

Experimental assessment of lipid yield and phycoremediation potential of five indigenous microalgae under various nutrient regimes (N:P) in BBM

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Research Article

Keywords: Microalgae, productivity, nutrient removal, N:P in medium, algal-lipid, bio-fuel

Posted Date: September 22nd, 2023

DOI: <https://doi.org/10.21203/rs.3.rs-3344643/v1>

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Version of Record: A version of this preprint was published at BioEnergy Research on January 23rd, 2024. See the published version at <https://doi.org/10.1007/s12155-024-10722-x>.

Abstract

The success of the emerging algal fuel industry depends on identifying high-quality species and standardization of their culture protocols for lipid yield and nutrient removal efficiency. The primary objective of the study was to determine the phycoremediation cum lipid-rich biomass production potential of five green microalgae isolated from bloomed freshwaters of Kerala. Three experiments were carried out under various nitrogen and phosphorus content with different N:P. The first experiment included four different treatment levels of N, the second experiment included four different treatment levels of P, and the third experiment had two different treatment levels of N and P together in BBM. Regular BBM was the control. The parameters measured were biomass and lipid productivity, fatty acid composition, biodiesel properties, Nitrogen and Phosphorus removal rate, and efficiency. *Chlorolobion braunii* appeared productive in N-rich media with high N:P, whereas *Monoraphidium contortum* in P-rich media with low N:P. Among the five species tested, the latter shows the highest lipid yield and productivity at low N:P in culture media. The lipid content of four species has demonstrated high-quality biofuel properties. The optimum nutrient regimes and N:P for Nitrogen Removal and Phosphorus removal rates were found to be species-specific. Overall, the experiments stand as a model for understanding the influence of variations in N and P concerning N:P on biomass productivity, lipid content, lipid productivity, and N and P removal rate and efficiency of algae for initial standardization of the culture protocols for further industrial trials.

Introduction

Increasing the share of renewable energy in the global energy matrix is goal 7 (SDG 7) of the sustainable development goals for 2030 (SDG30) of the UN [1]. Since the UN started focusing on sustainable development, the share of renewables in the energy sector has been increasing, reaching 13–14% of the entire energy sector worldwide today. But fossil fuels remain 82% of global primary energy needs, causing 86% of total CO₂ emissions globally [2]. However, the development, which is rooted in a circular economy, depending on the extent of utilization of renewable resources for energy needs of human developmental progress [3], can only be sustainable. It is well known that among renewable energy resources, biomass resources are the largest and most important option for renewable energy. Accordingly, novel biomass resources are essential to achieve more advanced bioeconomic sustainability [4], dependent on biomass resources for a more stable and viable energy-sustaining economy. Since biomass resources can support developmental sustainability from multiple perspectives, a new circular bioeconomy concept is emerging, emphasizing biomass resources for various human needs to attain sustainability.

Bioeconomy integrates circular economy principles and bioeconomic concepts using biobased renewable resources [5] for multiple purposes, including energy requirements. Therefore, identifying measures for accessible and fast-yielding biomass sources [6], especially lipid-rich biomass, has become imperative in achieving goal 7 of SDG 30. Microalgae, especially many species of freshwater green microalgae, are well known for their high lipid content and high biomass yield [7]. The lipid-rich microalgal biomass is fuel grade, suitable for biodiesel manufacture [8] already industrially explored [9].

In the above context, research on generating microalgal biomass resources is highly significant as a multi-edged technological process. Microalgae being photosynthetically most efficient (20% better photosynthetic efficiency than terrestrial plants), their culture is the best biological carbon sequestration process and solar energy capture [10]. Algal culture can be an industrially enabled massive biomass production suitable for several bioenergy forms such as biodiesel, bioethanol, biobutanol, and biocrude [11]. Still, global algal biomass utilization remains relatively meager because of the high production cost [12] and many other limitations [13]. However, comparing the benefits of microalgal culture, it has become imperative to invest in microalgal research for augmenting algal biomass production as an economically feasible industrial process [14]. Growing microalgae in nutrient-rich wastewater can be a double-edged technology for simultaneously cleaning water and biomass generation [15]. It is also an effective strategy to lower the cost of production of algal biomass [16].

Algal wastewater culture enables nutrient recovery through helpful biomass [17] and is a tertiary treatment (phycoremediation) of nutrient-rich effluents [18]. Microalgae may also remove hazardous chemicals or toxins in water [19]. Algal water treatment enables the formation of suitable biomass and the release of oxygen into the medium of growth [20]. Still, the absence of suitable feedstocks for specific geo-climatic zones and cost-effective cultivation methods [21] limit indigenous microalgal utilizations for multiple industrial needs. Therefore, finding suitable indigenous microalgal strains of high-yield and quality biomass [22] and standardizing methods of exploitation of such microalgal wealth [23] remain crucial for advancing algal technology locally and globally.

In selecting algae for industrial water treatments combined with biomass for biofuel, fast growth rate, and nutrient removal are crucial criteria. The fast growth rate is frequently positively correlated with a high nutrient removal rate and strain robustness [24]. The rapid growth rate can make up even low lipid content because the high biomass yield per unit of time can improve the overall biomass performance and compensate for algae's low lipid productivity [17]. Among the natural environments for searching for fast-growing algae, eutrophic waters can be the most suitable places as the water-blooming species are often fast-growing [15]. The screening of lipid content and lipid productivity of the alga are significant for assessing the commercial exploitation of microalgal biomass for biofuel purposes [25–27]. In addition, optimization of growth parameters is also required, especially in selecting potential local microalgal strains for phycoremediation and biomass production because the effectiveness of phycoremediation is highly dependent on the species chosen [28] and their growth environment [10].

In the above contexts, a systematic experimental study with five indigenous microalgae collected from the eutrophic freshwater of Kerala was carried out. The primary objectives of the study included (1) Estimation of the biomass production potential of five green indigenous microalgae of Kerala

concerning varying nutrient regimes in the Bolds Basal Medium (BBM), (2) Understanding the lipid content and productivity of the species, (3) Evaluation of the biodiesel feasibility of the lipid content of the species and (4) Assessing the nutrient removal efficiency of the species.

Materials and Methods

Microalgal strains

The five indigenous microalgae used in the present study are *Monoraphidium contortum* (Thuret) Komarkova-Legnerova 1969 (MK045307.1), *Halochlorella rubescens* P.J.L.Dangeard, nom. inval. 1966 (MK106023.1), *Coccomyxa simplex* Mainx 1928 (MK024328.1), *Chlorolobion braunii* (Nägeli) Komárek 1979 (OR131025), and *Kirchneriella obesa* var. *contorta* Schmidle 1894. The first three species are already molecularly characterized species kept as pure cultures in the algal repository at the School of Biosciences, Mahatma Gandhi University, Kottayam, Kerala. The remaining two indigenous microalgae were collected afresh from a bloomed rural pond near the Mahatma Gandhi University Campus in Kottayam District, Kerala, India (coordinates: 9.659 N, 76.531 E). The first was morphologically and molecularly characterized (OR131025), whereas the second was morphologically identified per Algae Base (Guiry and Guiry, 2017). All five species' cellular details were obtained using a digital compound microscope (Carl Zeiss, model Axio Imager M2). Microphotographs were obtained using a digital camera (Axiocam 305) on the microscope. Morphological measurements (n = 30) were obtained from microphotographs using Zen BLUE 3.6 software. The details are shown in Fig. 1

Molecular Characterization of Algae

A genomic DNA kit (Origin Diagnostics and Research, Kerala) separated the total genomic DNA from the monoculture samples. DNA was extracted from 1 mL of each alga's pure monoculture according to the isolation kit's technique. Before further investigation, the extracted DNA was kept at minus 20°C in a deep freezer (Samsung, model SRS600NLS, Korea).

Polymerase Chain Reaction and 18SrRNA Gene Sequencing

The amplification and sequencing of PCR products were conducted at Eurofins Genomics Bangalore, India. The 18S rRNA gene region of the isolated algal DNA samples was amplified and sequenced using the following primers: forward primer: 5'CCTGGTTGATCCTGCCAG-3' and reverse primer: 5'TTGATCCTTCTGCAGGTTCA-3'. The resulting PCR product had a length of approximately 1500 bp. In each reaction, a total volume of 50 µL was prepared. This included 25 µL of PCR smart mix, five µL of template DNA, two µL (10 pmol µL⁻¹) of both the forward and reverse primers, and 16 µL of sterile distilled water to reach the final Volume. The PCR process consisted of 35 cycles, starting with a 5-minute incubation at 95°C, then denaturation for 30 seconds, annealing for 45 seconds, extension for 30 seconds, and a final 5-minute termination step [29]. To visualize the PCR products, they were subjected to electrophoresis on a 1.5% agarose gel in 1x Tris-borate-EDTA (TBE) buffer for 1 hour and 30 minutes. The gel was then stained with ethidium bromide (1%), and the DNA bands were visualized under UV light. A DNA ladder was used as a reference to ensure the correct size of the amplified DNA. The obtained nucleotide sequences were compared to the sequences in the GenBank nucleotide collection. This comparison was performed using the Basic Local Alignment Search Tool (BLAST) available at the National Center for Biotechnology Information website (www.ncbi.nlm.nih.gov).

Experimental design

Three separate experiments were conducted to examine the impact of variations in N:P by varying N or P individually or N and P together in the BBM. The first experiment examined variations in N:P by varying the amount of N (as NaNO₃) in the BBM while maintaining P constant at the BBM level (increasing N:P). In the second experiment, N:P variations were introduced using varying additions of P (as KH₂PO₄) while N was kept constant at the BBM level (decreasing N:P). The third experiment examined the influence of constant N:P on algal parameters but at varying N and P together in the BBM. All the experiments were carried out in triplicate. Altogether, four varied treatments were done in the first and second sets of experiments, whereas only two were done in the third. The details of the three experiments and the treatments at each experiment are given in Table 1.

Table 1
Concentration of Nitrogen, Phosphorus, and N:P ratio in Experimental cultures

Experimental trial 1 (Variation in N content at BBM level of P)					
Treatments			N (ppm)	P (ppm)	N:P
NaNO ₃	1	BBM1/2N	20.58	53.29	0.85
	2	BBM2N	82.32	53.29	3.42
	3	BBM3N	123.48	53.29	5.13
	4	BBM4N	164.64	53.29	6.84
Experimental trial 2 (Variation in P at BBM level of N content)					
KH ₂ PO ₄	1	BBM1/2P	41.16	33.32	2.72
	2	BBM2P	41.16	93.24	0.98
	3	BBM3P	41.16	133.19	0.68
	4	BBM4P	41.16	173.14	0.53
Experimental trial 3 (Variation in N, P & K together)					
NaNO ₃ & KH ₂ PO ₄	1	BBM1/2NP	20.58	33.32	1.47
	2	BBM2NP	82.32	93.24	1.95
Control (Normal BBM)					
Control – Normal BBM			41.16	53.29	1.71

Experimental conditions

The pH of the culture medium was 7.3, adjusted using 0.1M HCl/NaOH with the support of a pH meter (Hanna HI 1131). The experimental cultures were maintained under the light intensity of 6000 lux, 16-hour light, and 8-hour dark cycle at 24°C. The cultures were maintained in 1L Erlenmeyer flasks containing 1000 ml of culture medium. In all cases, at the beginning, approximately 200 mg of wet biomass (measured using an electronic balance - Sartorius BSA224S-CW) was added to the medium as seed culture. The initial cell densities of algae in the culture media were determined using a hemocytometer. The initial cell densities for the five different species of algae were 9.9×10^5 (*Monoraphidium contortum*), 3.57×10^5 (*Halochlorella rubescens*), 4.6×10^6 (*Coccomyxa simplex*), 8.025×10^5 (*Chlorolobion braunii*), and 10.076×10^5 (*Kirchneriella obesa*), respectively. All three culture flasks (triplicate cultures) of treatment for each alga had approximately similar initial cell densities. The cultures received continuous and filtered (0.2 µm PTFE 1N Sartorius) aeration through 5mm plastic tubes connected to an air pump (Hailea HAP 80 80L/M 60W). The duration of cultures was twenty days.

