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Ciprofloxacin toxicity and its co-metabolic removal by a freshwater microalga *Chlamydomonas mexicana*

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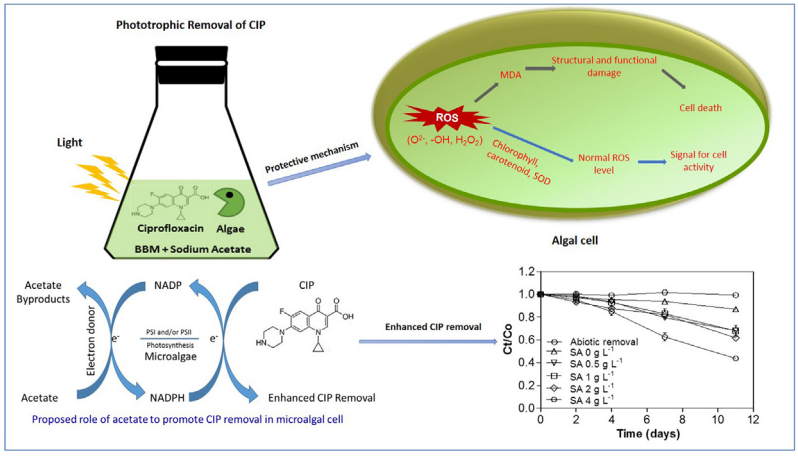
HIGHLIGHTS

- Toxicological effects of ciprofloxacin on *C. mexicana* was studied.
- 96 h EC_{50} of ciprofloxacin for *C. mexicana* was 65 mg L^{-1}.
- Ciprofloxacin influenced the biochemical characteristics of microalgal cells.
- MDA and SOD of *C. mexicana* were significantly increased by ciprofloxacin.
- Sodium acetate acts as an electron donor and enhanced ciprofloxacin removal.

GRAPHICAL ABSTRACT



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ABSTRACT

This study evaluated the toxicity and cellular stresses of ciprofloxacin (CIP) and its co-metabolic removal in a freshwater microalga *Chlamydomonas mexicana*. The toxicological effects of CIP on *C. mexicana* were assessed by studying the growth and biochemical characteristics of the microalga including total chlorophyll, carotenoid content, malondialdehyde (MDA) and superoxide dismutase (SOD) activity. The calculated effective concentration (EC_{50}) of CIP on *C. mexicana* was 65 ± 4 mg L^{-1} at 96 h. The growth of *C. mexicana* was significantly inhibited at increased concentrations of CIP, showing 36 ± 1, 75 ± 3. and $88 \pm 3\%$ inhibition at 40, 60 and 100 mg L^{-1} CIP, respectively, compared to the control after 11 days of cultivation. The total chlorophyll, carotenoid, MDA and SOD activity were significantly increased as a result of relatively high concentrations of CIP stress. *C. mexicana* showed $13 \pm 1\%$ removal of CIP (2 mg L^{-1}) after 11 days of cultivation; however, the addition of an electron donor (sodium acetate, 4 g L^{-1}) highly enhanced the removal of CIP (2 mg L^{-1}) by >3-fold after 11 days. Kinetic studies showed that removal of CIP followed a first-order model (R^2 0.94–0.97) with the apparent rate constants (k) ranging from 0.0121 to 0.079 d^{-1}.

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1. Introduction

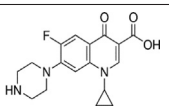
The sources, occurrence, fate, effects and risks of pharmaceutical compounds (PCs) in the environment are emerging concerns as their ubiquitous distribution is revealed by recent improvements in analytical methods [1]. Extensive use of antibiotics for disease prevention, treatment of microbial infections and promotion of animal and plant growth have led to the frequent detection of antibiotics and their degradation products in the environment [2,3]. The antibiotics released in the environment can increase the resistance of bacteria and subsequently compromise public health by preventing treatment of infections caused by these bacteria [4]; this is one of the major challenges for human and veterinary medicine. Bioaccumulation of antibiotics in humans is evident through biomagnification in the food chain, attributed to their recalcitrance and lipophilic properties [5].

Ciprofloxacin (CIP), a third-generation of fluoroquinolones, is one of the most widely used broad-spectrum antibiotics in human and veterinary medicine. It works against gram-negative and gram-positive bacteria through inhibition of bacterial DNA unwinding and duplicating. Ineffective removal of CIP by conventional water treatment technologies has caused its continuous discharge into the environment and it has been widely detected in surface water, groundwater, wastewater and even in raw milk [2,3,6–8]. The worldwide median concentration of CIP in freshwater ecosystem has been reported to be 0.164 mg L^{-1}, and its maximum detected concentration was 6.5 mg L^{-1} [1]. Its ecological effects are also of increasing concern as it has been found to harm a wide range of microorganisms such as bacteria, algae, crustaceans and invertebrates even at low concentrations [9–12].

