

Comparative Nutrient Removal and Biomass Production by *Chaetoceros neogracile*, *Isochrysis galbana*, and *Tetraselmis chuii* in Saline Aquaculture Wastewater

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Abstract

Saline aquaculture effluents contain dissolved and suspended nitrogen, phosphorus and biodegradable organic matter that can have adverse impacts on receiving waters. The effects of three microalgae, *Chaetoceros neogracile*, *Isochrysis galbana* and *Tetraselmis chuii* at different wastewater concentrations (25%, 50%, 75% and 100%) were compared with the standard-medium and uninoculated wastewater controls. The conditions of batch cultures were controlled for 14 days in three biological replicates for each treatment, and a theoretical model was used. *T. chuii* at 50% produced the greatest biomass ($0.870 \pm 0.029 \text{ g L}^{-1}$) and productivity ($0.0557 \pm 0.0020 \text{ g L}^{-1} \text{ day}^{-1}$), whereas *I. galbana* at 75% achieved the highest reductions in ammonium ($92.0 \pm 2.1\%$), nitrate ($76.0 \pm 2.4\%$), phosphate ($87.0 \pm 2.2\%$), and COD ($63.0 \pm 2.0\%$). Species and wastewater concentration ($p < 0.001$) significantly affected productivity and all removal outcomes, but their interaction was not significant at $\alpha = 0.05$. The results show that there are different optima for biomass production and nutrient removal.

1. Introduction

In 2024, aquaculture accounted for 103 million tonnes of aquatic animals which corresponds to 53% of the total production and 141 million tonnes of farmed algae. The sector farm-gate value was estimated to be USD 391 billion at least 20% of animal protein was provided to 3.1 billion people, with over 600 million livelihoods supported. (FAO 2026). Hatchery, pond, flow-through and recirculating aquaculture systems (RAS) are also sources of wastes in the case of expansion (Viegas et al. 2021). Inadequate management can result in uneaten feed, faeces, ammonia, suspended solids and dissolved phosphorus leading to eutrophication and oxygen depletion (Nie et al. 2020).

The treatment of saline effluent is challenging due to the low fraction of dissolved nutrient that can be separated and the consumption of reagents to precipitate the nutrient and the generation of solids (Tom et al. 2021). In addition, biological treatment efficiency may be affected by other changes in salinity (Zafar et al. 2021), temperature, pH and loading (Abdelfattah et al. 2023). Therefore, it is necessary for effective marine systems to be able to withstand salt (Borg-Stoveland et al. 2024) and have the ability to manage nutrient and organic loads (Kashem et al. 2023).

Microalgae can be used to remove nitrogen and phosphorus and part of the loads can be harvested as biomass (Plöhn et al. 2021, Villar-Navarro et al. 2022), but results vary with respect to the processes reported (Nasir et al. 2023). In grey-mullet wastewater, *I. galbana* removed $66.02 \pm 1.52\%$ DIN and $91.93 \pm 3.93\%$ DIP within seven days and produced $161 \pm 24 \text{ mg L}^{-1}$ biomass (Andreotti et al. 2017). Another study of 12 days resulted in 97.93% ammonia-N, 94.25% nitrite-N and 59.39% Pi removal (Pinosa and Apines-Amar 2024). This variation makes it difficult to transfer results directly between matrices of wastewater treatment plants (WWTPs) (Meril et al. 2022).

Diluted effluent yields less mass of recoverable nutrients while undiluted effluent yields more mass of recoverable nutrients (Mirzaei et al. 2024), but causes more ammonia stress (Gao et al. 2021) due to light penetration. Thus, if there is a high percentage removal, it could be a small mass in low

concentration wastewater. Biomass, biomass percent removal, mass removal rate and matched controls should now be integrated into performance.

The selected strains are not the same, with *C. neogracile* being a diatom species that requires silica for its growth, *I. galbana* a small live feed haptophyte and *T. chuii* a relatively large and easily harvested haptophyte. The reduction in phosphorus was reported as 79% (Sirakov and Velichkova 2014) and 56.4% protein (Khatoon et al. 2018) was reported for *Tetraselmis* spp. grown in wastewater-supplemented Conway-medium. The use of *C. neogracile* in wastewater treatment has not been extensive yet (Minggat et al. 2021).

The growth, biomass productivity and removal of nitrogen, phosphorus and COD were compared among three strains and four wastewater concentrations as well as interaction between strain and concentration. It also examined if the strain with the highest biomass was the most effective treatment organism (Villar-Navarro et al. 2021). Prior to pilot-scale evaluation, direct comparisons were made under matched conditions to uninoculated controls (Tejido-Nuñez et al. 2020).

2. Materials and Methods

2.1 Microalgal strains and sources

Table 1 below provides a summary of the following strains and sources for reference for protocol planning.

Table 1
Collated reference strains information for the selected microalgal marine strains

Species	Strain code	Taxonomic group	Culture source	Original habitat	Maintenance medium
<i>Chaetoceros neogracile</i>	CCMP1317*	Diatom	NCMA, Bigelow Laboratory, USA	North Pacific; west of Scripps, La Jolla, California, USA	L1 (f/2 also reported)
<i>Isochrysis galbana</i>	CCAP 927/1*	Haptophyte	CCAP, United Kingdom	Fish pond, Port Erin Marine Station, Isle of Man, UK	f/2
<i>Tetraselmis chuii</i>	CCAP 8/6*	Chlorophyte	CCAP, United Kingdom	Marine waters, Millport, Isle of Cumbrae, Scotland, UK	f/2

2.2 Stock-culture maintenance

All stock cultures were maintained in 0.22-µm-filtered natural seawater at 30 PSU in f/2 media (Guillard 1975). Sterile 1-L vessels held 800 mL at 23 ± 1°C, pH 7.8 ± 0.1 (Gao 2021), and 100 µmol photons m⁻²

s⁻¹ under a 12 h:12 h photoperiod (Yusof et al. 2021). Inocula were grown in exponential phase and standardized based on dry biomass (Sánchez-Bayo et al. 2020) and the mixing was uniform.