Microalgal growth-assessment

Every third day, the growth rate was measured using a hemocytometer (from 1 ml of media pipetted out after the manual mixing) to ensure normal growth of the algae and the absence of contamination. The entire biomass of a flask was extracted after 20 days of incubation using an Eppendorf centrifuge set to 8000 rpm and 20°C for 10 minutes. The N and P content of the supernatant was determined to measure the Nutrient Removal Rate (NRR) and Nutrient Removal Efficiency (NRE) of each alga. The algal biomass pellet extracted from a flask was cleaned of media components by rinsing three times in distilled water before freeze-drying with a Lyophilizer (Labogene Scanvac Cool Safe). The freeze-dried biomass was kept at minus 20°C in a deep freezer (Samsung, model SRS600NLS, Korea) until further investigation. The biomass yield was calculated as percentage increase of biomass per litre each day, per the formula [30]:

$$\% \text{ Increase in Biomass yield of algae (L}^{-1}\text{day}^{-1}) =$$

$$\frac{\text{Final dry weight (mg)} \times 100}{\text{Initial dry weight (mg)} \times \text{Volume of culture (L)} \times \text{total number of culturing days}}$$

$$\text{Initial dry weight (mg)} \times \text{Volume of culture (L)} \times \text{total number of culturing days}$$

The volumetric biomass productivity P_{Biomass} of algae was calculated as per the equation [31]

$$\text{Biomass Productivity (mg l}^{-1}\text{day}^{-1}) = (X_2 - X_1) \cdot (t_2 - t_1)^{-1}$$

Where X_1 and X_2 were the biomass dry weight concentrations (mg l⁻¹) on days t_1 (the starting point of cultivation) and t_2 (the end point of cultivation), respectively.

Measurement of N and P in the medium

Total Phosphorus (TP) was calculated using the Vanadium Molybdate Yellow method per standard guidelines (APHA 1995), and Total Nitrogen (TN) was measured as nitrate nitrogen using the HANNA (HI 5315) probe. The following formulae were used to compute the removal rate ($\text{mg l}^{-1} \text{ day}^{-1}$) and removal efficiency (%) for nutrients [32]

$$\text{Removal efficiency} = C_i - C_H / C_i \times 100$$

$$\text{Removal rate} = C_i - C_H / t_H - t_0$$

where C_i is the initial concentration (as mg l^{-1}) at time (t_0), while C_H is the concentration after time (t_H).

Estimation of Lipid content

Bligh and Dyer's method [33] extracted crude lipids from the lyophilized algal biomass. 100 mL of chloroform: methanol (2:1 vol vol⁻¹) was added into 1g of lyophilized biomass and was kept for continuous shaking at 150 rpm for four hours in a rotary shaker (RRS-06; Roteck, India). The mixture was centrifuged at 4000rpm at room temperature for 5 min, and the residual biomass was separated from the extract. The extract was then poured into a separating funnel, adding 40 mL of distilled water. The bottom layers containing the organic phase were collected into a bottle. The residual biomass and the oil were kept in a rotary evaporator (Heidolph, model Hei-VAP; Germany) to remove excessive solvent from the lipid. The solvent-free residual biomass was further subjected to a hot extraction method to collect the remaining lipids. The biomass was placed in a Soxhlet extractor and extracted with 75 mL of hexane, refluxed under 70°C for two hours. The extracted lipid component was collected and made solvent-free using a rotary evaporator. Finally, the two extracted lipid components were mixed to get the total lipid content. The percentage of lipids in algae (%) was calculated using the following Eq. [27]

$$\% \text{ of lipid in the algae} = \frac{\text{Weight of Lipid (mg)} \times 100}{\text{Importance of sample (mg)}}$$

Fatty acid profile

The biochemical composition of total extracted fatty acids was done per AOAC [34] methods using GC-FID analysis with Shimadzu GC-2010 Plus instrument (Japan). Exactly 50 mg of algal oil was saponified with 2 mL of 0.5 M KOH-CH₃OH solution, followed by methylation with 1 mL of Methanolic HCl (as a catalyst) at 55°C for 20 min for obtaining the fatty acid methyl esters (FAMES). The FAME was extracted from the mixture using 5 mL of heptane. The methylated sample was injected into the GC column (Restek-Stabilwax; polyethylene glycol, 30 m length, 0.25 mm internal diameter) for the profiling of FAME. The column temperature was programmed to ramp from 100°C for 2.5 min to 240°C. The flame ionization detector's (FID) temperature was fixed at 250°C. Nitrogen and hydrogen were used as the carrier gases, flowing at 30 and 40 mL per minute, respectively. The recorded chromatographic data were compared to fatty acid standards with known retention times (Supelco FAME – 37 Mix, Sigma-Aldrich). Considering the analysis cost, lipids' percentage fatty acid composition was characterized after thoroughly mixing the triplicate samples into a single composite sample.

Assessment of biodiesel properties

Biodiesel quality was estimated using empirical equations from the fatty acid profile, including iodine value (IV g I₂ 100 g⁻¹ oil), saponification value (SV, mg KOH g⁻¹), cetane number (CN), degree of unsaturation (DU, wt %), high heating value (HHV, MJ kg⁻¹), oxidation stability (OS, h), long-chain saturated factor (LCSF, % wt), cold filter plugging point (CFPP, °C), cloud point (CP, °C), and pour point (PP, °C). Using Eqs, (1)–(9) [30, 35]

$$IV = \sum 254 \times D_i \times A_i / MW_i \text{ — 1}$$

$$SV = \sum (560 \times A_i) / MW_i \text{ — 2}$$

D_i is the number of double bonds in the i th component, A_i is the percentage of each fatty acid, MW_i is the component's molecular weight, and 254 is the atomic weight of the two iodine atoms theoretically added to one double bond.

$$CN = 43.3 + \frac{5458}{SV} - 0.225 \times IV \text{ — 3}$$

$$DU = MUFA + 2 \times PUFA \text{ — 4}$$

$$HHV = 49.43 - 0.041 (SV) - 0.015 (IV) \text{ — 5}$$

$$OS = \frac{117.9295}{X} + 2.5905$$

where the X = content of linoleic and linolenic acids (wt%) ($0 < X < 100$).

$$LCSF = (0.1 \times C_{16:0}) + (0.5 \times C_{18:0}) + (1 \times C_{20:0}) + (2 \times C_{24:0}) \text{ — 6}$$

CFPP = (3.1417 x LCSF) -16.477-7

CP = (0.526 x C16)- 4.992-8

PP= (0.571 x C16) - 12.2400-9

Statistical Analysis

GraphPad Prism 5.0 and SPSS version 21 were used for the statistical analysis and data processing. The results are represented as the mean and standard deviation of three replicates of all the treatments. Percentage is expressed as weight/weight on a dry biomass basis. One-way analysis of variance (ANOVA) and Tukey post hoc analysis was used to determine significant differences at a 95% confidence interval among the treatments to compare the differences between media composition and algal species regarding biomass productivity, N and P removal rate, removal efficiency and lipid composition of different microalgal species.

Result and Discussion

The biomass productivity, lipid content, lipid productivity, N-removal rate (NRR), N-removal efficiency (NRE), P-removal rate (PRR), and P-removal efficiency (PRE) of five different green algal species concerning varying N:P (0.53 to 6.84) in BBM are found out. The experiments examined variations in N and P concerning N:P in BBM on biomass productivity, lipid content, lipid productivity, and N and P removal rate and efficiency concerning the five species of algae. The highest Biomass Productivity (BP), Lipid Yield (LY), and Lipid Productivity (LP) of the five species of algae concerning N or P or N and P together, and N:P are shown in Table 2. The details of the best NRE, NRR, PRE, and PRR concerning N or P or N and P together, and N:P in BBM by the five species are shown in Tables 3 and 4, respectively.

Table 2
The highest Biomass Productivity (BP), Lipid Yield (LY), and Lipid Productivity (LP) of the five species of algae

Algae	Exp	Treatment	N:P	Biomass productivity (BP)	Treatment	N:P	Lipid yield (LY)	Treatment	N:P	Lipid productivity (LP)
<i>Monoraphidium contortum</i>	1	BBM &BBM2N	1.71to 3.42	77.69 ± 1.53to 80.72 ± 1.55	BBM 1/2N	0.85	44.38 ± 0.81	BBM	1.71	31.52 ± 1.06
	2	BBM2P,BBM3P&BBM4P	0.98 to 0.53	102.22 ± 1.07 to 103.66 ± 4.19	BBM	1.71	39.07 ± 1.58	BBM to BBM4P	1.71 to 0.53	30.59 ± 2.12 to 31.58 ± 0.86
	3	BBM &BBM1/2NP	1.71 to 1.47	80.72 ± 1.55 to 83.72 ± 2.53	BBM 1/2NP	1.47	42.96 ± 1.75	BBM 1/2NP	1.47	35.94 ± 1.04
<i>Halochlorella rubescenes</i>	1	BBM3N	5.13	105.04 ± 2	BBM 1/2N	0.85	30.57 ± 0.82	BBM2N	3.42	25.66 ± 0.11
	2	BBM & BBM2P	1.71 to 0.98	82.2 ± 2.03	BBM to BBM4P	1.71 to 0.53	25.41 ± 1.63 to 26.95 ± 0.65	BBM	1.71	21.48 ± 1.05
	3	BBM1/2NP & BBM2NP	1.95 & 1.47	86.79 ± 2.55 to 90.77 ± 2.54	BBM 1/2NP	1.47	30.96 ± 0.22	BBM 1/2NP	1.47	26.87 ± 0.84
<i>Coccomyxa simplex</i>	1	BBM1/2N	0.85	72.94 ± 2.61	BBM 1/2N	0.85	30 ± 1.34	BBM 1/2N	0.85	21.86 ± 0.68
	2	BBM2P & BBM3P	0.68 to 0.98	71.1 ± 3.01 to 73.18 ± 3.02	BBM4P	0.65	27.89 ± 0.7	BBM 1/2P	2.72	19.04 ± 0.99
	3	BBM1/2NP	1.95	70.18 ± 2.02	BBM 1/2NP	1.47	32.62 ± 0.52	BBM 1/2NP	1.47	22.9 ± 0.74
<i>Chlorolobion braunii</i>	1	BBM3N & BBM4N	5.13 to 6.84	107.62 ± 8.02 to 109.23 ± 3.03	BBM 1/2N	0.85	34.32 ± 0.89	BBM 1/2N	0.85	34.14 ± 1.59
	2	BBM1/2P	2.72	109.49 ± 4.82	BBM 1/2PtoBBM4P	2.72 to 0.53	26.15 ± 0.47 to 27.89 ± 0.45	BBM 1/2P to BBM	2.72 to 1.71	27.49 ± 0.44 to 28.64 ± 1.39
	3	BBM	1.71	102.18 ± 2.02	BBM 1/2NP	1.47	32.96 ± 0.22	BBM 1/2NP	1.47	30.03 ± 1.01
<i>Kirchneriella obesa</i>	1	BBM & BBM2N	1.71 to 3.42	86 ± 1 to 87.09 ± 2.01	BBM 1/2N	0.85	22.99 ± 1.16	BBM &BBM 1/2N	0.85 to 1.71	17.02 ± 0.55 to 17.13 ± 0.86
	2	BBM	1.71	86 ± 1	BBM 4P	0.53	24.44 ± 0.66	BBM	1.71	17.13 ± 0.86
	3	BBM & BBM2NP	1.71 to 1.47	84 ± 3.61 to 86 ± 1	BBM 1/2NP	1.47	25.76 ± 0.86	BBM & BBM 2NP	1.71 & 1.47	17.13 ± 0.86 & 17.47 ± 0.59

Table 3
The highest Nitrogen Removal Rate (NRR), Nitrogen Removal Efficiency (NRE) of the five species of algae

