PhotoBioValue project: Linking N:P Ratios and Nitrogen Sources to Microalgae-Based Wastewater Bioremediation and Biomass Valorisation

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Abstract: Microalgae-based systems offer potential for wastewater bioremediation by extracting nutrients while producing biomass for various uses. However, variations in wastewater properties, particularly the source of nitrogen and phosphorus, their concentration, and the N:P ratio, impact treatment efficiency. This study aimed to evaluate the combined effects of different N:P ratios and nitrogen sources on biomass composition, wastewater treatment, and *Chlorella vulgaris* growth. Three sources of nitrogen (NO_3^-, NH_4^+, and both) have been studied alongside four N:P ratios (9, 14, 20, and 27). All synthetic effluents were suitable for *C. vulgaris* growth, and the N:P ratio of 9 with NH_4^+ as the only nitrogen source had the greatest specific growth rate. Nitrogen was entirely removed at a N:P ratio of 9, with NH_4^+ being the preferred source. When both sources were available in the media, NH_4^+ was consumed first. The removal of phosphorus reached 99 %. Nitrogen constraints reduced the biomass's protein concentration while increasing its lipid and carbohydrate contents. Combining NO_3^- and NH_4^+ boosted protein content compared to the use of NO_3^- and NH_4^+ alone. These results show that the microalga can effectively treat diverse wastewaters with different compositions, reaching optimum growth and nutrient removals at a N:P ratio of 9.

Keywords: Biomass composition; *Chlorella*; Nitrogen to phosphorus ratio; Nutrient removal; Wastewater treatment.

1. Introduction

The discharge of untreated wastewater poses a major environmental threat, with nitrogen and phosphorus being major contaminants that negatively impact water quality (Tang et al., 2023). To address this, wastewater treatment plants must efficiently remove these nutrients, as regulated by EU legislation (Directive 2024/3019).

Conventional activated sludge treatment is widely used, but it is energy-intensive and produces greenhouse gases (Winkler and Straka, 2019). Microalgae-based systems offer a sustainable alternative by removing nutrients while providing valuable biomass for biofertilisers, biofuels, and even biohydrogen production (Atashkar and Saidi, 2024). However, microalgae-based wastewater bioremediation faces challenges due to effluent variability, particularly fluctuations in nitrogen and phosphorus content. These nutrients vary in concentration, N:P ratio, and nitrogen form, impacting microalgal growth and treatment efficiency (Dalvi et al., 2021). Additionally, since nitrogen and phosphorus drive microalgal metabolism, their availability also affects biomass composition (Haider et al., 2022). While previous studies have examined the individual effects of N:P ratios and nitrogen sources on microalgae, a comprehensive analysis of their combined impact is lacking. This study addresses that gap by assessing how different N:P ratios (9, 14, 20, 27) and nitrogen sources (NH_4^+, NO_3^-, and both) influence *Chlorella vulgaris* growth, nutrient removal efficiency, and biomass composition. These findings contribute to optimise microalgal wastewater treatment and biomass valorisation.

2. Materials and methods

Twelve synthetic wastewater compositions were studied, varying the N:P ratio (9, 14, 20, 27) and nitrogen source (NH_4^+, NO_3^-, or both), with phosphate (PO_4^{3-}) as the phosphorus source.

Chlorella vulgaris, provided by the Culture Collection of Algae and Protozoa (Scotland, United Kingdom), was cultured in 1 L photobioreactors under batch conditions for 11 days at 22 ± 2 ºC, with 203 ± 9 µmol m^{-2} s^{-1} illumination and continuous aeration (1.7 L min^{-1}). The initial biomass concentration was 82 ± 2 mg DW L^{-1}, and the pH was maintained at 7.2 ± 0.1.

Microalgal biomass and nutrient concentrations were monitored throughout the assays to assess microalgal growth and nutrient removal. At the end of the assays, the biomass was harvested, frozen at -80 ºC, lyophilised, and macerated. The lipid, carbohydrate, and protein contents were then analysed.

3. Results and discussion

C. vulgaris exhibited successful growth under all studied conditions. In assays with NO_3^- as the sole nitrogen source, the adaptation phase lasted until day 2, delaying exponential growth, whereas with NH_4^+, exponential growth began immediately on day 0 with negligible adaptation. When NO_3^- was the nitrogen source, no significant variations ($p > 0.05$) were found in specific growth rate (μ) across N:P ratios, ranging between 0.31 ± 0.03 and 0.36 ± 0.03 d^{-1}. In contrast, when NH_4^+ was the only nitrogen source, as the N:P ratio decreased, μ increased, reaching its highest value at a ratio of 9 (0.74 ± 0.07 d^{-1}).

Nitrogen removal efficiency (RE) varied across assays, with complete removal achieved only at an N:P ratio of 9, regardless of the nitrogen source. When using NO_3^- alone, maximum RE reached 9 % at N:P ratio of 9, while higher N:P ratios led to incomplete removal despite greater nitrogen uptake. A similar trend was observed for combined NO_3^- and NH_4^+ assays, though NH_4^+ removal was more rapid, especially in the first days of cultivation. Assays with only NH_4^+ achieved complete removal at the N:P ratios of 9 and 14, confirming the microalga's preference for NH_4^+ due to its lower assimilation energy requirements. The highest consumption rate (k) was observed at an N:P ratio of 9 using NH_4^+ (1.8 ± 0.2 d^{-1} for nitrogen, 1.0 ± 0.2 d^{-1} for phosphorus). Phosphorus removal exceeded 99% across all assays. Ultimately, an N:P ratio of 9 with NH_4^+ as the nitrogen source provided optimal nutrient removal, diverging from the Redfield ratio (16), likely due to strain-specific characteristics and environmental factors.

In this study, the carbohydrate content ranged from 18 ± 1 to 42.5 ± 0.7% (w/w), protein content from 12.5 ± 0.6 to 22.3 ± 1% (w/w), and lipid content from 9.9 ± 0.1 to 16.7 ± 0.5% (w/w). Higher nitrogen levels favoured protein accumulation, while nitrogen deprivation led to increased lipid and carbohydrate storage. The increase in protein content with increasing N:P ratios reflects nitrogen's role in protein synthesis. The nitrogen source also affected biomass composition. In general, assays using NO_3^- as the sole nitrogen source resulted in higher protein content compared to those using NH_4^+ alone ($p <$ 0.05). However, at intermediate N:P ratios (14 and 20), the combination of NO_3^- and NH_4^+ led to higher protein content ($p < 0.05$).

4. Conclusion

C. vulgaris successfully grew under all studied conditions, with the highest growth rate (0.74 ± 0.07 d^{-1}) observed when NH_4^+ was the sole nitrogen source. The microalga preferred NH_4^+, consuming it faster than NO_3^- and achieving effective nutrient removal, particularly at an N:P ratio of 9. Biomass composition varied with nutrient availability, with lower N:P ratios increasing carbohydrate and lipid content, while protein levels were higher when both NO_3^- and NH_4^+ were present. These findings highlight the adaptability of C. vulgaris for wastewater treatment. However, biomass composition is affected by growth conditions, and thus its suitability for specific applications must be simultaneously addressed.

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