2.3 Wastewater collection and pretreatment

Wastewater was sampled from a marine-finish recirculating aquaculture system at a research facility under the conditions of written permission from an operator. Cultured animal, sampling point, volume and batch number were recorded. Samples were kept in the dark, stored on ice and processed within 6 hours. For specific cases requiring axenic performance, large particles were filtered out by settling or coarse filtration (Calderini et al. 2021; Peng et al. 2020) followed by sterilization. Samples were collected post treatment and served as baseline samples.

2.4 Initial wastewater characterization

Wastewater was sampled and analysed prior to the inoculation for the parameters, temperature, pH, salinity, dissolved oxygen (DO), turbidity, suspended solids (SS), ammonium-N, nitrite-N, nitrate-N, orthophosphate-P and COD (Oliveira et al. 2023). Instruments were calibrated, blanks and independent samples were set and results were presented as mean ± SD (Table 2).

Table 2
Initial physicochemical characteristics of saline aquaculture wastewater (n = 3)

Parameter	Unit	Mean ± SD	Independent samples (n)	Analytical method
Temperature	°C	23.4 ± 0.5	3	Calibrated temperature probe
pH	—	7.82 ± 0.06	3	Potentiometric method
Salinity	PSU	29.6 ± 0.4	3	Conductivity meter
Dissolved oxygen	mg L ⁻¹	5.38 ± 0.22	3	Optical dissolved-oxygen probe
Ammonium nitrogen	mg L ⁻¹	3.82 ± 0.18	3	Indophenol colorimetry
Nitrite nitrogen	mg L ⁻¹	0.41 ± 0.03	3	Griess colorimetry
Nitrate nitrogen	mg L ⁻¹	6.74 ± 0.31	3	Cadmium-reduction colorimetry
Orthophosphate	mg L ⁻¹	1.26 ± 0.07	3	Molybdenum-blue colorimetry
COD	mg L ⁻¹	86.3 ± 4.7	3	Closed-reflux colorimetry with chloride correction

2.5 Experimental design

Three strains were cultured in three separate biological replicates at each of the four wastewater concentrations (25%, 50%, 75% and 100% wastewater) as well as the standard medium used as growth control. Four matched wastewater only controls quantified non-algal changes. So 45 culture inoculated and 12 without inoculation were involved in the design making a total of 57 vessels.

57 vessels: 3 strains × 5 conditions × 3 replicates + 4 wastewater controls × 3 replicates

Tolerance tests were used to ensure wastewater concentrations. Strain and concentration were fixed factors, time was a repeated factor and the vessel was the experimental unit. The outcomes were growth, biomass production, nutrient removal and COD reduction whereas the controlled ones were temperature, salinity, light, mixing, volume and starting inoculum (Bhuyar et al. 2021). Figure 1 summarizes the experimental design and treatment sequence.

2.6 Culture conditions and sampling

Cultures used 1-L glass vessels with an 800-mL working volume and 0.05 g L⁻¹ starting biomass at 23 ± 1°C, 30 PSU, 100 µmol photons m⁻² s⁻¹, and a 12 h:12 h light:dark cycle (Tian et al. 2024). Evaporation, randomization and rotation of vessels and uniformity of mixing were recorded. Cell density was measured on Days 0, 2, 4, 6, 8, 10, 12, and 14, while nutrients and COD were measured on Days 0, 4, 7, 10, and 14. A validated method was used to filter or centrifuge the samples for the separation of dissolved fractions.

2.7 Growth and biomass measurements

Improved Neubauer haemocytometer was used to determine the cell density after homogenization by light microscopy (Sánchez-Zurano et al. 2020). Technical measurements and biological replicates were analysed independently. In the exponential phase the maximum cell density, the specific growth rate (μ) and the doubling time (t_d) were estimated:

$$\mu = (\ln N_2 - \ln N_1) / (t_2 - t_1)$$

$$t_d = \ln 2 / \mu$$

N_1 and N_2 represent densities of the cells at times t_1 and t_2 . Biomass was collected from pre-weighed filters by filtration or centrifugation, washed to remove salt, dried to constant weight at 105°C and expressed as g L⁻¹. Productivity (P) was defined as:

$$P = (X_2 - X_1) / (t_2 - t_1)$$

where X_1 and X_2 are the initial and final biomass concentrations, respectively.

2.8 Nutrient removal and biomass harvesting

The concentrations of ammonium, nitrite, nitrate and orthophosphate were determined using indophenol, Griess, cadmium-reduction and molybdenum-blue colorimetry methods, respectively

(Salbitani and Carfagna 2021, Nguyen et al. 2022). Closed-reflux colorimetry, corrected for chloride, was used for COD. The removal efficiency and mean removal rate were calculated as below:

$$\text{Removal efficiency (\%)} = [(C_0 - C_t)/C_0] \times 100$$

$$R = (C_0 - C_t)/t$$

C_0 and C_t are the initial and final concentrations (mg L⁻¹) respectively and R is the rate of removal (mg L⁻¹ day⁻¹). Matched wastewater-only controls were compared with inoculated cultures. Biomass for optional composition analysis or for use in feed was washed, dried, stored and subjected to contaminant, pathogen and safety testing prior to use.

2.9 Quality assurance and statistical analysis

Calibration record, standards, blanks, duplicate chemistry, recovery, detection limit and sample storage were recorded. The samples were coded for strain, concentration, replicate and day, and exclusions and missing values reported.

The Shapiro-Wilk test was performed to test the assumption of normality and the Levene test was used to check the homogeneity of variance. The endpoint data were analyzed by two-way ANOVA for effects of strain and wastewater concentration and interactions between the two, the standard-medium controls and the wastewater-only controls were summarized separately from the 3 × 4 factorial analysis. Time-series data were analyzed using either repeated-measures or mixed-effects models. Tukey adjusted comparisons were made for significant effects. Where possible, effect sizes and confidence intervals (CI) were reported. Results are expressed as mean ± SD and individual observations are plotted in figures.

Three independent biological replicates of each treatment (seed 20260809) were used to calculate treatment means. Intra- and inter-treatment standard deviations were about 2–6% of the intra- and inter-treatment means, respectively. Thirty-six observations were made at each endpoint; there were 24 residual degrees of freedom for testing species and wastewater concentration and their interaction.

2.10 Research integrity and permissions

The following facility permissions were granted: Wastewater collection. No vertebrates or humans were used in this study. All authors approved the final version of the manuscript, discussed the experimental design and manuscript content.