Algae	Exp	Treatment	N:P	Nitrogen Removal Rate (NRR)	Treatment	N:P	Nitrogen Removal Efficiency (NRE)
<i>Monoraphidium contortum</i>	1	BBM3N	5.13	3.53 ± 0.19	BBM 1/2N to BBM	0.85 to 1.71	91.09 ± 3.58 to 94.4 ± 4.83
	2	BBM1/2P to BBM4P	2.72 to 0.53	1.65 ± 0.01 to 1.86 ± 0.08	BBM1/2P to BBM	1.71	94.4 ± 4.23
	3	BBM2NP	1.95	2.41 ± 0.74	BBM	1.71	94.4 ± 2.28
<i>Halochlorella rubescenes</i>	1	BBM2N &BBM3N	3.42 to 5.13	3.47 ± 0.07 to 3.87 ± 0.36	BBM	1.71	96.05 ± 0.19
	2	BBM1/2P to BBM4P	2.72 to 0.53	1.88 to 1.99 ± 0.7	BBM1/2P to BBM3P	2.72 to 0.68	94.84 ± 0.12 to 96.34 ± 0.29
	3	BBM2NP	1.95	3.08 ± 0.7	BBM	1.71	96.05 ± 0.19
<i>Coccomyxa simplex</i>	1	BBM3N	5.13	2.91 ± 0.11	BBM	1.71	96.05 ± 0.19
	2	BBM1/2P to BBM3P	2.72 to 0.68	1.89 ± 0.01 to 1.97 ± 0.8	BBM1/2P to BBM3P	2.72 to 0.68	94.84 ± 0.12 to 96.34 ± 0.29
	3	BBM	1.71	1.99 ± 0.06a	BBM	1.71	96.05 ± 0.38
<i>Chlorolobion braunii</i>	1	BBM2N	3.42	3.23 ± 0.26	BBM	1.71	95.85 ± 0.1
	2	BBM1/2P to BBM4P	2.72 to 0.53	1.87 ± 0.3 to 2.02	BBM 1/2P to BBM3P	2.72 to 0.68	95.08 ± 1.71 to 96.87 ± 0.91
	3	BBM2NP	1.95	2.05 ± 0.4	BBM 1/2NP &BBM	1.47 to 1.71	93.45 ± 3.32 to 95.85 ± 0.1
<i>Kirchneriella obesa</i>	1	BBM3N &BBM4N	5.13 to 6.84	2.31 ± 0.38 to 2.45 ± 0.62	BBM 1/2N to BBM	0.85 to 1.71	93.88 ± 1.48
	2	BBM1/2P to BBM3P	2.72 to 0.68	1.74 ± 0.63 to 1.94 ± 0.06	BBM	1.71	93.3 ± 2.92
	3	BBM2NP	1.95	2.28 ± 0.52	BBM 1/2NP to BBM	1.47 to 1.71	87.95 ± 5.47 to 93.3 ± 6.92

Table 4
The highest Phosphorus Removal Rate (PRR) and phosphorus Removal Efficiency (PRE) of the five species of algae

Algae	Exp	Treatment	N:P	Phosphorus Removal Rate (PRR)	Treatment	N:P	Phosphorus Removal Efficiency (PRE)
<i>Monoraphidium contortum</i>	1	BBM1/2N	0.85	2.67 ± 0.15	BBM 1/2N	0.85	79.33 ± 4.35
	2	BBM2P	0.98	2.45 ± 0.15	BBM1/2P	0.85	82.74 ± 0.7
	3	BBM2NP	1.95	3.57 ± 0.11	BBM2NP	1.95	84.89 ± 2.63
<i>Halochlorella rubescens</i>	1	BBM1/2N	0.85	2.21 ± 0.18	BBM 1/2N to BBM4N	0.85 to 6.84	66.35 ± 2.75 to 68.66 ± 5.34
	2	BBM to BBM4P	1.71 to 0.53	1.94 ± 0.13	BBM1/2P	0.85	80.9 ± 0.12
	3	BBM	1.71	1.94 ± 0.13	BBM 1/2NP	1.47	74.31 ± 0.68
<i>Coccomyxa simplex</i>	1	BBM1/2N	0.85	1.93 ± 0.05	BBM 1/2N	0.85	63.99 ± 1.41
	2	BBM to BBM4P	1.71 to 0.53	0.79 ± 0.51 to 1.03 ± 0.09	BBM1/2P toBBM	0.85 to 1.71	37.51 ± 5.53 to 33.53 ± 3.35
	3	BBM2NP	1.95	0.63 ± 0.07	BBM	1.71	37.51 ± 0.53
<i>Chlorolobion braunii</i>	1	BBM1/2N	0.85	2.57 ± 0.23	BBM1/2N	0.85	76.25 ± 4.78
	2	BBM2P to BBM4P	0.98 to 0.53	2.46 ± 0.07 to 2.6 ± 0.04	BBM 1/2P toBBM	0.85 to 1.71	71.84 ± 5.4 to 75.39 ± 2.59
	3	BBM	1.71	2.15 ± 0.31	BBM 1	1.71	71.84 ± 10.4
<i>Kirchneriella obesa</i>	1	BBM 1/2N to BBM	0.85 to 1.71	1.97 ± 0.1 to 2.05 ± 0.16	BBM 1/2N to BBM4N	0.85 to 6.84	57.29 ± 1.06 to 68.72 ± 5.43
	2	BBM2P to BBM4P	0.98 to 0.53	2.37 ± 0.21 to 2.83 ± 0.25	BBM1/2P	0.85	94.19 ± 0.75
	3	BBM1/2NP to BBM	1.95 to 1.71	1.95 ± 0.05 to 2.05 ± 0.16	BBM 1/2NP	1.95	95.84 ± 0.56

Biomass productivity at varying N and P regimes in BBM

The details of biomass productivity of the five different species at varying treatments of the three experiments are given in Supplementary Table 1. The regular BBM was the control in all three experiments. Figure 2a, b, and c demonstrate the responses of the five species to the diverse treatments in the three experiments.

Figure 2a shows the biomass productivity of the five species at increasing N:P from half the level to four times N in BBM, causing an increase in N:P in BBM. All the algae showed unique responses in biomass productivity to the varying N regimes in BBM. In general, the biomass productivity of these species at varying N regimes in BBM was significantly different from each other except for *Chlorolobion braunii* and *Halochlorella rubescens* at specific treatments (Supplementary Table 1). The alga *Coccomyxa simplex* showed its highest biomass productivity at half the level of N, whereas *Kirchneriella obesa* showed its lowest at this level. However, in both of them, biomass productivity remained the same as that of the control at all other treatments. *Halochlorella rubescens* showed a significant increase in productivity compared to the control, up to three times N in BBM. In contrast, *Monoraphidium contortum* showed a similar increase in productivity, up to only two times N in BBM. *Chlorolobion braunii* showed a significant increase in its productivity from the control at 3 to 4 times N in BBM.

Figure 2b demonstrates the biomass productivity of the five species from half the BBM level to four times P (KH₂PO₄) in BBM, causing a decrease in N:P in BBM. In general, responses in productivity of the five algae to P variations in BBM were also unique. While *Chlorolobion braunii* and *Coccomyxa*

simplex significantly increased biomass productivity compared to control at half the level of P in the BBM, all the other species significantly decreased biomass productivity at the same treatment.

In *Chlorolobion braunii*, the productivity decreased significantly from control up to 2 times P in BBM but remained constant afterward. In *Coccomyxa simplex*, the biomass productivity remained the same as that of control till three times P and then went significantly lower than control at four times P in BBM. In *Halochlorella rubescens*, biomass productivity was significantly lower than control from two to 4 times P in BBM. *Kirchneriella obesa* biomass productivity was significantly lower than control in all the consecutive higher P treatments. However, biomass productivity of *Monoraphidium contortum* increased significantly from half the level of P to 2 times P in BBM and remained the same up to 4 times P in BBM. Thus, it became clear that *Monoraphidium contortum* is a productive green alga suitable for P-rich media with an N:P lower than BBM's.

Figure 2c shows the biomass productivity of the five species at two varied N and P together treatments in BBM while N:P remains constant. Like the other two experiments, all species' biomass productivity was unique at varying amounts of N and P, keeping N:P the same in the medium. While *Kirchneriella obesa* and *Chlorolobion braunii* had their productivity significantly lower than the control at half the level of N and P together in BBM, the others had their productivity same as that of the control at this treatment. In the second treatment of N and P together at two times that of BBM, the productivity of *Kirchneriella obesa* was the same as that of control, whereas that of *Halochlorella rubescens* was significantly higher than that of control. However, productivity was substantially lower in all the other three species than the control at this second treatment.

Overall, the biomass productivity of *Chlorolobion braunii* (highest productivity of $109.23 \text{ mg l}^{-1} \text{ day}^{-1}$ at 3-4N in BBM) stands significantly higher than all the other algae at all the tested concentrations of N in BBM, revealing it as a productive green alga suitable for N-rich growth media with a broad increase in N:P (0.85 to 6.84). On the other hand, the biomass productivity of *Monoraphidium contortum* (highest productivity of $103.66 \text{ mg l}^{-1} \text{ day}^{-1}$ at 2-4P in BBM) stands significantly higher than the other algae at high concentrations of P in BBM, revealing it is a productive species suitable for P-rich growth media with a narrow range of low N:P (0.53 to 0.98). However, the biomass productivity of *Halochlorella rubescens* (highest productivity of $90.77 \text{ mg l}^{-1} \text{ day}^{-1}$) stands significantly higher than all others at two times N and P in BBM (N:P = that of BBM).

In general, green algal growth and yield are related to the total amount of N or P in the medium [36], and a low N:P ratio (lower than that of BBM) is considered unfavorable for the growth of green algae [37]. In the current experimental study, low N:P could be created by increasing P in the BBM or decreasing N in the BBM while keeping P constant. Accordingly, in the current study, *Monoraphidium contortum* showed significantly higher biomass productivity at a low N:P (0.98 to 0.53) by increasing P in the medium (2–4 times KH_2PO_4 in the BBM). The P content in BBM is 0.053 g/L , and BBM with 2–4 times P (KH_2PO_4) in the medium is 0.093 to 0.173 gL^{-1} . In the current study, *Monoraphidium contortum* showed a significant increase in biomass productivity and a rise in P in medium from 0.093 gL^{-1} to 0.173 gL^{-1} .

In contrast, its biomass productivity decreased significantly along with a decrease in P content at 0.053 gL^{-1} (BBM level) to 0.033 gL^{-1} (half the P in BBM) in the medium. Dhup et al. (2017) [38] observed a decline in the growth of *Monoraphidium contortum* at 0.1 gL^{-1} P. In contrast, in the current study, a decrease in productivity was observed for the species at 0.053 gL^{-1} P only. However, an increase in growth of the species at P content at 0.093 (two times KH_2PO_4 in BBM) for this species is found while keeping N at the BBM level in the medium. Moreover, the species showed no significant difference in productivity at higher N and P together in BBM. Thus, it became clear that P in the medium positively influences this species' growth. Therefore, *Monoraphidium contortum* may be suitable for treating P-rich waste waters. However, the optimum P regime needs to be standardized through further experimentation.

Overall, the biomass productivity of all five algae was unique in the BBM and showed individual responses to variations in N:P concerning N and P. Therefore, it may be concluded that specific N:P and the amount of N or P in the medium are decisive to the productivity of each algal species in a growth media. The primary significance of the current experiment is that it enabled simultaneous identification of the specific influence of N:P and N and P content on the growth and productivity of five species by adding varying amounts of N or P and N and P together into growth media. Thus, the current study emerged as a model experiment to identify the optimum nutrient requirement of indigenous species and to check whether they are suitable for p-rich or N-rich wastewaters.

The positive response of algae to low N in the medium is attributed to a lack of initial lag phase [39] in the growth cycle of such species of algae. In the present experiment with five indigenous species, *Coccomyxa simplex* is identified as such a species. In laboratory culture studies, limiting light availability for individual cells may affect growth above a critical culture density [40]. However, the difference in cell size of specific algal species determines their specific limit to light from high population density in the cultures. Accordingly, in the present experiment, light limitation on biomass productivity was observed for large cell-sized *Halochlorella rubescens* at three times N in the medium. In contrast, light limitation on biomass productivity of small cell-sized *Chlorolobion braunii* did not arise up to 4 times N in the growth medium.