Various advanced oxidation processes (AOPs) have been investigated to remove CIP from surface water and wastewater, such as Fenton oxidation [13] and UV/H_2O_2 [14]. High operational and maintenance costs of these methods restrict their utilization for long-term applications. Moreover, the incomplete mineralization of PCs during AOPs can generate transformation products that exhibit toxicity comparable to or greater than that of the parent compound [15]. Bioremediation of contaminated waters by mixotrophic microalgae is recently attracting research communities. The microalgal bioremedial system is a solar power-driven, ecologically comprehensive and sustainable reclamation strategy. Mixotrophic microalgae are the native species in freshwater and act as the primary producers in food webs, and detrimental effects on microalgae may elicit significant effects on the entire food chain [16]. They are key indicator organisms for assessing water quality and eco-toxicity of pollutants. Studies have demonstrated that mixotrophic microalgae are able to bioaccumulate and remove environmental contaminants such as nutrients (nitrogen and phosphorous), heavy metals and emerging contaminants from wastewater [17–22]. As such, they have been suggested as a promising candidate for the removal of contaminants [16]. PCs in the wastewater show persistence against degradability via microbial actions. Augmenting wastewater with organic substrates (e.g., glucose and sodium acetate) or other nutrient substrates (e.g., nitrogen and phosphorus sources) would be an effective strategy to enhance the biodegradation of persistent organic pollutants. The additional organic substrates not only serve to sustain biomass production, but also act as an electron donor for the co-metabolism of the non-growth substrate, which can ultimately improve the degradation of pollutants [23–25]. Despite these findings, studies investigating the removal of PCs by microalgae are rare, as are studies investigating the influence of co-metabolic substrates on their removal, to the best of our knowledge.

Most of previous studies mainly focused on the ecotoxicological effects of CIP on microalgal species without any information on its removal [9,10]. Therefore, this study aimed to investigate the removal of CIP at relative high concentration by freshwater green microalgae. Four different microalgal species (*Chlamydomonas mexicana*, *Chlamydomonas pitschmannii*, *Chlorella vulgaris* and *Ourococcus multisporus*) were screened to choose the most effective species for the removal of CIP, and *C. mexicana* was selected for further assessment. The toxicological effects of CIP on *C. mexicana* were determined by assessing its growth rate, total chlorophyll and carotenoid content, malondialdehyde (MDA) and superoxide dismutase (SOD) activity. Furthermore, the positive influence of sodium acetate as a co-metabolic organic substrate on the total removal of CIP by *C. mexicana* was established and thoroughly assessed.

Table 1
Physico-chemical properties of ciprofloxacin.

Properties	Ciprofloxacin
Chemical Structure	
Molecular Formula	$C_{17}H_{18}FN_3O_3$
CAS Reg. No.	85721–33-1
Molecular Mass	331.34
Water Solubility	30 mg mL^{-1} at 20 °C
logKow	0.28
pKa	5.90; 8.89
Therapeutic Class	fluoroquinolones

2. Experimental

2.1. Chemicals

All chemicals used in this study were of analytical grade. Ciprofloxacin (purity, 98%) was purchased from Sigma-Aldrich (St. Louis, MO, USA), and its physico-chemical properties are presented in Table 1. Glucose, sodium acetate and sodium formate were also purchased from Sigma-Aldrich. Acetonitrile (HPLC grade) was purchased from Thermo Fisher Scientific (USA). Methanol and other chemicals were obtained from Duksan (Seoul, South Korea).

2.2. Preparation of the microalgal culture

Chlamydomonas mexicana GU732420, *Chlamydomonas pitschmannii* GU732416, *Chlorella vulgaris* GU732416 and *Ourococcus multisporus* GU732424 were used in this study. The microalgal strains were individually inoculated in 250 mL Erlenmeyer flasks containing 150 mL Bold's Basal Medium (BBM) at 10% concentration ($V_{inoculum}/V_{media}$). The microalgal cultures were cultivated in a shaker incubator at 150 rpm and 27 °C under continuous illumination of white fluorescent light (45–50 μmol photon m^{-2} s^{-1}) for 7 days. The microalgal suspension (cultivated for 7 days) was diluted using sterilized BBM to achieve the optical density (OD) of ~1.0 at 680 nm using a visible spectrophotometer (DR/3900, Hach, USA) for further experiments. All flasks in the following experiments were cultivated in a shaker incubator at 150 rpm and 27 °C under white fluorescent light illumination (alternate light/dark periods of 16/8 h) of 45–50 μmol photon m^{-2} s^{-1} for 11 days.

2.3. Microalgal screening and growth inhibition assay

An initial experiment was conducted to screen microalgal species (*C. mexicana*, *C. pitschmannii*, *C. vulgaris* and *O. multisporus*) for their capacity to degrade CIP. Screening experiments were carried out in 250 mL Erlenmeyer flasks containing 150 mL sterilized BBM, 2 mg CIP L^{-1} and 1.5% of the microalgal suspension (optical

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