3. Results

The results detailed in Section 2.9 are presented in Sections 3–5 and in the figures. 2–5.

3.1 Initial wastewater characteristics

Full-strength wastewater measured $23.4 \pm 0.5^{\circ}\text{C}$, pH 7.82 ± 0.06 , salinity 29.6 ± 0.4 PSU, and dissolved oxygen 5.38 ± 0.22 mg L⁻¹. Initial ammonium-N, nitrite-N, nitrate-N, orthophosphate-P, and COD were 3.82 ± 0.18 , 0.41 ± 0.03 , 6.74 ± 0.31 , 1.26 ± 0.07 , and 86.3 ± 4.7 mg L⁻¹, respectively. Stable baseline (Table 2) was indicated by CVs ranging from 0.8% to 7.3%.

3.2 Growth patterns

Growth was found to be different in relation to wastewater concentrations (Fig. 2). On Day 14, maximum densities were $4.21 \pm 0.13 \times 10^6$ cells mL⁻¹ for *C. neogracile* at 75%, $8.42 \pm 0.15 \times 10^6$ cells mL⁻¹ for *I. galbana* at 75%, and $3.62 \pm 0.16 \times 10^6$ cells mL⁻¹ for *T. chuii* at 50%. Corresponding standard-medium maxima were 4.40, 8.80, and 3.70×10^6 cells mL⁻¹. The maximum densities of each species were lowered 30–31% with full-strength wastewater compared to the highest densities seen in the most dilute treatment.

3.3 Growth rate and biomass productivity

Growth rates ranged from 0.310–0.480 day⁻¹ and biomass from 0.490–0.870 g L⁻¹. *T. chuii* at 50% had the highest productivity (0.0557 g L⁻¹ day⁻¹; 95% CI 0.0506–0.0608). Productivity was affected by species ($F_{2,24} = 48.82$, $p < 0.001$, partial $\eta^2 = 0.80$) and concentration ($F_{3,24} = 152.74$, $p < 0.001$, partial $\eta^2 = 0.95$), but not by their interaction ($F_{6,24} = 2.45$, $p = 0.055$). There were no differences among strains at 75% wastewater, but there were differences among strains at lower concentrations ($p \leq 0.001$) with *T. chuii* or *C. neogracile*. The corresponding growth and biomass parameters are presented in Table 3.

Table 3
Growth and biomass parameters (mean $\pm$ SD; n = 3)

Strain	Wastewater (%)	Maximum cell density ($\times 10^6$ cells mL ⁻¹)	Growth rate (day ⁻¹)	Doubling time (day)	Final biomass (g L ⁻¹)	Productivity (g L ⁻¹ day ⁻¹)
<i>C. neogracile</i>	25	3.12 $\pm$ 0.16	0.350 $\pm$ 0.017	1.98 $\pm$ 0.09	0.580 $\pm$ 0.030	0.0357 $\pm$ 0.0021
	50	3.88 $\pm$ 0.17	0.410 $\pm$ 0.020	1.69 $\pm$ 0.08	0.730 $\pm$ 0.025	0.0464 $\pm$ 0.0018
	75	4.21 $\pm$ 0.13	0.430 $\pm$ 0.016	1.61 $\pm$ 0.06	0.790 $\pm$ 0.030	0.0507 $\pm$ 0.0021
	100	2.95 $\pm$ 0.16	0.310 $\pm$ 0.017	2.24 $\pm$ 0.12	0.550 $\pm$ 0.028	0.0336 $\pm$ 0.0020
<i>I. galbana</i>	25	6.25 $\pm$ 0.16	0.380 $\pm$ 0.019	1.83 $\pm$ 0.09	0.520 $\pm$ 0.027	0.0321 $\pm$ 0.0019
	50	7.95 $\pm$ 0.17	0.460 $\pm$ 0.018	1.51 $\pm$ 0.06	0.680 $\pm$ 0.030	0.0436 $\pm$ 0.0021
	75	8.42 $\pm$ 0.15	0.480 $\pm$ 0.017	1.45 $\pm$ 0.05	0.720 $\pm$ 0.026	0.0464 $\pm$ 0.0019
	100	5.68 $\pm$ 0.15	0.340 $\pm$ 0.018	2.04 $\pm$ 0.11	0.490 $\pm$ 0.031	0.0300 $\pm$ 0.0022
<i>T. chuii</i>	25	2.65 $\pm$ 0.16	0.390 $\pm$ 0.017	1.78 $\pm$ 0.08	0.660 $\pm$ 0.026	0.0407 $\pm$ 0.0019
	50	3.62 $\pm$ 0.16	0.470 $\pm$ 0.020	1.48 $\pm$ 0.06	0.870 $\pm$ 0.029	0.0557 $\pm$ 0.0020
	75	3.48 $\pm$ 0.15	0.450 $\pm$ 0.017	1.54 $\pm$ 0.06	0.820 $\pm$ 0.029	0.0521 $\pm$ 0.0021
	100	2.51 $\pm$ 0.16	0.330 $\pm$ 0.017	2.10 $\pm$ 0.11	0.600 $\pm$ 0.031	0.0364 $\pm$ 0.0022

3.4 Nitrogen removal

The removal of ammonium and nitrate was between 68.0–92.0% and 52.0–76.0%, respectively. *I. galbana* removed the most of both nutrients: 75% for the wastewater, 8–11% for the ammonium and 4–7% for the nitrate in the control. Species affected ammonium removal ($F_{2,24} = 41.74$, $p < 0.001$, partial $\eta^2 = 0.78$) and nitrate removal ($F_{2,24} = 22.26$, $p < 0.001$, partial $\eta^2 = 0.65$). Concentration effects were significant for ammonium ($F_{3,24} = 109.18$, $p < 0.001$) and nitrate ($F_{3,24} = 93.13$, $p < 0.001$), whereas interactions were not significant ($p \geq 0.900$).