According to Li et al. (2008) [40], sodium nitrate concentrations above ten mM in the media are toxic to green microalgae. Our experiments have shown that all algae except *Halochlorella rubescens* and *Monoraphidium contortum* are nitrate-tolerant species capable of tolerating Sodium nitrate content up to about 12 mM in the media. After all, their productivity remained the same at 3 to 4 times N in the BBM. In contrast, *Halochlorella rubescens* and *Monoraphidium contortum* are observed as nitrate-sensitive species because their growth or biomass productivity at three times N was significantly lower than that at two times N (10 mM) in the growth media. Dhup and Dhawan (2014) [41] also observed the Nitrate sensitivity of *Monoraphidium*

spp. in their experiment. A decrease in the growth and productivity of algae at high Nitrate content in growth media can be due to an increase in nitrate reductase activity, which enhances the production of nitrite and ammonia that are accumulated *in vivo* [42].

According to [36], an N:P above five or more is favorable for the growth of microalga *Coelastrum morus*. In contrast, in the current study, a higher N:P, up to 6.84, is advantageous for *Chlorolobion braunii*. In comparison, P-varied treatments enabled us to show that *Monoraphidium contortum* and *Coccomyxa simplex* have the potential to grow at shallow N:P (0.98). Thus, The current experiments helped us explain how N:P concerning N or P in the growth media is significant to specific algal biomass productivity in the growth media, which is essential to utilize them for the phycoremediation of specific industrial wastewaters.

The productivity of *Chlorolobion braunii* ($109.23 \pm 3.03 \text{ mg l}^{-1} \text{ day}^{-1}$ at four-times N in BBM), *Halochlorella rubescens* ($105.04 \pm 2 \text{ mg l}^{-1} \text{ day}^{-1}$ at three-times N in BBM) and *Monoraphidium contortum* ($103 \pm 2.01 \text{ mg l}^{-1} \text{ d}^{-1}$ at two to four-times P in BBM) discovered in the current study is higher than that of some other indigenous productive species of green microalgae previously reported from eutrophic waters of Kerala [25, 30].

Lipid yield and productivity

One of the most significant goals of the current investigation was to assess the lipid yield and productivity of the five species of algae under varied nutrient regimes of N and P concerning N:P ratios in the BBM. The details of the lipid yield and lipid productivity of the five species in all three experiments and the varied treatments are shown in Supplementary Table 2. Figure 3 and Fig. 4 depict specific trends in lipid yield and lipid productivity of the five species, respectively, concerning variations in N, P, and N:P. Since lipid productivity is the product of biomass productivity and lipid yield, it also varies in species per biomass productivity. Accordingly, the lipid productivity of all the species was unique, with significant differences between them at all the treatments.

In the first experiment of N-varied treatments, all five species showed significantly higher lipid yield at the first treatment of half the level of N in BBM than the control and all the other treatments. The exciting trend was decreased lipid yield and increased N or N:P. However, the highest lipid productivity among all the five species of algae was shown by *Chlorolobion braunii* at half the N level (0.85 N:P) in BBM. The other species showed their specific productivity lower than that of *Chlorolobion braunii* at all the other treatments. *Kirchneriella obesa* showed the lowest productivity among all species at all the treatments.

In the second experiment of P-varied treatments, the five algal species showed unique lipid yield responses to each tested level of P in the BBM. Among all the species and all the treatments, *Monoraphidium contortum* showed the highest lipid yield at the control. In contrast, *Kirchneriella obesa* showed the lowest yield at two times P in BBM. In the P-varied experimental treatments, all the algae showed lipid productivity per each algae's biomass productivity. Accordingly, *Monoraphidium contortum* showed the highest lipid productivity at the half level of P, two times P, and four times P in BBM without any significant difference in lipid productivity between these treatments. However, *Kirchneriella obesa* showed the lowest lipid productivity at four times P in BBM.

In the third experiment of N and P together treatments in BBM, all five species showed significantly higher lipid yield in the first treatment of half the level of N and P in BBM than the control and at all other treatments.

As in the previous experiments of individual N treatments, all the species showed their highest lipid yield at half the N and P together treatments. In this experimental treatment, *Monoraphidium contortum* showed significantly higher lipid yield and productivity than all other species at half N and P in BBM. Similarly, *Kirchneriella obesa* showed the lowest lipid yield at two times N and P treatment. However, the lowest lipid productivity among all species over the different treatments was shown by *Coccomyxa simplex* at two times N and P in the BBM. Overall, *Monoraphidium contortum* emerged as a green alga with the highest lipid yield and productivity at a lower nutrient regime (N or P or N and P together) than the BBM.

The details of lipid productivity for all five species in various treatments of all three experiments are presented in Fig. 4. Indigenous microalgae with efficient photosynthetic systems are well known for their rapid growth rate and lipid yield compared to traditional plants [43]. Such microalgae with consistent growth and strong adaptability are to be carefully screened for their protein and lipids content in their commercial applications [44], especially for phycoremediation cum biomass production. Lipid productivity is the product of biomass productivity and lipid yield; it depends on the biomass productivity of algae. A decreasing trend of lipid content with an increase in N in the current study was similar to the observation of [40]. Usually, lipid synthesis within algae increases as an adaptation to environmental stress, such as lack of nutrients [45]. Moreover, reducing nitrogen levels can stimulate lipid production in algae because nitrogen scarcity leads to the buildup of carbon precursors in the form of acetyl-CoA, which serves as a fundamental building block for synthesizing lipids [46]. Additionally, it is well-known that low N content induces a decline in algal growth and a shift in cell metabolism, mainly towards lipid accumulation, which can produce more lipids in some algae species [26].

Unlike the positive influence of a reduction of N on lipid productivity, a decrease in P in the medium didn't stimulate lipid productivity in any of the studied species in the current study. However, P deprivation induces excessive lipid content in some algae [47, 48]. On the contrary, in the present study, some species showed a significant increase in lipid content at four times P in the medium than at regular BBM. While excessive P in the medium may act negatively on biomass productivity, the cell metabolism in such species shifts towards lipid production.

It may be noted that *Monoraphidium contortum*, *Halochlorella rubescens*, and *Coccomyxa simplex* showed their highest lipid content and lipid productivity at half N and P together in BBM treatment. It agrees with the observation of [38] that a decrease in P and N below the optimum level can boost biomass productivity because of better nutrient utilization. It is well known that under nutrient stress, microalgal cells accumulate more cellular lipids in cells, up to 50 to 70% of the biomass, with neutral lipids (TAGs) making up the most significant proportion of this accumulation [49].

Another factor influencing lipid productivity is the N:P ratio of the medium. All the three species mentioned above showed their highest lipid productivity at N:P of 1.4, similar to the optimal nutrient regime for achieving the highest lipid yield in *Chlorella surukiana* [44]. However, N and P content and proportions may vary significantly for various microalgae species [50]. In the current study, different microalgae species also showed variations in maximum lipid productivity at varied N:P.

Fatty acid profile of the five species

The fatty acid profile of all five species of algae was carried out at their highest lipid productivity. The details of the same are presented in Table 5. The fatty acids of the five species of algae studied range from C8:0 to C24:0. The predominant saturated fatty acids are palmitic acid (C16: 0) and stearic acid (C18: 0) in these species. However, oleic acid (C18:1) was these algae's primary monounsaturated fatty acid. The major polyunsaturated fatty acids in these microalgae were linoleic and (C18:2) and linolenic acid (C18:3). The fatty acids such as C8:0, C15:0, C20:0, C21:0, C22:0, C24:0, C16:1, C18:3n6, C20:5n3 were present only in some of the selected species (Table 5).

Table 5
Fatty acid profile (% of total FAME) of five algae at its highest lipid-productivity

Fatty Acid	<i>Monoraphidium contortum</i>	<i>Halochlorella rubescenes</i>	<i>Coccomyxa simplex</i>	<i>Chlorolobion braunii</i>	<i>Kirchneriella obesa</i>
C8:0	ND	0.138528	ND	0.014857	ND
C12:0	0.179802	0.190476	0.541272	0.148566	0.125122
C14:0	0.988912	0.519481	1.623816	0.282276	0.569999
C15:0	0.25472	ND	0.383401	0.074283	0.375365
C16:0	27.58466	45.78355	32.00271	23.78547	24.09287
C17:0	0.269703	0.380952	0.473613	ND	0.236341
C18:0	4.225352	2.233766	2.796572	2.124499	2.113166
C20:0	ND	0.363636	0.270636	ND	ND
C21:0	0.25472	ND	ND	ND	ND
C22:0	0.149835	ND	ND	0.267419	0.597803
C24:0	0.674258	ND	1.082544	0.267419	ND
Σ% SFA	34.582	49.61	39.175	26.965	28.111
C16:1	0.869044	0.225108	ND	ND	5.755596
C18:1n9t	41.62421	25.78355	30.35634	50.79483	45.04379
C20:1	ND	0.658009	0.721696	1.188531	1.014876
C22:1n9	0.25472	0.900433	1.195309		0.125122
Σ% MUFA	42.748	27.567	32.273	51.983	51.939
C18:2n6c	11.13275	13.43723	9.855661	7.829446	8.563882
C18:3n3	11.01289	9.264069	18.49346	13.2224	11.20534
C18:3n6	0.344621	0.121212	0.202977	ND	0.180731
C20:5n3	0.179802	ND	ND	ND	ND
Σ% PUFA	22.67	22.823	28.552	21.052	19.95
n-6	11.47737	13.55844	10.05864	7.829446	8.744613
n-3	11.19269	9.264069	18.49346	13.2224	11.20534
n-6/n-3	1.025435	1.463551	0.543902	0.592135	0.780397
ND not detected					

The total saturated fatty acid (SFA) ranged from 26.96 (*Chlorolobion braunii* at half the N in BBM) to 49.61% (*Halochlorella rubescens* at half the N & P in BBM). The monounsaturated fatty acid (MUFA) content ranged from 27.567% (*Halochlorella rubescens* at half the N & P in BBM) to 51.983% (*Chlorolobion braunii* at half the N in BBM). The polyunsaturated fatty acids (PUFA) in these algae ranged from 19.95 (*Kirchneriella obesa* at 4 N in BBM) to 28.55% (*Coccomyxa simplex* at half the N & P in BBM). Therefore, among the tested microalgal species, *Chlorolobion braunii* (50.79%), *Kirchneriella obesa* (45.04%), and *Monoraphidium contortum* (41.62%) showed the highest oleic acid content, making it the most suitable for the production of good quality biodiesel.

Biodiesel properties of the algal lipids

In evaluating the caliber of the five microalgae species for their biodiesel quality, empirical equations were used to estimate the same from their respective fatty acid profile (Table 6). All five species studied showed the Cetane Number (CN) ranging from 51.31 to 55.65 and matched the American standard for biodiesel biodiesel (ASTM D6751) and European biodiesel standards (EN14214).

Table 6
Fuel properties of biodiesel produced from the five green microalgal species at its highest lipid-productivity

SI No	Biodiesel quality parameter	<i>Monoraphidium contortum</i>	<i>Halochlorella rubescens</i>	<i>Coccomyxa simplex</i>	<i>Chlorolobion braunii</i>	<i>Kirchneriella obesa</i>
1	IV (g I ₂ 100g ⁻¹)	90.268	74.784	97.983	97.018	93.868
2	SV (mg KOH g ⁻¹)	204.668	208.48	206.039	203.386	204.915
3	CN	52.657	55.654	50.744	51.307	51.815
4	DU (wt%)	88.088	73.212	89.378	94.087	91.839
5	HHV (MJ kg ⁻¹)	40.424	40.338	40.362	40.449	40.409
6	OS (h)	7.792	7.758	6.721	8.192	8.502
7	LCSF (wt%)	6.954	6.059	7.034	4.377	4.363
8	CFPP (°C)	5.37	2.558	5.623	-2.727	-2.771
9	CP (°C)	9.518	19.09	11.841	7.519	7.681
10	PP (°C)	3.511	13.902	6.034	1.342	1.517

IV iodine value, SV saponification value, CN cetane number, DU degree of unsaturation, HHV higher heating value, OS oxidative stability, LCSF long-chain saturation factor, CFPP cold filter plugging point, CP cloud point, PP pour point.

