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EVALUATING THE EFFECTS OF AMMONIUM ON THE GROWTH AND PHYSIOLOGICAL RESPONSES OF MICROALGAE

Tatiana Buse[1,2*], Olga Tiron[1], Mihai Nita-Lazar[1]

[1]National Research and Development Institute for Industrial Ecology – ECOIND, 57-73 Drumul Podu Dambovitei, district 6, 060652, Bucharest, tatiana.buse@ecoind.ro, Romania
[2]Faculty of Biotechnical Systems Engineering, National University of Science and Technology Politehnica, Bucharest, Romania.

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Introduction

The increasing degree of urbanization leads to the generation of ever-larger volumes of municipal wastewater rich in ammonium, frequently exceeding the limits imposed by environmental legislation. This highlights the need for sustainable technologies capable of eliminating excess ammonium while reducing the use of chemicals. Microalgae represent an ecological alternative to conventional treatment methods due to their high capacity to utilize ammonium while simultaneously producing biomass with biotechnological potential. In this context, the integration of microalgae into wastewater treatment processes aligns with the principles of the circular economy, reducing chemical consumption and promoting biological depollution. Among them, Chlorella spp. are recognized for their strong nitrogen assimilation capacity, although their tolerance to high ammonium levels varies depending on the strain. The present study aims to evaluate the impact of two ammonium concentrations on the development of the *Chlorella vulgaris* strain, with the aim of identifying an inhibitory effect on microalgae. The results obtained may provide directions for the controlled integration of microalgae in effluents contaminated with high ammonium concentrations, and for the development of sustainable biotechnologies with a dual role: purification and biomass valorization.

Materials and methods

The study was structured in two main stages: (i) cultivation of the target microalga and (ii) testing the effects of different ammonium concentrations on the *Chlorella vulgaris* inoculum.

(i) Cultivation of the target microalga. The species used, obtained from the Culture Collection of Algae & Protozoa, was *Chlorella vulgaris* CCAP 211/11B, a unicellular eukaryotic microalga. It was cultivated for 14 days in a specific culture medium, 3N-BBM+V, under controlled conditions: a photoperiod of 16 h light : 8 h dark, light intensity of 342 µmol/m²/s, temperature of 25 ± 1 °C, continuous agitation at 70 rpm, and pH control through the addition of carbon dioxide.

(ii) Ammonium testing. To assess the effect of ammonium, synthetic wastewater was used, adjusted to two concentrations: 30 mg/L NH_4^+, the maximum value permitted by legislation according to NTPA 002, and 90 mg/L NH_4^+, a value above the limit, applied to test tolerance. The experimental duration was 4 days. The impact on growth and removal performance was monitored by periodically determining the following parameters: biomass concentration (g/L), specific growth

rate (day^{-1}), specific ammonium uptake rate (mg NH_4^+/g biomass/day), chlorophyll „a" content (mg/L), and residual ammonium concentration (%).

Results and conclusions

Chlorella vulgaris exhibited physiological variations dependent on NH_4^+ concentration. At 30 mg/L NH_4^+, the microalgal culture showed the following trend: ammonium removal efficiency (fig.1) increased from 37.9% (day 2) to 93.1% (day 4) over the course of the experiment. In this case, the ammonium assimilation rate (17 mg NH_4^+/g biomass/day) confirmed that the cells were metabolically active. In contrast, at 90 mg/L NH_4^+, the removal efficiency ranged between 32.4% (day 2) and 53.2% (day 4). Interestingly, the ammonium assimilation rate per unit of biomass was slightly higher (20.1 mg NH_4^+/g biomass/day), suggesting that although the overall efficiency was lower, the cells were able to uptake more ammonium per biomass unit.

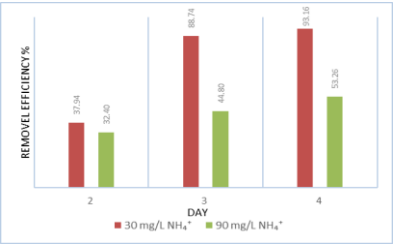

Figure 1. Variation ammonium removal efficiency for 30 and 90 mg/L NH_4^+

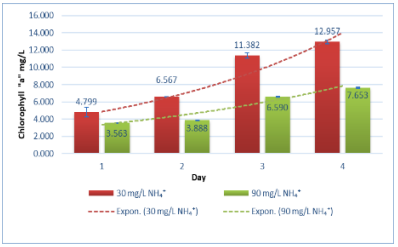

Figure 2. Variation of chlorophyll "a" during ammonium testing

The chlorophyll „*a*" content in the first sample increased steadily from 4.8 ± 0.5 to 12.9 ± 0.2 mg/L (Figure 2), corresponding to a biomass concentration that rose from 0.16 to 0.49 g/L, reflecting active photosynthesis and a healthy physiological state. In the culture exposed to 90 mg/L NH_4^+, chlorophyll „*a*" also increased, from 3.5 ± 0.03 to 7.6 ± 0.04 mg/L, associated with a biomass growth from 0.13 to 0.43 g/L. When analyzing chlorophyll „*a*" proportion relative to dry weight, in the first treatment this ratio was 28.7 mg Chl „*a*"/g d.w. (day 1) and decreased to 26.3 mg Chl „*a*"/g d.w. (day 4), while in the second treatment it started at 26.5 mg Chl „a"/g d.w. (day 1) and declined to 17.6 mg Chl „*a*"/g d.w. (day 4). For the 30 mg/L NH_4^+ concentration, the specific growth rate was 0.392 day^{-1}, whereas for the 90 mg/L treatment it was lower, at 0.27 day^{-1}.

These results indicate that *Chlorella vulgaris* can effectively assimilate both low and moderate ammonium concentrations. Photosynthetic activity was sustained in both cases, without apparent toxic effects on the microalgal culture. Future research could explore higher ammonium levels, correlated with increased biomass concentrations, in order to determine the maximum tolerance threshold of this strain.

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