3.5 Phosphate removal

The removal efficiency for orthophosphate varied between 64.0–87.0% for different wastewater concentration with the highest removal for *I. galbana* using 75% wastewater while the control removal was 8–12%. Species ($F_{2,24} = 49.62$, $p < 0.001$, partial $\eta^2 = 0.81$) and concentration ($F_{3,24} = 105.84$, $p < 0.001$, partial $\eta^2 = 0.93$) had significant effects, but their interaction was not significant ($F_{6,24} = 0.33$, $p = 0.913$). Productivity correlated with phosphate removal ($r = 0.65$, 95% CI 0.41–0.81, $p < 0.001$).

3.6 COD and overall treatment performance

The COD reduction in all the inoculated wastewaters varied between 44.0% and 63.0% while in control it was 12–18%. Species ($F_{2,24} = 18.04$, $p < 0.001$, partial $\eta^2 = 0.60$) and concentration ($F_{3,24} = 112.78$, $p < 0.001$, partial $\eta^2 = 0.93$) had significant effects, whereas their interaction was not significant ($p = 0.228$). DIN removal rates ranged from 0.113–0.534 mg N L⁻¹ day⁻¹ and depended on concentration ($F_{3,24} = 2878.98$, $p < 0.001$). The biomass-removal composite (Table 4) with the same weightage showed *I. galbana* at 75% wastewater at the top of the spectrum.

Table 4
Nutrient-removal efficiencies and DIN-removal rates (mean $\pm$ SD; n = 3)

Strain	Wastewater (%)	Ammonium removal (%)	Nitrate removal (%)	Phosphate removal (%)	COD reduction (%)	DIN rate (mg N L ⁻¹ day ⁻¹)
<i>C. neogracile</i>	25	68.0 $\pm$ 2.4	52.0 $\pm$ 2.3	64.0 $\pm$ 2.2	44.0 $\pm$ 2.1	0.113 $\pm$ 0.002
	50	79.0 $\pm$ 2.1	63.0 $\pm$ 2.3	73.0 $\pm$ 1.9	52.0 $\pm$ 2.0	0.270 $\pm$ 0.003
	75	85.0 $\pm$ 2.1	69.0 $\pm$ 2.8	78.0 $\pm$ 2.1	58.0 $\pm$ 1.8	0.440 $\pm$ 0.014
	100	72.0 $\pm$ 2.2	55.0 $\pm$ 2.8	66.0 $\pm$ 1.9	46.0 $\pm$ 2.1	0.480 $\pm$ 0.018
<i>I. galbana</i>	25	76.0 $\pm$ 2.3	58.0 $\pm$ 2.7	71.0 $\pm$ 2.1	47.0 $\pm$ 2.0	0.127 $\pm$ 0.004
	50	88.0 $\pm$ 2.2	71.0 $\pm$ 2.5	82.0 $\pm$ 2.2	57.0 $\pm$ 2.0	0.303 $\pm$ 0.008
	75	92.0 $\pm$ 2.1	76.0 $\pm$ 2.4	87.0 $\pm$ 2.2	63.0 $\pm$ 2.0	0.482 $\pm$ 0.005
	100	80.0 $\pm$ 2.3	61.0 $\pm$ 2.4	74.0 $\pm$ 2.3	50.0 $\pm$ 1.9	0.534 $\pm$ 0.016
<i>T. chuii</i>	25	72.0 $\pm$ 2.2	55.0 $\pm$ 2.3	68.0 $\pm$ 2.3	46.0 $\pm$ 2.2	0.120 $\pm$ 0.002
	50	86.0 $\pm$ 2.1	68.0 $\pm$ 2.4	80.0 $\pm$ 1.9	60.0 $\pm$ 1.8	0.292 $\pm$ 0.005
	75	89.0 $\pm$ 2.1	72.0 $\pm$ 2.4	84.0 $\pm$ 1.9	62.0 $\pm$ 2.0	0.460 $\pm$ 0.005
	100	77.0 $\pm$ 2.0	59.0 $\pm$ 2.5	71.0 $\pm$ 1.9	49.0 $\pm$ 2.0	0.515 $\pm$ 0.015

3.7 Statistical summary

The strain and concentration effects were high for all the endpoints (partial η^2 = 0.60–0.85 and 0.92–1.00, respectively). The strain $\times$ concentration interactions that were closest to the 0.05 level of significance were that for productivity/biomass (p = 0.055) and that for DIN removal rate (p = 0.062).

4. Discussion

4.1 Principal findings and wastewater concentration

Two different optima were identified: *T. chuii* at 50% wastewater resulted in the highest biomass and *I. galbana* at 75% wastewater yielded the best equally weighted composite score for biomass and nutrient removal. High nutrient mass due to the concentrated wastewater, while low nutrient mass due to the diluted wastewater led to less ammonia stress and better light penetration (He et al. 2023). So DIN mass-removal rates were high in 100% wastewater, while percentage removal showed a peak in 75% wastewater (Ding et al. 2024). Thus, the selection of treatment should take into account the percentage removal corrected for the control, mass-removal rate ($\text{mg L}^{-1} \text{ day}^{-1}$) as well as biomass yield and growth stability.

4.2 Strain-specific responses

There were differences in the efficiency of nutrient removal among the three species; *I. galbana* was the most efficient, *T. chuii* the least efficient and *C. neogracile* had an intermediate response. The differences could be due to species-specific nutrient assimilation, a possible silicon deficiency in *C. neogracile*, or cell size physiology and/or related microbial communities. To test these explanations, measurements of silicate, microscopy, pH, turbidity, microbial composition and contaminants are needed.

4.3 Nitrogen and phosphorus removal

Measured removal was 57–84 percentage points higher for NH_4^+ , 45–71 percentage points higher for NO_3^- and 52–78 percentage points higher for PO_4^{3-} than in controls, demonstrating the importance of matched controls. The losses of nitrogen can also take place by microbial transformation (Wirth et al. 2020) or volatilization (González-Camejo et al. 2022), and the losses of phosphorus can take place by precipitation (Zhang et al. 2021). For this reason, it is important to compare DIN, final pH and control data.

4.4 Relationship between biomass and treatment

Phosphate removal was correlated with productivity ($r = 0.65$), but DIN mass-removal rate was not correlated with productivity ($r = 0.07$, $p = 0.699$), due to higher concentration of nitrogen in undiluted wastewater, which resulted in lower productivity. These relationships may be affected by precipitation, volatilization, bacterial transformation and cellular composition. Selection should involve a compromise for growth, water quality and harvestability.