Iodine value (IV), which measures the total unsaturation within a mixture of fatty acids, is another crucial data. The calculated IV for five species ranged from 74.78 to 97.983; all showed < 120 that matched EN14214. All five microalgal species had higher heating value (HHV), within the predetermined range (40.26 to 42.65 MJ kg⁻¹) for ordinary biodiesel, which is typically 7–12% less than that of diesel made from petroleum (46 MJ kg⁻¹).

For fuel applications requiring low temperatures, the cloud point (CP), pour point (PP), and cold filter plugging point (CFPP) are crucial. While the PP is the temperature at which the wax-formed fuel can flow, the CP is the temperature at which a cloud of wax crystals initially forms when the fuel is cooled. All species except *Halochlorella rubescens* (CP = 19.09°C) exhibited CP values ranging between 7.52 to 11.841°C and PP values 1.342 to 13.902°C, matching the ASTM D6751 standard. The CFPP values ranged from – 2.727 to 5.62°C, demonstrating the suitability of biodiesel for all five algae at low temperatures.

The EN 14214 set the oxidative stability as anything above six h. All species recorded 6.72 to 8.502 h oxidative stability in the present case. The saponification value, which measures the average molecular weight of all the fatty acids in algal lipids, ranged from 203.386 to 208.48. In the present study, the Degree of Unsaturation (DU) value ranges from 73.212 to 94.09%, making it suitable for long-term storage.

The fatty acid profile is an essential criterion for selecting a microalgal species for phycoremediation cum biomass utilization as a nutraceutical or biofuel resource [51]. Chlorophyceae algal species predominantly have C16:0, C18:0, C18:1n9c, and C18:2n6 fatty acids [26, 52]. The unsaturated fatty acid content generally makes microalgae extremely stress-responsive to flourish in nutrient-deficient environments. Unsaturated fatty acid content also enables them to have improved membrane fluidity for thriving under adverse conditions [53].

In general, an ideal balance of saturated and unsaturated FAMES in cells determines the good biodiesel quality of microalgae. The primary fatty acids determining the fuel qualities are palmitic, oleic, and linoleic acids [54], present in the desired proportion in all the studied algae. Higher concentrations of C16 and C18 fatty acids are advantageous for biodiesel quality [55], and the findings of this study met the requirement for high-quality biodiesel. Usually, vegetable oils with a high oleic acid content have a decent fuel balance, including ignition quality, combustion heat, cold filter plugging point

(CFPP), oxidative stability, viscosity, and lubricity [56]. The highest oleic acid content was found in *Chlorolobion braunii* (50.79%), followed by *Kirchneriella obesa* (45.04%), and the least oleic acid was found in *Halochlorella rubescens* (25.78%)

Additionally, the fatty acid composition of algal oil determines biodiesel quality, such as oxidation stability, cetane rating, melting point, viscosity, and fluidity at low temperatures. The low level of polyunsaturated fatty acids and a large number of saturated fatty acids act as crucial markers of biodiesel quality because unsaturated bonds decrease the oxidative stability of biodiesel [57]. However, oils with high unsaturated fatty acid content and a low melting point are preferred in biodiesel production for low-temperature uses [58]. The CN is a frequently used indicator for determining fuel quality because it is decisive in the combustion behaviour of the fuel's readiness to self-ignite when injected [35]. The minimal CN levels for biodiesel are 47.0 and 51.0, respectively, per ASTM D6751 and EN14214 standards. Therefore, the lipids of all five algae with above 51 CN satisfy such criteria for biofuels. However, *Halochlorella rubescens* did not meet the CP limit of -3 to 12 set by EN 14214 among the five algae tested.

The NRR, PRR, NRE, and PRE of the five microalgae

The details of the NRR and NRE of all five algae are given in Supplementary Table 3, whereas PRR and PRE are given in Supplementary Table 4. The trends in their responses concerning NRR are shown in Fig. 5a, b, and c, whereas PRR is demonstrated in Fig. 6a, b, and c, respectively. Overall, the NRR, PRR, NRE, and PRE of the five species of green algae concerning variations in N or P and N and P together enabled us to check the potentials of five indigenous green algae in response to different regimes of N, P concerning N:P in the growth medium. The details of the best NRE, NRR, PRE, and PRR concerning N or P or N and P together, and N:P in BBM by the five species are shown in Tables 3 and 4, respectively.

The NRE and NRR of the five microalgae

In the first experimental treatments of variations of N in BBM, keeping P constant, all five species showed their highest NRE at the control (93.3 to 96.05%) without any significant difference among them. At half the N in the BBM (N:P = 0.85), the NRE was significantly lower than the control in *Halochlorella rubescens*. The NRE of all other species remained the same as that of the control at half the level of N in the medium. However, in all species, at all levels of increase of N in the BBM (N:P 1.71 to 6.84), the NRE remained lower than that of the control. In the second experiment of varying P keeping N constant in the BBM, the NRE of all species at half the P in BBM (N:P = 2.72) remained the same as that of the control. However, on increasing P in the BBM (N:P 1.71 to 0.53), the NRE of all the species remained the same or decreased only. In the third experiment, at half the level of N and P together in BBM (N:P = 1.71), the NRE of the three species significantly decreased from that of the control. However, in *Chlorolobion braunii* and *Kirchneriella obesa*, the NRE remained the same as that of the control during this treatment. The NRE of all species showed a significant decrease from that of the control at two times N and P in BBM (N:P = 1.71).

In general, in all species, the NRR at half the N in BBM was significantly lower than that at the control (Fig. 5a). On increasing N in the BBM, *Halochlorella rubescens*, *Monoraphidium contortum* and *Coccomyxa simplex* showed a significant increase in NRR from the control (regular BBM) up to 3 times N in BBM (N:P 1.71 to 5.13); but at 4N (N:P = 6.84), the NRR decreased significantly from that of the control (Fig. 5a). In *Chlorolobion braunii* the NRR increased significantly from the control up to 2N (N:P = 3.42), and it remained the same at 3N. However, its NRR significantly decreased at 4N, like the first three species. In *Kirchneriella obesa*, NRR remained the same at control and 2N but increased significantly at 3N but remained the same on further increase up to 4N. Overall, the highest NRR was exhibited by *Halochlorella rubescens* (3.87mg l⁻¹ day⁻¹) and *Monoraphidium contortum* (3.53 mg l⁻¹ day⁻¹) at 123.48 ppm N without any significant difference in NRR by the two species.

In the second experimental condition of varying P in the regular BBM (Fig. 5b) keeping N constant, except *Coccomyxa simplex* and *Kirchneriella obesa*, all the other three species showed the same NRR at all variations of P in BBM. However, in *Coccomyxa simplex* and *Kirchneriella obesa*, NRR remained the same up to 3 times P in BBM, significantly decreasing at four times P in the medium.

In the third experiment of varying N and P together in BBM without a change in N:P, the NRR of all species at half the N and P in BBM was significantly lower than that of the control. At an increase of NP up to two times the level of BBM (Fig. 5c), the NRR of all algae except *Coccomyxa simplex* and *Chlorolobion braunii* significantly increased from that of the control. In *Coccomyxa simplex*, NRR decreased significantly, whereas in *Chlorolobion braunii*, NRR remained the same without any significant difference from control in two times N and P in BBM.

All species demonstrated the highest NRE at the control and half the N in BBM without any significant difference between the treatments. Moreover, at a constant level of phosphorus (P), NRR and NRE of the five tested species varied based on the nitrogen (N) content and nitrogen-to-phosphorus (N:P) ratio in the medium.

It is well-known that at low nitrogen content in medium, NRE is almost complete [59]. This is because microalgae utilize both nitrogen and phosphorus together for their growth. Naturally, an excessive supply of one major nutrient element exhausts the other essential nutrients from the growth medium, leading to no further change in NRE [60].

The significance of N:P on algal growth and nutrient removal was first proposed by Redfield (1958) [61], and the optimal N:P for marine phytoplankton per Redfield is 16 mol-N/mol-P. Subsequent studies reveal that the optimal N:P of specific microalgae varies according to the species and may also be

influenced by the particular conditions of cultivation depending on their adaptation to culture conditions [62]. Although N:P has no influence on NRR by certain species [36], the present study revealed that an increase in N:P positively influences the NRR of microalgae, and the effect is species-specific.

In the present study, the optimum N:P for the maximum NRR was 5.13 in *Halochlorella rubescens*, *Monoraphidium contortum*, and *Coccomyxa simplex*. However, the optimum N:P for *Chlorolobion braunii* was 3.42, and for *Kirchneriella obesa*, 6.84. The N:P ratio significantly impacts nutrient usage by microalgae, the optimum of which depends on the species [63]. However, according to Dhup and Dhawan (2014) [41], the highest nutrient uptake rate is associated with an N:P ratio of 0.153 for certain algae.

PRE and PRR of the five microalgae

In N treatments, *Monoraphidium contortum*, *Chlorolobion braunii*, and *Coccomyxa simplex* showed significantly higher PRE at half the level of N in the BBM than at all other treatments. *Halochlorella rubescens* and *Kirchneriella obesa* PRE were the same as the control at half the level of N in BBM. However, towards an increase of N in the BBM, the PRE remained the same as that of the control. In general, *Monoraphidium contortum* (with PRE 62–79%) and *Chlorolobion braunii* (with PRE 66–76%) are found to be the best among the five species for removal of P from N-rich media. In P treatments, *Monoraphidium contortum*, *Halochlorella rubescens*, and *Kirchneriella obesa* showed significantly higher PRE at half the level of P in the BBM than all other treatments. In contrast, the PRE of *Chlorolobion braunii* and *Coccomyxa simplex* remained equal to that of control. However, in all species, the PRE remained lower than that of the control towards an increase in P in BBM.

In N and P together treatments, the PRE of the three species, *Monoraphidium contortum*, *Chlorolobion braunii*, and *Coccomyxa simplex*, was significantly lower than that of the control, whereas that of *Halochlorella rubescens* and *Kirchneriella obesa* remained same as that of control. The PRE of all species except *Monoraphidium contortum* showed a significant decrease from that of the control at two times N and P in BBM. *Monoraphidium contortum* showed a significantly higher PRE than the control at two times N and P together in BBM.

In the N treatments, all the species except *Kirchneriella obesa* showed significantly higher PRR than the control at half the level of N in BBM. In contrast, *Kirchneriella obesa* remained the same as that of control. However, on increasing N in the BBM, the PRR responses of all the species remained the same as that of the control or decreased only (Fig. 6a).

In the P treatments, the different species showed a unique increase in PRR. The algae *Halochlorella rubescens* and *Kirchneriella obesa* showed a rise in PRR to 3P in BBM. In contrast, *Monoraphidium contortum* and *Chlorolobion braunii* showed a surge in PRR up to 2 P in BBM. The PRR of *Coccomyxa simplex* remained the same as that of control without any significant difference up to 4 times P in BBM (Fig. 6b). It may be noted that each species is suited best for growth media of different phosphorus regimes. *Chlorolobion braunii* has its best PRR ($2.5 \text{ mg l}^{-1} \text{ day}^{-1}$ PRR at 0.98 N:P), *Kirchneriella obesa* ($2.83 \text{ mg l}^{-1} \text{ day}^{-1}$ at 0.68 N:P) and *Monoraphidium contortum* ($2.45 \text{ mg l}^{-1} \text{ day}^{-1}$ at 0.98 N:P).

In the N and P together treatment, PRR decreased significantly for all the species from the control in the first treatment of half the level of N and P in BBM; however, on increasing N and P together to two times, PRR either remained the same (*Kirchneriella obesa*) or decreased (*Coccomyxa simplex*, *Chlorolobion braunii* & *Halochlorella rubescens*). However, in *Monoraphidium contortum*, PRR increased exponentially to $3.57 \text{ mg l}^{-1} \text{ day}^{-1}$ at the third treatment (N:P = 1.71) (Fig. 6c).

