continued to add carbon, while nitrogen levels remained constant [35]. *P. wilhelmii* and *N. gaditana* showed high intracellular carbon accumulation relative to nitrogen, reaching C: N ratios of 18-22 mol/mol, while *Synechococcus* sp. had 40.9% lower final C:N ratio of 13 mol/mol (Fig. 2c). The observed large increase in C:N ratio is an indicator of nitrogen limitation in the tested strains. As nitrogen in the medium is depleted, microalgae cells accumulate carbon storage compounds, such as lipids.

Standard initial content of dissolved inorganic nitrogen (1.3 mM) was removed in exponential growth phase (between days 5-7) in all tested strains. After inorganic nitrogen depletion,

intercellular nitrogen consumption declined while intercellular carbon accumulation continued to rise towards the experiment's end (Fig. 2 d). The fastest DIN removal was observed by *N. gaditana* by day 5, i.e., during the exponential growth phase (Fig. 2d). Other strains exhibited prolonged DIN removal while remaining in the exponential growth phase, with *P. wilhelmmii* completing DIN removal by day 6 and *Synechococcus* sp. by day 7 (Fig. 2d). Interestingly, although carbon accumulation through photosynthesis is expected to continue for more than 10 days after nitrogen depletion [37,38]. *P. wilhelmii* exhibited an unexpected continuous increase in cellular nitrogen following the removal of dissolved nitrogen (Fig. 2b and 2d), which may suggest that oil refinery WW had some nitrogen of organic and inorganic origin taken up by the cell [19].

Since WW samples contained inorganic and organic nitrogen, the observed nitrogen uptake implies that *P. wilhelmii* could employ a mixotrophic strategy. While previous studies indicated that marine *Nannochloropsis* species, including our strain, are obligate photoautotrophs [39], recent study by [40] reported evidence of mixotrophy in novel *Picochlorum* sp. (Trebouxiophyceae). Consequently, *P. wilhelmii* may also exhibit mixotrophy. The apparent ability of *P.wilhelmii* to remove organic nitrogen-containing contaminants from wastewater in addition to the proven ability to remove inorganic nitrogen is a significant feature for the use of this alga in the bioremediation of WW.

3.2. Biomass and lipid production

N. gaditana exhibited the highest growth rate (0.576 g/gday) and lipid productivity (29.45 mg/Lday) (Table 3). As a result, *N. gaditana* also showed the highest final lipid content (37% d.w.), while *P.wilhelmi* had a slightly lower final lipid content (28% d.w.). Despite a slower growth rate (0.432 g/gday), and lower final lipid content (26.30 mg/Lday) *P. wilhelmii* achieved the highest volumetric productivity (93.9) and the highest rate of WW toxicity removal (76.5 %) among the tested strains (Table 3). *Synechococcus* sp. displayed a much slower growth rate then both *P. willhelmii* (22% slower) and *N. gaditana* (41.67% slower). It also had the highest hydraulic retention time (HRT) of 3.02, compared to *P. wilhelmii* and *N. gaditana*, which had lower HRT values of 2.28 and 1.72, respectively (Table 3). Among the studied species, *P. wilhelmii* demonstrated the highest efficiency in nitrogen removal and a notable reduction in toxicity in the wastewater (Table 3). Among the picoukaryotes, *N. gaditana* and *P. wilhelmii*, and a cyanobacteria, *Synechococcus* sp. there was a marked difference in C:N ratios, with *N. gaditana* showing the highest affinity for carbon incorporation and, consequently, lipid production.

Table 3. Summary of the observed growth indices in experimental cultures.

	μ g/(gday)	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 %
P. wilhelmii	0.432	0.895	93.9	2.28	28	26.30	76.5
Synechococcus sp.	0.336	0.531	71.0	3.02	21	14.91	12.4
N. gaditana	0.576	0.698	79.6	1.72	37	29.45	51.0

N. gaditana exhibited the highest specific growth rate, lipid production, final lipid content, and overall biomass yield, as well as the shortest retention time required for nitrogen removal (Table 2). It also showed the fastest DIN removal (Figure 2d), resulting in high intracellular carbon accumulation, which contributed to the increased biomass and final lipid content (Table 3). These characteristics make *N. gaditana* one of the most promising species for both valuable biomass production and wastewater remediation.

Synecochoccus sp., cyanobacterial strain, exhibited the lowest maximum specific growth rate, lowest maximum specific DIN uptake rate and, consequently, the lowest toxicity reduction rate among the tested strains (Table 3). Based on these results, *Synechoccocus* sp. was determined to be unsuitable for bioremediation applications within the context of this study, primarily due to its limited capacity for toxicity reduction. However, it is important to note that other studies have identified specific strain of *Synechococcus, S. elongatus*, as a promising candidate for WW bioremediation [41]. The highest nitrogen demand, and consequently the lowest C:N ratio was observed in *Synechococcus* sp (Fig. 2 c) resulting in the lowest specific DIN uptake rate of 0.531 mmol/ (gday) and the lowest lipid content (21%) (Table 3).

Although it had a slower N uptake, *N. gaditana* showed the highest potential for biofuel production based on lipid proportions and lipid productivity, even though its lipid yield was lower. *P. wilhelmii* demonstrated the highest specific nitrogen uptake rate and highest efficiency in toxicity reduction.

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 (μMAX, day-1) were calculated from accumulated biomass in batch mode using the logistic Verhulst model [31,42].

Table 4. presents the calculated parameters for optimum open raceway pond (ORP) cultivation and biomass production (t/year) [31,42]. Growth parameter was used to find optimal dilution rate, i.e. wastewater flow in the continuous operation of the bioreactor.