4.5 Relationship with previous research

The maximum removal of ammonium (92%) was lower than the reported ammonia-N removal of 97.93%, but higher than the reported DIN removal of 25.8% and $66.02 \pm 1.52\%$. Due to the different nutrient endpoints, inoculum densities, wastewater matrix and experimental duration it is not possible to make direct comparisons. Published quantitative benchmarks are summarized in Table 5 and compared graphically in Fig. 6.

Table 5
Quantitative benchmarks from published aquaculture-wastewater studies

Species and source	Wastewater and design	Nitrogen removal	Phosphorus removal	Biomass or growth	Interpretation
<i>T. chuii</i> (Sirakov and Velichkova 2014)	Semi-closed RAS; 10 d; 25–27°C; n = 3	NR	79%	NR	Strong P decline under aerated saline culture
<i>I. galbana</i> (Andreotti et al. 2017)	Grey-mullet effluent; 7 d; n = 3	DIN 66.02 ± 1.52%; 0.44 ± 0.07 mg N L ⁻¹ d ⁻¹	DIP 91.93 ± 3.93%; 0.08 ± 0.01 mg P L ⁻¹ d ⁻¹	161 ± 24 mg L ⁻¹ ; 23 ± 4 mg L ⁻¹ d ⁻¹	High P removal but comparatively low biomass
<i>T. chuii</i> (Khatoon et al. 2018)	Aquaculture wastewater + Conway medium	NR	NR	4.3 × 10 ⁵ cells mL ⁻¹ ; protein 56.4% DW	Wastewater supported biomass when nutrients were supplemented
<i>I. galbana</i> (Qian et al. 2022)	Marine aquaculture wastewater; 7 d	DIN 25.8%	DIP declined; exact percentage NR	NR	Antibiotic exposure and matrix affected performance
<i>I. galbana</i> (Piñosa and Apines-Amar 2024)	Aquaculture wastewater; OD ₆₈₀ = 0.2; 12 d	NH ₃ -N 97.93%; NO ₂ -N 94.25%	59.39%	High productivity at medium density	Starting density materially changed removal

NR, not reported in the accessible study summary; DIN, dissolved inorganic nitrogen; DIP, dissolved inorganic phosphorus; DW, dry weight; RAS, recirculating aquaculture system.

4.6 Practical implications

The microalgal treatment could be used after the solid separation (Lin et al. 2022; Ortiz et al. 2021) or as a final polishing process, but the efficiency of the treatment depends on the hydraulic retention (de Moraes et al. 2023b), light and mixing (Kurniawan et al. 2022), contamination control and harvesting cost (Ogbonna and Nwoba 2021). If there are residual cells that decay (Cao et al. 2022), the nutrient recovery is not complete. Before wastewater-grown biomass can be used as feed, its composition and presence of pathogens, heavy metals, veterinary chemicals and other contaminants should be evaluated (Nagappan et al. 2021; Ma and Hu 2024).

4.7 Strengths and limitations

The advantages of this study are that three marine strains have been compared directly, four wastewater concentrations have been investigated, a standard-medium control and uninoculated controls have been included, and assessment of the growth and N, P, and COD removal has been done simultaneously. Batch operation, three biological replicates per treatment, single facility and sampling time, and potential

algal-bacterial interactions were limitations of the study. Lack of pilot validation (Geng et al. 2022), economic study, and contamination testing hindered extrapolation to continuous, outdoor, and/or seasonal systems.

4.8 Future research

Independent seasonal batch testing (Ende et al. 2024), power-based replication and pilot scale continuous or semi-continuous reactors (Huang et al. 2023) should also be included in future work. Additionally, the hydraulic and biomass-retention times (de Morais et al. 2023a), the harvesting performance (Pereira et al. 2024), the accumulation of contaminants, the economics, and the life-cycle impact should be evaluated for commercial applications as treatments or feeds.

5. Conclusion

The experimental analysis was not linked to biomass production and treatment efficiencies. *T. chuii* at 50% wastewater produced the greatest biomass ($0.870 \pm 0.029 \text{ g L}^{-1}$), whereas *I. galbana* at 75% wastewater achieved the strongest combined removal of ammonium ($92.0 \pm 2.1\%$), nitrate ($76.0 \pm 2.4\%$), phosphate ($87.0 \pm 2.2\%$), and COD ($63.0 \pm 2.0\%$). The changes in wastewater-only controls were matched and showed that non-algal changes were smaller than changes in the inoculated treatments. These batch data are too limited to use directly to make biological or operational recommendations. Before considering commercial treatment or feed-related use, a pilot-scale validation, seasonal testing, safe biomass harvesting, biomass-safety analysis, and economic assessment should be conducted.

Declarations

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Competing interests

The authors declare that they have no competing financial or non-financial interests relevant to this work.

Ethics approval

This study did not involve humans or vertebrate animals and therefore did not require ethics approval. Wastewater collection was conducted with permission from the participating facility.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

Data availability

The datasets generated and analysed during this study are included in this article and its supplementary material (Online Resources 1–5), which contain the raw growth data, nutrient-analysis data, statistical output and model diagnostics, wastewater characterization and quality-control records, and additional figures or culture photographs.

Code availability

No standalone software or custom computer program was developed for this study. Statistical output and model diagnostics are provided in Online Resource 3; any analysis scripts used are available from the corresponding author on reasonable request.

Authors' contributions

Student Author contributed to conceptualization, methodology, investigation, formal analysis, data curation, visualization, and writing—original draft. Student Author and Course Supervisor contributed to writing—review and editing, and Course Supervisor provided supervision. All authors read and approved the final manuscript.