Among the five species, only *Kirchneriella obesa* showed increased PRR and a decrease in N in the medium. A similar response is known earlier [41] in *Monoraphidium contortum*. [38] PRE decreases with increased P for all microalgae species, but the tolerance limit is species-specific. According to the author, an elevated phosphorus content usually harms the culture, hindering algal growth because of inefficient resource utilization. The nutrient removal efficiency declines because the cells avoid uptake beyond a certain threshold. In the present study, PRR increased along with P in the medium, but the response was species-specific. A similar demonstration of an increase in PRR, along with an increase in phosphorus in the media, is known [36].

Conclusion

The present experiments with five microalgae concerning changes in N, P, and NP levels in the medium and consequent variations in N:P ratios enabled the primary screening of their optimum culture protocols for maximum biomass and lipid yield and their best nitrogen and phosphorus removal efficiency. All these algae exhibited distinct species-specific responses to each alteration in N or P variations and the consequent changes in N:P ratios in the growth media. In general, the nutrient removal rate and biomass productivity of all the tested five algae appear suitable for further undergoing an industrial trial with them for phycoremediation in a range of eutrophic scenarios; some for N-rich media, some others for P-rich or NP-rich situations at varying N:P ratios. *Chlorolobion braunii* and *Monoraphidium contortum* are identified as suitable for N-rich and P-rich conditions, respectively. *Halochlorella rubescens* and *Kirchneriella obesa* are ideal for nutrient removals; the former is for N removal from N-rich media, whereas the latter is for P removal from P-rich media. *Monoraphidium contortum* is a promising species for lipid yield and productivity. Most of the species' lipid content is excellent for biofuel conversions. Identifying the industrial prospects of such species is crucial to conserving them for future growth of algal technology for various bioindustries. In this context, the present preliminary experimental screening model is highly significant as it enables simultaneous identification of algae for multiple industrial uses within a limited time and using limited resources.

Declarations

Funding: *The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.*

Competing Interests: *The authors have no relevant financial or non-financial interests to disclose.*

Author Contributions: The first author carried out the experiments and laboratory analysis per the second author's plan and guidance and prepared the manuscript's first draft. The second author critically evaluated the content, revised and edited the same thoroughly, and both authors have approved submitting the article to your esteemed journal.

Data Availability: All the supporting data related to the submission are available in the supplementary file.

References

1. UN (2023) Progress towards the Sustainable Development Goals: UN
2. Tai H, Samandari H, Pachthod D, et al (2022) The energy transition: A region-by-region agenda for near-term action. Mc Kiensey & Company
3. DGNB, GBCE, RFBB, et al (2023) Circular economy. In: Circular Economy Taxonomy Study – Assessing the market-readiness of the proposed Circular Economy EU Taxonomy Criteria for buildings
4. Giuntoli J., Ramcilovic-Suominen S, Kallis G, et al (2023) Exploring new visions for a sustainable bioeconomy. European Commission
5. Tan ECD, Lamers P (2021) Circular Bioeconomy Concepts—A Perspective. *Front Sustain* 2:1–8. <https://doi.org/10.3389/frsus.2021.701509>
6. Ladanai S, Vinterbäck J (2009) Global Potential of Sustainable Biomass for Energy. In: SLU, Institutionen för energi och teknik Swedish University of Agricultural Sciences, Department of Energy and Technology. p 32
7. Rodríguez-Palacio MC, Cabrera-Cruz RBE, Rolón-Aguilar JC, et al (2022) The cultivation of five microalgae species and their potential for biodiesel production. *Energy Sustain Soc* 12:1–6. <https://doi.org/10.1186/s13705-022-00337-5>
8. Kumar L, Anand R, Shah MP, Bharadvaja N (2022) Microalgae biodiesel: A sustainable source of energy, unit operations, technological challenges, and solutions. *J Hazard Mater Adv* 8:100145. <https://doi.org/10.1016/j.hazadv.2022.100145>
9. DOE US (2010) National algal biofuels technology roadmap. In: Energy USD of (ed) National Algal Biofuels Technology Roadmap. U.S. Department of Energy. <http://biomass.energy.gov>, pp 1–174
10. Zielinski M, Debowski M, Kazimierowicz J, Izabela Swica (2023) Microalgal Carbon Dioxide (CO₂) Capture and Utilization from the European Union Perspective. *Energies* 16:
11. Miranda AM, Hernandez-Tenorio F, Ocampo D, et al (2022) Trends on CO₂ Capture with Microalgae: A Bibliometric Analysis. *Molecules* 27:1–14. <https://doi.org/10.3390/molecules27154669>
12. Makareviciene V, Sendzikiene E (2022) Application of Microalgae Biomass for Biodiesel Fuel Production. *Energies* 15:. <https://doi.org/10.3390/en15114178>
13. Li L, Gao K, Yang M, et al (2023) Challenges and potential solutions of microalgae-based systems for wastewater treatment and resource recovery. 1–4. <https://doi.org/10.3389/fbioe.2023.1210228>
14. McGowen J, Knoshaug EP, Laurens LML, Forrester J (2023) Outdoor annual algae productivity improvements at the pre-pilot scale through crop rotation and pond operational management strategies. *Algal Res* 70:102995. <https://doi.org/10.1016/j.algal.2023.102995>
15. Ray JG, Santhakumaran P, Kookal S (2021) Phytoplankton communities of eutrophic freshwater bodies (Kerala, India) in relation to the physicochemical water quality parameters. *Environ Dev Sustain* 23:259–290. <https://doi.org/10.1007/s10668-019-00579-y>
16. Wang Y, Ho SH, Cheng CL, et al (2016) Perspectives on the feasibility of using microalgae for industrial wastewater treatment. *Bioresour Technol* 222:485–497. <https://doi.org/10.1016/j.biortech.2016.09.106>
17. Li K, Liu Q, Fang F, et al (2019) Microalgae-based wastewater treatment for nutrients recovery: A review. *Bioresour Technol* 291:121934. <https://doi.org/10.1016/j.biortech.2019.121934>
18. Quiroz Arita CE, Peebles C, Bradley TH (2015) Scalability of combining microalgae-based biofuels with wastewater facilities: A review. *Algal Res* 9:160–169. <https://doi.org/10.1016/j.algal.2015.03.001>
19. Abdel-Raouf N, Al-Homaidan AA, Ibraheem IBM (2012) Microalgae and wastewater treatment. *Saudi J Biol Sci* 19:257–275. <https://doi.org/10.1016/j.sjbs.2012.04.005>
20. Konstantin Bloch, Vardi P (2009) MICROALGAE AS PHOTOSYNTHETIC OXYGEN GENERATORS FOR POLLUTION CONTROL, LIFE SUPPORT SYSTEMS AND MEDICINE. In: *Algae: Nutrition, Pollution Control, and Energy Sources*. pp 1–21
21. Lizzul AM, Hellier P, Purton S, et al (2014) Combined remediation and lipid production using *Chlorella sorokiniana* grown on wastewater and exhaust gases. *Bioresour Technol* 151:12–18. <https://doi.org/10.1016/j.biortech.2013.10.040>
22. Nguyen LN, Aditya L, Vu HP, et al (2022) Nutrient Removal by Algae-Based Wastewater Treatment. *Curr Pollut Reports* 369–383. <https://doi.org/10.1007/s40726-022-00230-x>
23. Amaro HM, Salgado EM, Nunes OC, et al (2023) Microalgae systems - environmental agents for wastewater treatment and further potential biomass valorisation. 337:. <https://doi.org/10.1016/j.jenvman.2023.117678>

24. Ji L, Ge Q, Li Y, et al (2021) A comparative study of the growth and nutrient removal effects of five green microalgae in simulated domestic sewage. *Water (Switzerland)* 13:. <https://doi.org/10.3390/w13243613>
25. Santhakumaran P, Kookal SK, Ray JG (2018) Biomass yield and biochemical profile of fourteen species of fast-growing green algae from eutrophic bloomed freshwaters of Kerala, South India. *Biomass and Bioenergy* 119:155–165. <https://doi.org/10.1016/j.biombioe.2018.09.021>
26. Santhakumaran P, Kookal SK, Mathew L, Ray JG (2020) Experimental evaluation of the culture parameters for optimum yield of lipids and other nutraceutically valuable compounds in *Chloroidium saccharophilum* (Kruger) comb. Nov. *Renew Energy* 147:1082–1097. <https://doi.org/10.1016/j.renene.2019.09.071>
27. Kookal S, Santhakumaran P, Ray JG (2016) Chlorococcum humicola (Nageli) Rabenhorst as a Renewable Source of Bioproducts and Biofuel. *J Plant Stud* 5:48. <https://doi.org/10.5539/jps.v5n1p48>
28. Guleri S, Saxena A, Singh KJ, et al (2020) Phycoremediation: a Novel And Synergistic Approach in Wastewater Remediation. *J Microbiol Biotechnol Food Sci* 10:98–106. <https://doi.org/10.15414/jmbfs.2020.10.1.98-106>
29. Moro CV, Crouzet O, Rasconi S, et al (2009) New design strategy for development of specific primer sets for PCR-based detection of Chlorophyceae and Bacillariophyceae in environmental samples. *Appl Environ Microbiol* 75:5729–5733. <https://doi.org/10.1128/AEM.00509-09>
30. Santhakumaran P, Kookal SK, Mathew L, Ray JG (2019) Bioprospecting of Three Rapid-Growing Freshwater Green Algae, Promising Biomass for Biodiesel Production. *Bioenergy Res* 12:680–693. <https://doi.org/10.1007/s12155-019-09990-9>
31. Hempel N, Petrick I, Behrendt F (2012) Biomass productivity and productivity of fatty acids and amino acids of microalgae strains as key characteristics of suitability for biodiesel production. *J Appl Phycol* 24:1407–1418. <https://doi.org/10.1007/s10811-012-9795-3>
32. Eladel H, Abomohra AEF, Battah M, et al (2019) Evaluation of *Chlorella sorokiniana* isolated from local municipal wastewater for dual application in nutrient removal and biodiesel production. *Bioprocess Biosyst Eng* 42:425–433. <https://doi.org/10.1007/s00449-018-2046-5>
33. Bligh, E.G. and Dyer WJ (1959) Canadian Journal of Biochemistry and Physiology. *Can J Biochem Physiol* 37:
34. AOAC (2006) Official Methods of Analysis., 17th edn. AOAC International, Washington 33.
35. Hoekman SK, Broch A, Robbins C, et al (2012) Review of biodiesel composition, properties, and specifications. *Renew Sustain Energy Rev* 16:143–169. <https://doi.org/10.1016/j.rser.2011.07.143>
36. Figler A, Márton K, B-Béres V, Bácsi I (2021) Effects of nutrient content and nitrogen to phosphorous ratio on the growth, nutrient removal, and desalination properties of the green alga coelastrum morus on a laboratory scale. *Energies* 14:. <https://doi.org/10.3390/en14082112>
37. Mayers JJ, Flynn KJ, Shields RJ (2014) Influence of the N: P supply ratio on biomass productivity and time-resolved changes in elemental and bulk biochemical composition of Nannochloropsis sp. *Bioresour Technol* 169:588–595. <https://doi.org/10.1016/j.biortech.2014.07.048>
38. Dhup S, Kannan DC, Dhawan V (2017) Growth, lipid productivity and cellular mechanism of lipid accumulation in microalgae *Monoraphidium* sp . following different phosphorous concentrations for biofuel production. 112:539–548
39. Hawrot-Paw M, Koniuszy A, Galczyńska M, et al (2020) Production of microalgal biomass using aquaculture wastewater as growth medium. *Water (Switzerland)* 12:. <https://doi.org/10.3390/w12010106>
40. Li Y, Horsman M, Wang B, et al (2008) Effects of nitrogen sources on cell growth and lipid accumulation of green alga *Neochloris oleoabundans*. *Appl Microbiol Biotechnol* 81:629–636. <https://doi.org/10.1007/s00253-008-1681-1>
41. Dhup S, Dhawan V (2014) Effect of nitrogen concentration on lipid productivity and fatty acid composition of *Monoraphidium* sp. *Bioresour Technol* 152:572–575
42. Jeanfils J, Canisius MF, Burlion N (1993) Effect of high nitrate concentrations on growth and nitrate uptake by free-living and immobilized *Chlorella vulgaris* cells. *J Appl Phycol* 5:369–374. <https://doi.org/10.1007/BF02186240>
43. Zhu W, Liu J, Li Q, et al (2022) Effects of Nutrient Levels on Microbial Diversity in Sediments of a Eutrophic Shallow Lake. *Front Ecol Evol* 10:1–8. <https://doi.org/10.3389/fevo.2022.909983>
44. Montoya-Vallejo C, Guzmán Duque FL, Quintero Díaz JC (2023) Biomass and lipid production by the native green microalgae *Chlorella sorokiniana* in response to nutrients, light intensity, and carbon dioxide: experimental and modeling approach. *Front Bioeng Biotechnol* 11:1–16. <https://doi.org/10.3389/fbioe.2023.1149762>
45. Sun XM, Ren LJ, Zhao QY, et al (2018) Microalgae for the production of lipid and carotenoids: A review with focus on stress regulation and adaptation. *Biotechnol Biofuels* 11:1–16. <https://doi.org/10.1186/s13068-018-1275-9>
46. Huang CH, Tan CS (2014) A review: CO2 utilization. *Aerosol Air Qual Res* 14:480–499. <https://doi.org/10.4209/aaqr.2013.10.0326>
47. Xin L, Hong-ying H, Ke G, Ying-xue S (2010) Effects of different nitrogen and phosphorus concentrations on the growth, nutrient uptake, and lipid accumulation of a freshwater microalga *Scenedesmus* sp. *Bioresour Technol* 101:5494–5500. <https://doi.org/10.1016/j.biortech.2010.02.016>
48. Yaakob MA, Mohamed RMSR, Al-Gheethi A, et al (2021) Influence of Nitrogen and Phosphorus on Microalgal Growth, Biomass, Lipid, and Fatty Acid Production: An Overview. *Cell* 10,393:10–20. <https://doi.org/10.3390/cells10020393>
49. Chisti Y (2007) Biodiesel from microalgae. *Biotechnol Adv* 25:294–306. <https://doi.org/10.1016/j.biotechadv.2007.02.001>
50. Almomani F (2020) Kinetic modeling of microalgae growth and CO2 bio-fixation using central composite design statistical approach. *Sci Total Environ* 720:137594. <https://doi.org/10.1016/j.scitotenv.2020.137594>