Table 4. Calculated open raceway pond (ORP) parameters for *Synechococcus, N. gaditana,* and *P. wilhelmii.*

		Synechococcus sp.	*Nannochloropsis gaditana*	*Pseudochloris wilhelmii*
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
Θp	Day	6.05	3.43	4.56

The calculated volume of ORP was 30.241 m^3 for *Synechococcus* sp., 17.161 m^3 for *N. gaditana*, and 22.785 m^3 for *P. wilhelmii.* Depth of ORP was set at 0.3 m. The surface area needed for each strain was also determined. *N. gaditana* exhibited the smallest spacial requirement, only 5.72 ha (Table 4). The maximum productivity retention time was highest in *Synechococcus* sp. (6.05). Considering all calculated parameters, biomass production was estimated. The lowest biomass production (129.62 t/year) was recorded for *Synechococcus* sp., while *N. gaditana* and *P. wilhelmii* demonstrated higher biomass production rates.

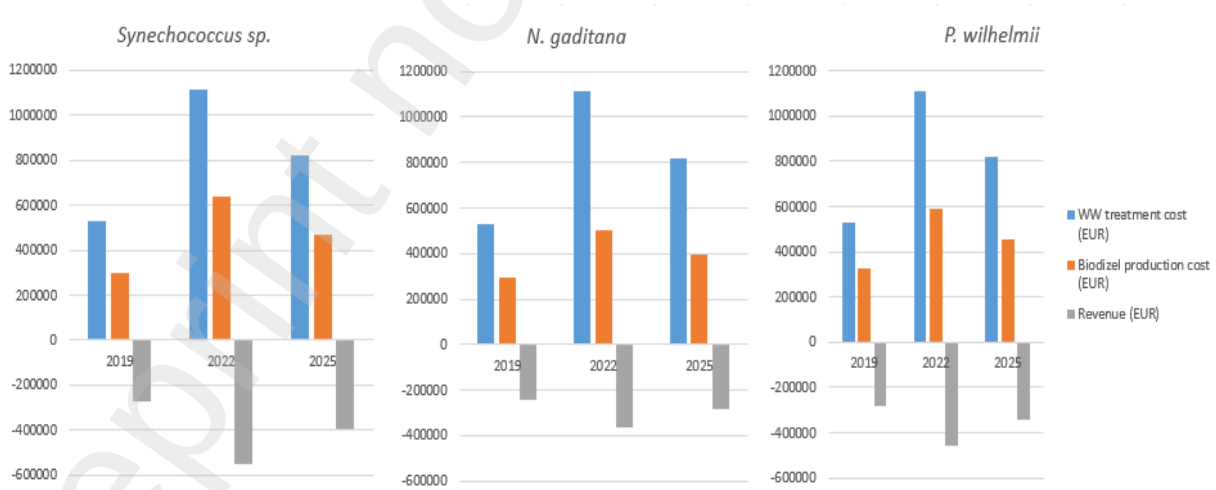

Figure 3. Comparison of treatment costs, production costs and revenue for *Synechococcus, N. gaditana,* and *P. wilhelmii*

WW treatment costs in 2019 amounted to 530,726 €. By 2022, a substantial increase was recorded, with costs rising to 1,112,178 €, effectively doubling. In 2025, WW treatment costs decreased significantly and stabilized at 821,452 € (Fig. 3).
The biodiesel production cost in 2019 was nearly identical for all tested strains. However, by 2022, a notable cost deviation was observed. For *Synechococcus* sp., the biodiesel production cost exceeded 600,000 €, whereas for *N. gaditana* and *P. wilhelmii*, the cost was 505 745 € and 590 815 €, respectively. In 2025, the biodiesel production cost for *Synechococcus* sp. declined to 467 470 €, while costs for *N. gaditana* and *P. wilhelmii* were recorded at around 400,000 € and 420,000 €. Revenue trends for *Synechococcus* sp. indicated a loss of -273 784 € in 2019, which worsened to -551 875 € in 2022. By 2025, the revenue loss increased to -395 537 €, representing an improvement of 27%. For *N. gaditana*, revenue was around -240,000 EUR in 2019, over -360 000 € in 2022, and returned to -284 467 € by 2025 (Fig. 3). *P. wilhelmii* exhibited a similar trend in revenue, from -280,000 € in 2019 to -456 471 € in 2022, further improving to below -350,000 € in 2025 (Fig. 2). 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 €/kWh (February 2025), compared to the earlier value of 0.2 EUR/kWh recorded in 2019, and value of 0.6 €/kWh in 2022 [43]. 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 [14,44,45]. 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].

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 4). Based on biomass production and biodiesel production economics estimated in this study, *N. gaditana* is the most productive and profitable strain for WW remediation (Table 4; Figure 3).

4. CONCLUSION

Three selected strains: *P. wilhelmii, Synechococcus* sp., and *N. gaditana* were tested for cultivation potential in oil refinery wastewater. Among the tested strains *Synechococcus* sp. had the lowestr metrics. Although *P. wilhelmii* demonstrated the highest efficiency in toxicity reduction, maximal volumetric productivity, and rapid nitrogen removal, *N. gaditana* proved to be a promising candidate in bioremediation of oil refinery WW. Biochemical analysis and growth kinetics revealed that *N. gaditana* exhibited the greatest biomass accumulation, the fastest nitrogen removal and highest lipid productivity. In terms of treatment costs, production expenses and revenue comparison, *N. gaditana* generated the highest revenue over the period from 2019-2022-2025.

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. As promising results of this study, it is important to note that further investigation should be undertaken to fully understand long-term resilience and stability of tested strains under real industrial-scale conditions. Future research should focus on pilot-scale validation of microalgal wastewater treatment systems, including the assessment of system robustness across varying industrial effluent types.

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