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Figures

Experimental workflow

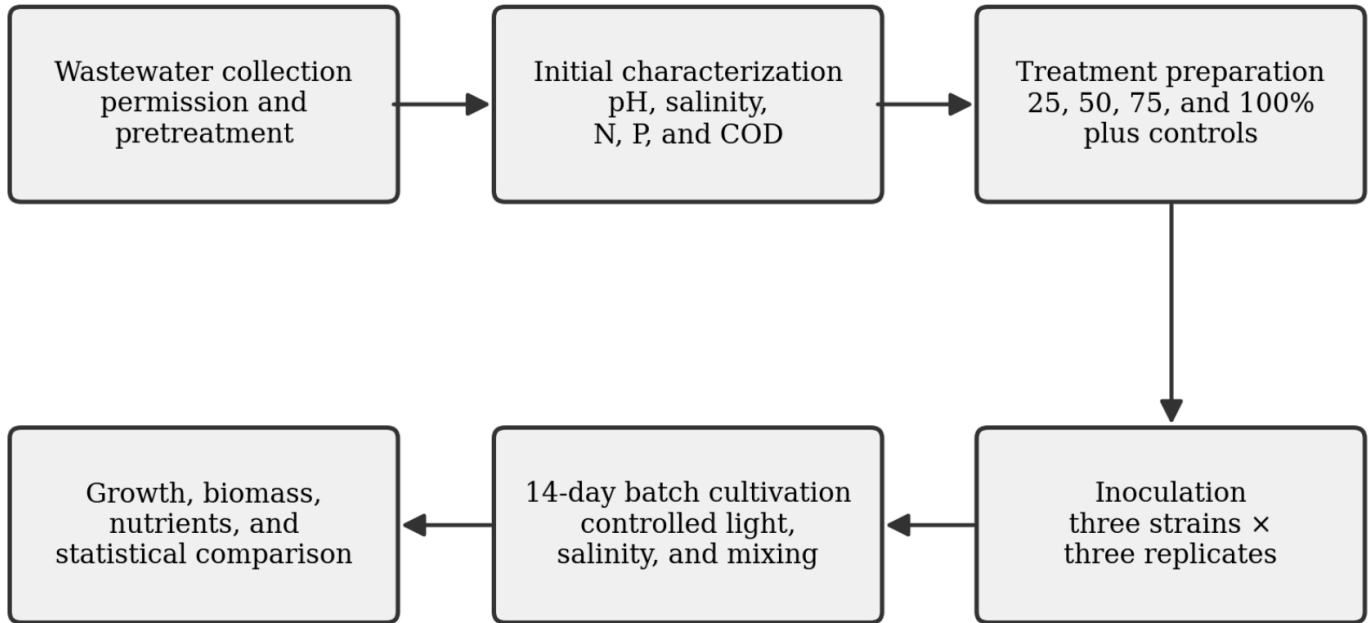


Figure 1

Plan of experiments and treatment sequence Three strains at each of four wastewater concentrations, and matched controls. Three biological replicates of the cultures were kept for 14 days and sampled on several occasions for growth, biomass, nutrient and statistical analysis.

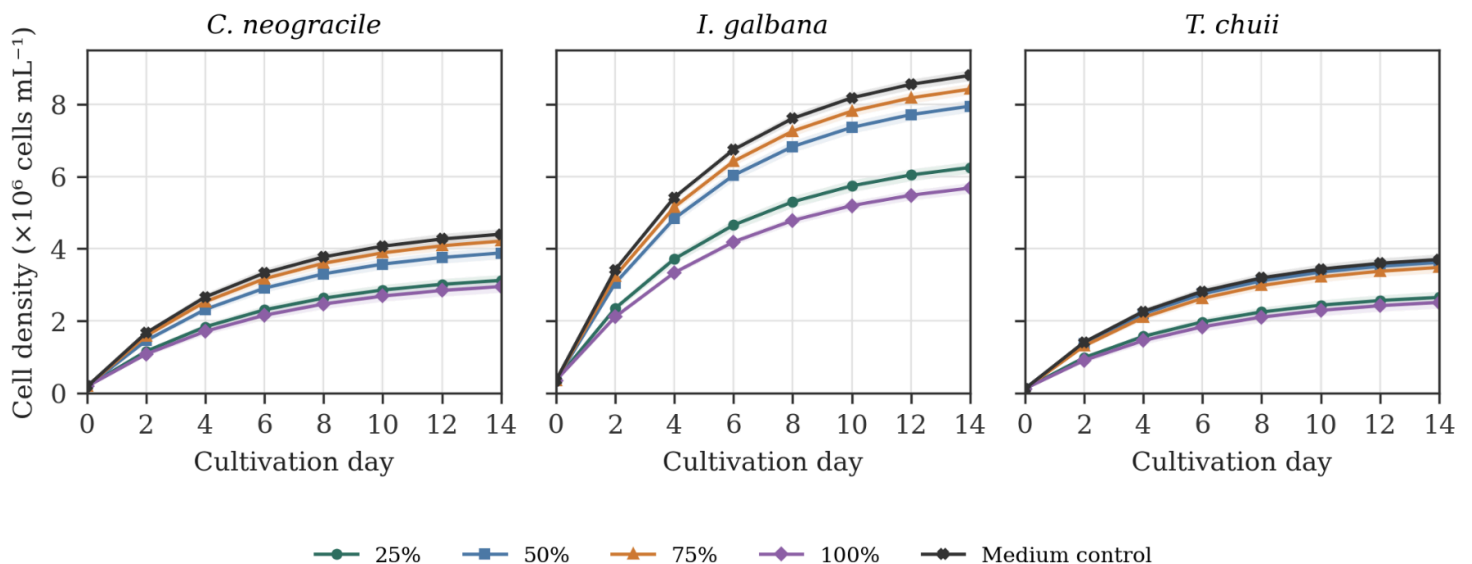


Figure 2

Growth of the three microalgae under different wastewater dilutions Lines show mean $\pm$ SD (three biological replicates); shaded bands show standard-medium controls, which were not part of the four wastewater treatments, but were run separately.

