

Responses of three species of microalgae to the presence of antibiotics commonly found in wastewater

Gabriele Frascaroli (✉ gabriele.frascaroli@gcu.ac.uk)

Glasgow Caledonian University <https://orcid.org/0000-0002-7743-2210>

Short Report

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Abstract

The study evaluated the reactions of three types of microalgae to seven antibiotics commonly detected in wastewater. It examined growth patterns, pigment production, alterations in the media's pH, and the elimination of both nutrients and antibiotics. The findings from this investigation have been summarised in a schematic presentation of the data.

Introduction

Since the early 1900s, antibiotics have transformed medicine, yet their increasing use and environmental release pose ecological and health concerns. Antibiotics persist in wastewater, even after standard treatment, potentially disrupting ecosystems and contributing to antimicrobial resistance. Due to their resilience, recent interest centres on microalgae for micropollutant removal from wastewater. However, the mechanisms behind microalgae's antibiotic removal remain unclear, and antibiotics can stress microalgae, affecting removal efficiency. This study focuses on three microalgae used in wastewater treatment (*A. protothecoides*, *T. obliquus*, and *C. acidophila*) and assesses their responses to a mixture of seven common antibiotics. It examines growth, pH, pigment production, and antibiotic removal to understand how microalgae respond to stress and its impact on antibiotic removal, addressing a crucial knowledge gap in the field.

Results Overview

pH variation and microalgae growth

In the presence of antibiotics, all three microalgae exhibited a gradual decrease in pH, with higher antibiotic concentrations leading to more significant pH drops (up to 3 units). However, no notable pH variations were observed in abiotic conditions or controls without antibiotics. This pH decline appears to result from a shift in algal metabolism triggered by antibiotics, where organic compounds in the media activate heterotrophic activity, increasing CO₂ production and lowering the media's pH. This response was directly proportional to antibiotic concentrations, suggesting that organic antibiotics induce a shift to a heterotrophic state, causing pH reduction in the media.

Chlorophylls and carotenoids

The presence of antibiotics influenced pigment production in the three microalgae studied. After 9 days, they showed increased chlorophyll A content, particularly when algae were exposed to the highest concentrations of antibiotics, with up to 36.9% increase in *A. protothecoides*. *A. protothecoides* also exhibited higher chlorophyll B content with antibiotics. Carotenoid levels increased in *T. obliquus* and *A. protothecoides* at higher antibiotic concentrations.

These pigment increases suggest a stress response to antibiotics, potentially aiding in antibiotic degradation or reactive oxygen species scavenging. *A. protothecoides* and *T. obliquus* were more sensitive to antibiotics than *C. acidophila*, which appeared more tolerant. While growth was not inhibited, the long-term effects of constant antibiotic exposure in real wastewater treatment plants need further investigation.

Antibiotics removal

Biosorption

This study found that microalgae can effectively remove certain antibiotics from wastewater through biosorption, with antibiotics like ciprofloxacin, metronidazole and ofloxacin being quickly adsorbed by the microalgae. However, clarithromycin behaved differently, showing desorption from the microalgae over time, which was influenced by pH changes in the media. *C. acidophila* and *A. protothecoides* exhibited higher biosorption potential for most antibiotics, but the process depends on both the algae and antibiotics involved. Biosorption is a physical process driven by chemical bonding between functional groups on the cell wall and antibiotics, without energy consumption, requiring further investigation into specific bonding mechanisms and algal responses to antibiotics.

Bioaccumulation and biodegradation

The study distinguishes between passive biosorption, occurring in the initial stages of microalgae-antibiotic interaction, and active removal processes like bioaccumulation and biodegradation. Active removal was observed only with *A. protothecoides* for four antibiotics at high concentrations. This suggests that *A. protothecoides* may engage in active removal when antibiotics reach specific levels, likely due to its ability to boost heterotrophic activity. Further research is needed to detect transformation products and better understand the relative contributions of bioaccumulation and biodegradation in active removal with microalgae.

Conclusions

The three microalgae species (*A. protothecoides*, *T. obliquus*, and *C. acidophila*) demonstrated effective pharmaceutical removal from contaminated wastewater without growth inhibition, especially for antibiotics ciprofloxacin, clarithromycin, metronidazole and ofloxacin. *A. protothecoides*, previously unexplored for pharmaceutical removal, showed active removal for sulfamethoxazole, ciprofloxacin, metronidazole, and ofloxacin due to induced heterotrophic activity. Stress responses included pigment overproduction, particularly Chlorophyll b in *A. protothecoides*, and pH changes affecting antibiotic removal, notably clarithromycin. This pioneering study highlights the link between physiological

responses to antibiotics and removal pathways in algae, emphasizing the need for long-term experiments to assess the impact on microalgae performance and potential inhibition.