51. Olmstead ILD, Hill DRA, Dias DA, et al (2013) A quantitative analysis of microalgal lipids for optimization of biodiesel and omega-3 production. *Biotechnol Bioeng* 110:2096–2104. <https://doi.org/10.1002/bit.24844>
52. Sahu A, Pancha I, Jain D, et al (2013) Phytochemistry Fatty acids as biomarkers of microalgae. *Phytochemistry* 89:53–58. <https://doi.org/10.1016/j.phytochem.2013.02.001>
53. SINGH SC, SINHA RP, Institut D-PH (2002) Role of Lipids and Fatty Acids in Stress Tolerance in Cyanobacteria. *Acta Protozool* 41:297–308
54. Mathimani T, Uma L, Prabakaran D (2018) Formulation of low-cost seawater medium for high cell density and high lipid content of *Chlorella vulgaris* BDUG 91771 using central composite design in biodiesel perspective. *J Clean Prod* 198:575–586. <https://doi.org/10.1016/j.jclepro.2018.06.303>
55. Sivaramakrishnan R, Suresh S, Incharoensakdi A (2019) *Chlamydomonas* sp. as dynamic biorefinery feedstock for the production of methyl ester and γ -polylysine. *Bioresour Technol* 272:281–287. <https://doi.org/10.1016/j.biortech.2018.10.026>
56. Abou-Shanab RAI, Hwang JH, Cho Y, et al (2011) Characterization of microalgal species isolated from freshwater bodies as a potential source for biodiesel production. *Appl Energy* 88:3300–3306. <https://doi.org/10.1016/j.apenergy.2011.01.060>
57. Piligaev A V., Sorokina KN, Shashkov M. V., Parmon VN (2018) Screening and comparative metabolic profiling of high lipid content microalgae strains for application in wastewater treatment. *Bioresour Technol* 250:538–547. <https://doi.org/10.1016/j.biortech.2017.11.063>
58. Qiu R, Gao S, Lopez PA, Ogden KL (2017) Effects of pH on cell growth, lipid production and CO₂ addition of microalgae *Chlorella sorokiniana*. *Algal Res* 28:192–199. <https://doi.org/10.1016/j.algal.2017.11.004>
59. Shrivastav A, Gupta SK, Ansari FA, et al (2014) Adaptability of growth and nutrient uptake potential of *Chlorella sorokiniana* with variable nutrient loading. *Bioresour Technol* 174:60–66. <https://doi.org/10.1016/j.biortech.2014.09.149>
60. Beuckels A, Smolders E, Muylaert K (2015) Nitrogen availability influences phosphorus removal in microalgae-based wastewater treatment. *Water Res* 77:98–106. <https://doi.org/10.1016/j.watres.2015.03.018>
61. Redfield AC (1958) Redfield_AmSci_1958.pdf. *Am. Sci.*
62. Wágner DS, Cazzaniga C, Steidl M, et al (2021) Optimal influent N-to-P ratio for stable microalgal cultivation in water treatment and nutrient recovery. *Chemosphere* 262:. <https://doi.org/10.1016/j.chemosphere.2020.127939>
63. Aslan S, Kapdan IK (2006) Batch kinetics of nitrogen and phosphorus removal from synthetic wastewater by algae. *Ecol Eng* 28:64–70. <https://doi.org/10.1016/j.ecoleng.2006.04.003>

Figures

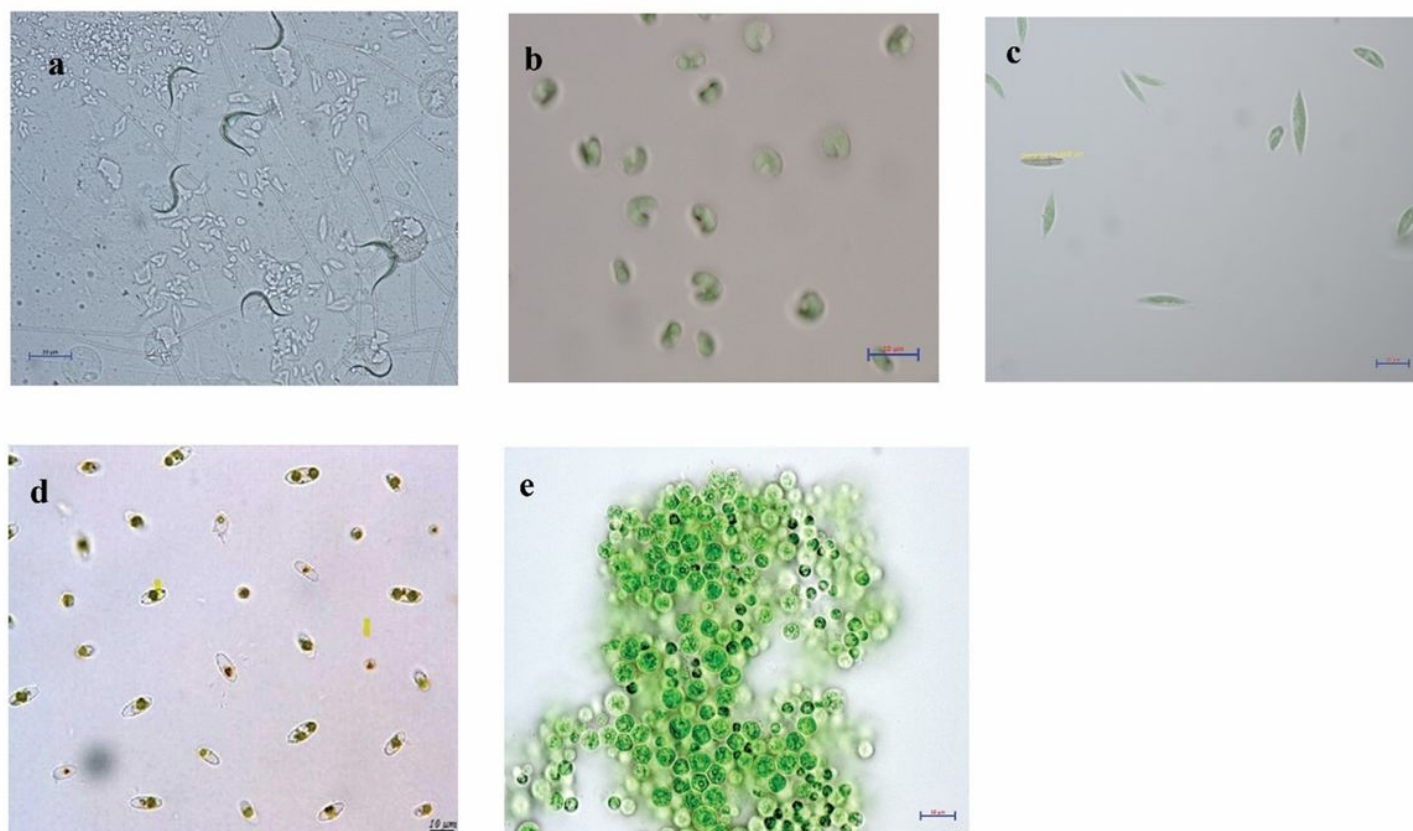


Figure 1

Microphotographs of five green algae. (a) *Monoraphidium contortum* (b) *Kirchneriella obesa* (c) *Chlorolobion braunii* (d) *Coccomyxa simplex* (e) *Halochlorella rubescens*

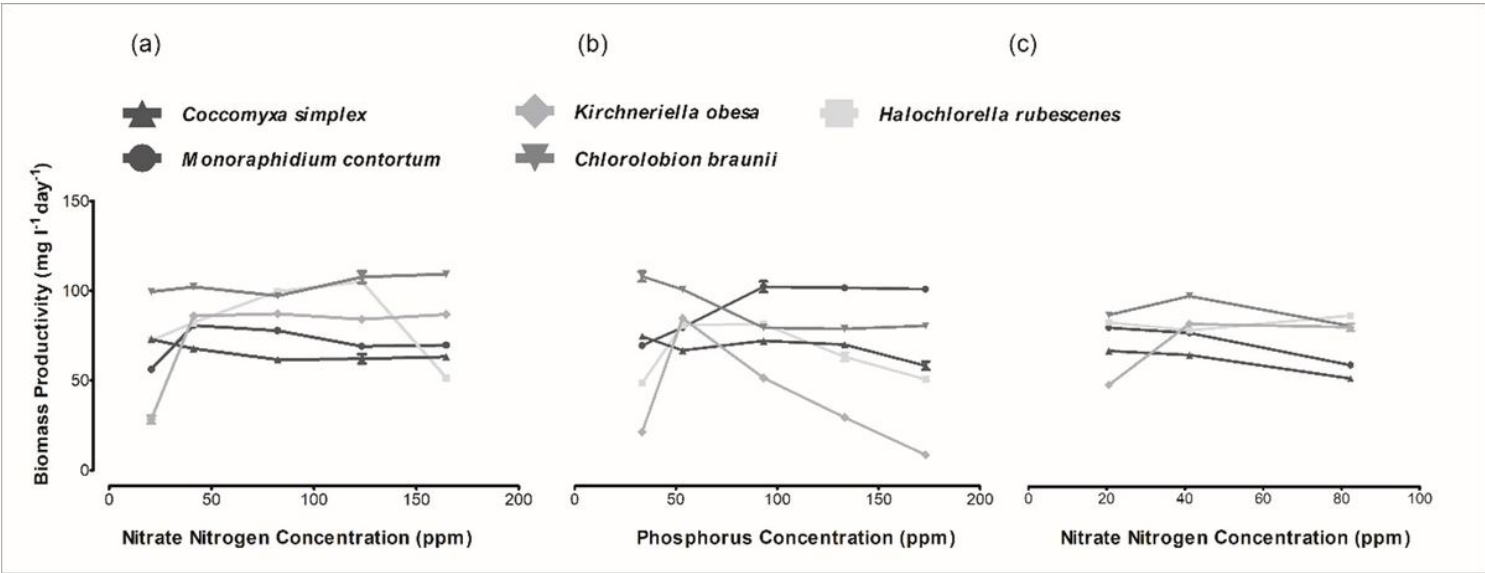


Figure 2

The trend in biomass productivity of five microalgae on the 20th day of the cultivation. The values are mean ± standard deviation (n = 3) (a) Five different N levels. (b) Five different P levels. (c) Three different N and P levels.