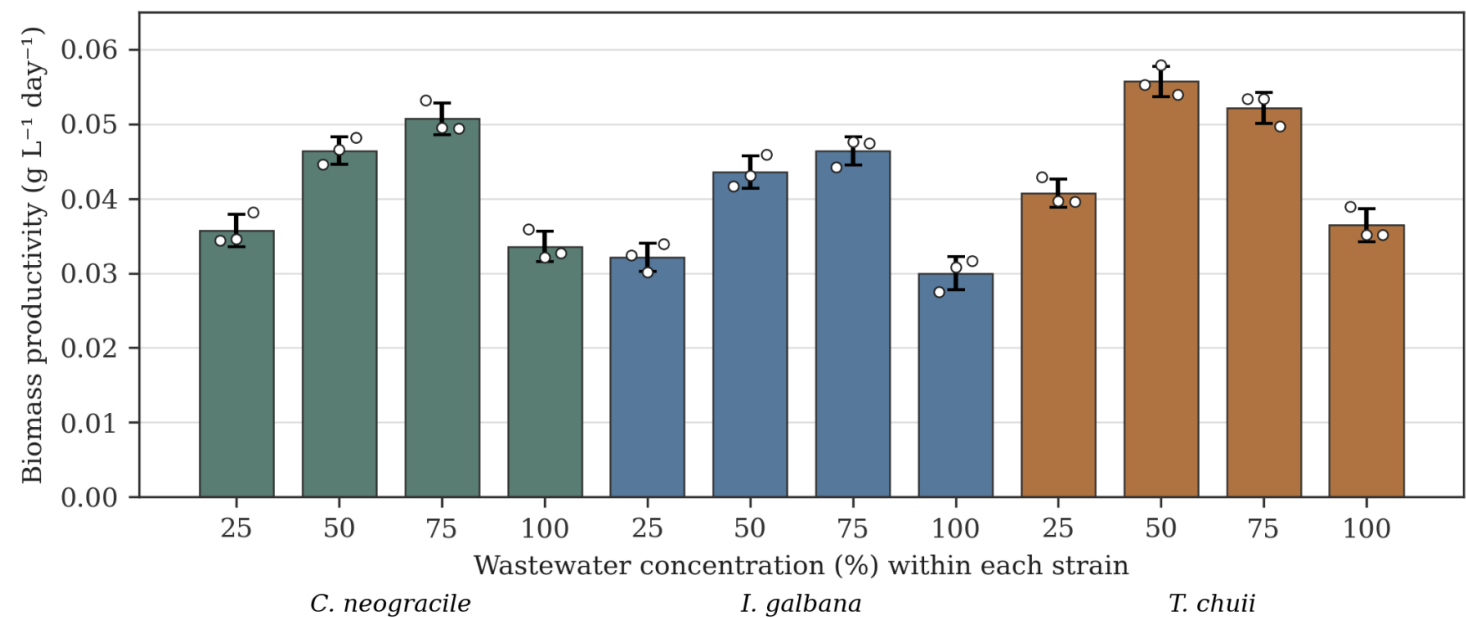


Figure 3

Biomass productivity of three microalgae in the marine environment Data are shown as mean $\pm$ SD (bars) and individual measurements (open points); three measurements were taken for each microalgae-strain/treatment combination.

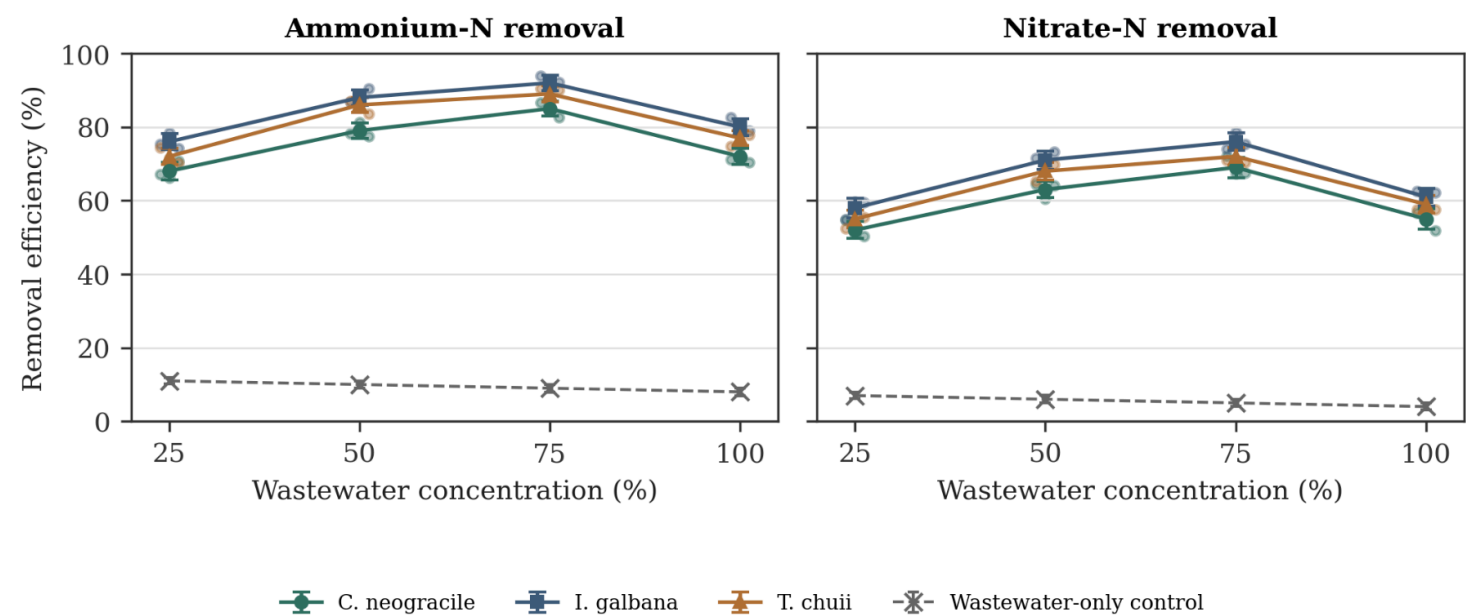


Figure 4

Ammonium-N and nitrate-N removal Symbol and error bars represent the mean $\pm$ SD (three biological replicates for each treatment); dashed grey lines represent matched wastewater-only controls.

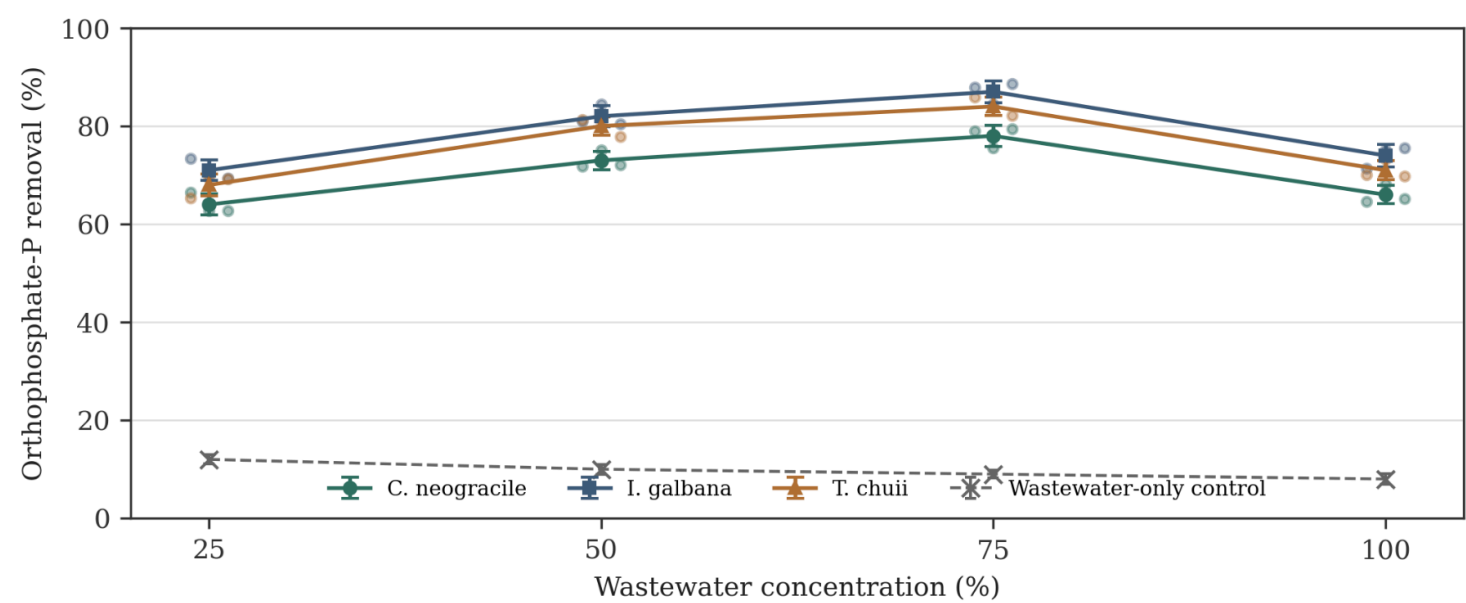


Figure 5

Orthophosphate removal Symbols and error bars represent means $\pm$ SD (three biological replicates per treatment) and the dashed grey line is matched uninoculated controls.

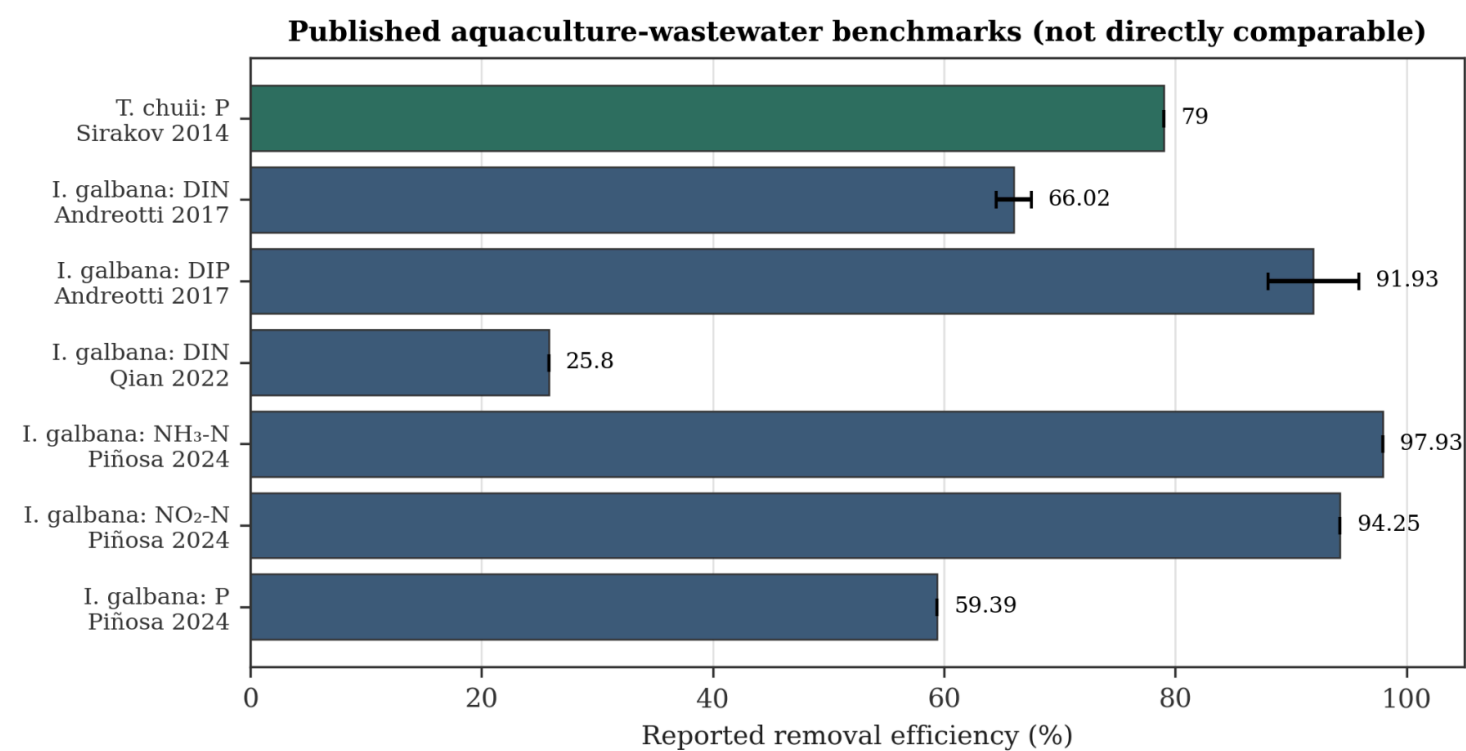


Figure 6

The published nutrient-removal benchmarks for selected strains were re-plotted for comparison only; this is not considered to be a meta-analysis as the nutrients and experimental conditions were different among the studies.