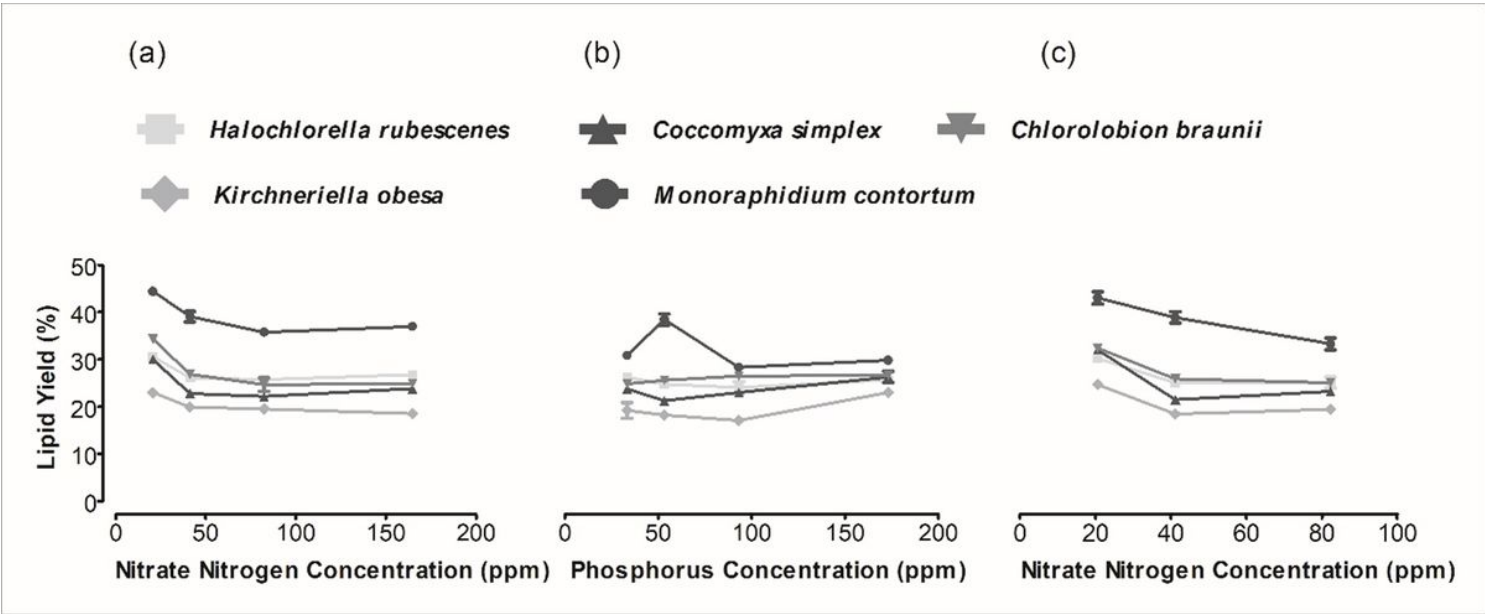


Figure 3

The trend in lipid yield of five microalgae on the 20th day of the cultivation. The values are mean ± standard deviation (n = 3) (a) Five different N levels. (b) Five different P levels. (c) Three different N and P levels.

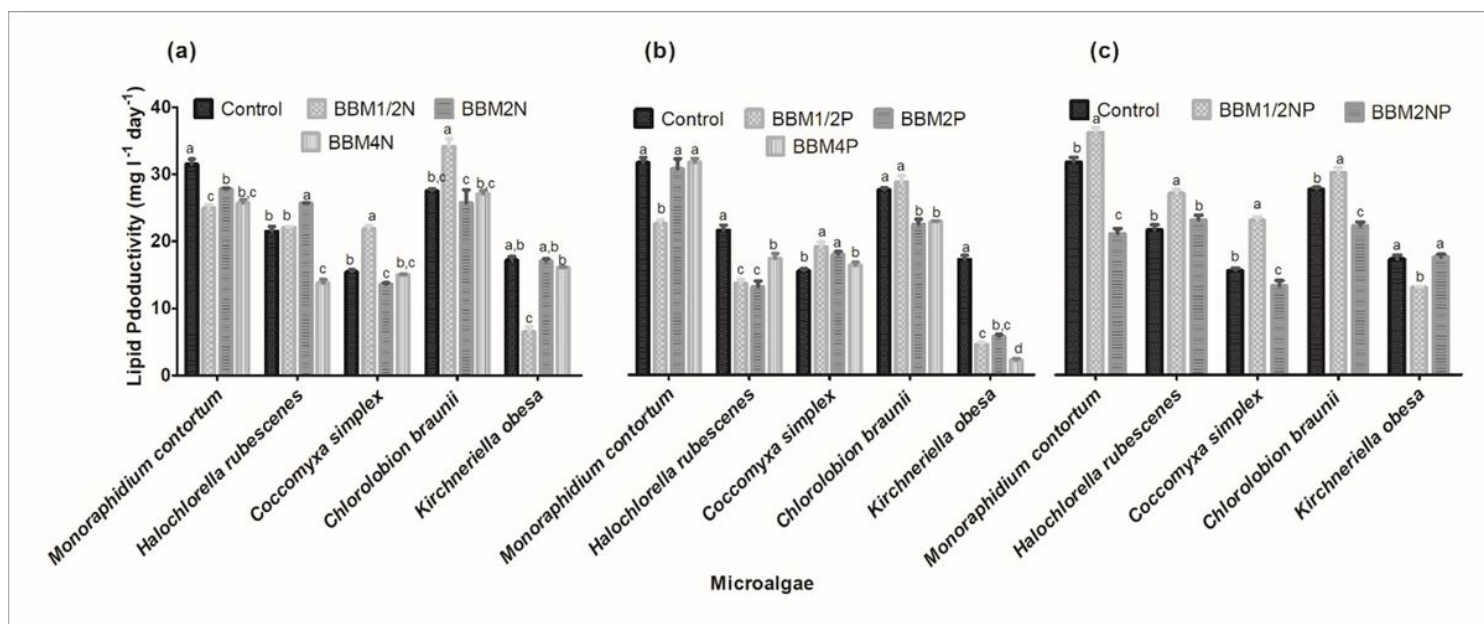


Figure 4

Lipid productivity of the five algae on the 20th day of the cultivation. The values are mean $\pm$ standard deviation (n = 3) (a) Five different N levels. (b) Five different P levels. (c) Three different N and P levels. The different small superscript letters on each bar with the same pattern indicate significant differences between samples at 5% level, $p < 0.05$; data are shown as mean $\pm$ S.D.; n = 3

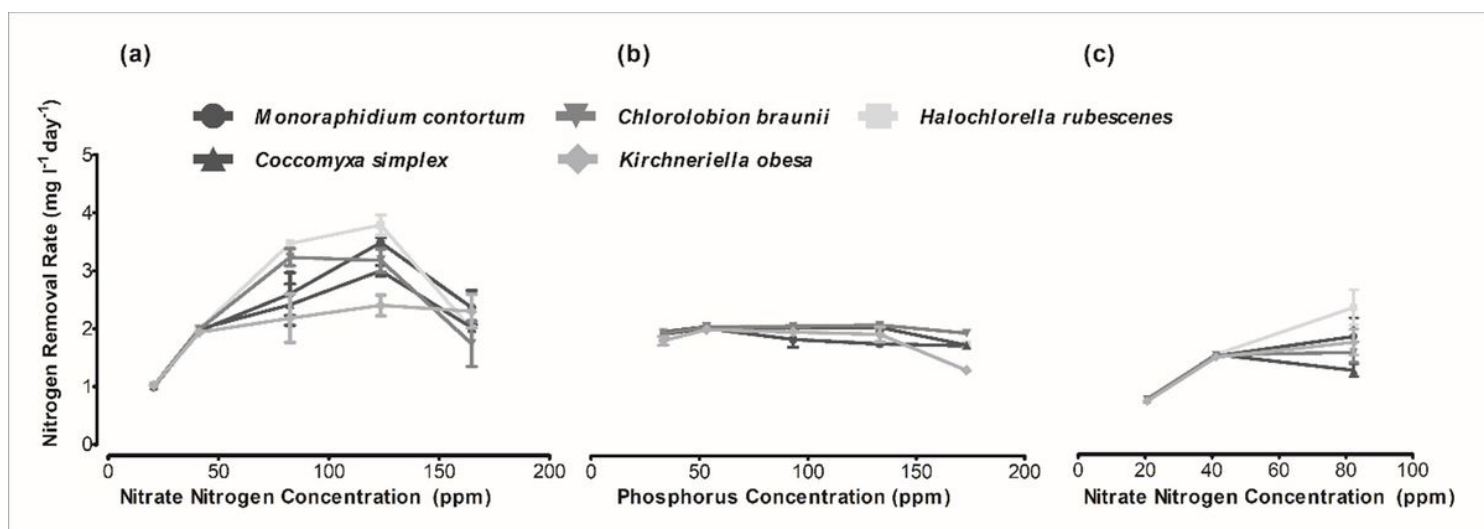


Figure 5

Nitrogen Removal Rate of the five algae on the 20th day of the cultivation. The values are mean $\pm$ standard deviation (n = 3) (a) Five different N levels. (b) Five different P levels. (c) Three different N and P levels.

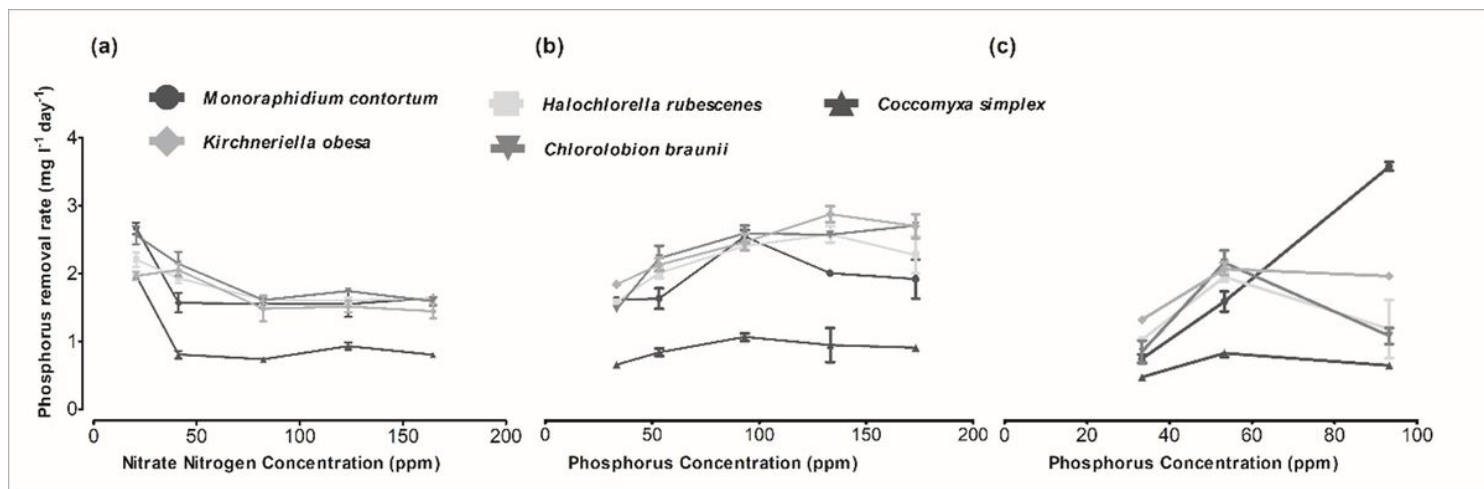


Figure 6

Phosphorus Removal Rate of the five algae on the 20th day of the cultivation. The values are mean $\pm$ standard deviation (n = 3) (a) Five different N levels. (b) Five different P levels. (c) Three different N and P levels.

Supplementary Files

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