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# Biodegradation and metabolic fate of levofloxacin via a freshwater green alga, *Scenedesmus obliquus* in synthetic saline wastewater

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ABSTRACT

Levofloxacin (LEV), a fluoroquinolone antibiotic has been frequently observed in water resources imposing ecotoxicological effects on aquatic microbiota. The biodegradation and metabolic fate of LEV via a microalga, *Scenedesmus obliquus* in synthetic saline wastewater were investigated in this study. LEV removal (1 mg $L^{-1}$) by *S. obliquus* was relatively low in the synthetic wastewater without the addition of sodium chloride (NaCl); however, its removal increased significantly from 4.5 to 93.4% with increasing of its salinity from 0 to 171 mM NaCl. Kinetic studies showed that the removal rate constant (k) increased from 0.005 to 0.289 $d^{-1}$ and degradation half-life decreased from 272 to 5 d in the presence of NaCl (0–856 mM). The removal mechanism analysis showed that the major mechanism of NaCl mediated enhancement of LEV removal was the bioaccumulation and subsequent intracellular biodegradation of LEV in microalgal cells. Six metabolites were identified via gas chromatography–mass spectrometry analysis after biodegradation of LEV. A metabolic pathway was postulated with regard to various cellular biocatalytic reactions in *S. obliquus*, including decarboxylation, demethylation, dehydroxylation, side chain breakdown, and ring cleavage.

## 1. Introduction

Clean water is an essential resource and factor for the safe livelihoods of human beings. Synthetic chemicals, including detergents, disinfectants, fragrances, fire retardants, nonprescription drugs, antibiotics, and pesticides have been continuously discharged into ground and surface water, and soil systems due to agricultural activities, domestic and industrial wastewater contamination, hospital effluents, and wastewater treatment plant disposal [1,2]. These chemicals are now being realized as emerging contaminants (ECs), presenting a large challenge for environmentalists to secure the health of human beings, because these ECs are imposing emerging ecotoxicological effects on target- and non-target organisms; for example, ECs are affecting the denitrification rates of bacteria in soil [3], the feminization of fish [4], and inhibiting the growth of human embryonic cells [5]. Harmful concentrations of ECs can be accumulated through biomagnification in food webs, which can induce adverse effects on high-trophic level organisms such as human beings, especially pregnant women, children, and the elderly, who possess weakened immune systems [6].

Levofloxacin (LEV), a third-generation fluoroquinolone antibiotic (FQ), has gained considerable concerns among researchers as being an emerging contaminant. LEV has been ubiquitously observed within surface water, groundwater, drinking water, and wastewater [7–9]. Ecotoxicological effects of LEV on aquatic organisms such as bacteria, algae, and invertebrates have also been observed [10,11]. The possibility of the antibiotic effects of LEV increasing the prevalence of bacterial resistance genes is currently under evaluation, having implications with regard to human health outcomes. The average LEV removal efficiency from current wastewater treatment plants (WWTPs) is usually below 10%, as WWTPs are not specially designed to remove trace concentrations of persistent ECs [7]. Other efforts to remove LEV from aqueous media such as advanced oxidative processes (AOPs) have been developed. However, the incomplete mineralization of LEV using AOPs has unexpectedly produced more persistent and toxic transformation products [12]. High operational and maintenance costs of AOPs also restrict their utilization in large-scale applications.

Bioremediation using microorganisms is considered an attractive option to remove ECs due to their low-cost and environmentally

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friendly characteristics. Mixotrophic microalgae have especially gained research interests due to their endogenous catabolic systems, heterotrophic capabilities, role in the fixation and turnover of carbon, and their recovered biomass to produce bioenergy. ECs are very difficult to be degraded under normal environmental conditions due to their hydrophobic and persistent nature. Therefore, the enhanced removal efficiency of ECs using a microalgae-bacteria consortium [13], with regard to the augmentation of organic substrates [14,15], and the addition of metallic ions [16] in microalgal cultures has been investigated. The altered removal efficiency of organic pollutants induced by salinity has rarely been evaluated. The discharge of saline wastewater from agro-food, petroleum and leather industries containing at high salinity (10–70 g $L^{-1}$ NaCl) and high organic content without prior treatment has the adverse effects on the aquatic life, water potability and agriculture. Highly saline wastewater is very toxic to biological remediation; and the costs of physico-chemical treatments are particularly high. Previous studies demonstrated that the salinity can enhance the production of valuable biochemical characteristics (lipid and FAMEs) of microalgae and can influence the toxicity of ECs on microalgae [17,18]. However, very rare studies have reported the biodegradation and biotransformation of organic pollutants using microalgae in highly saline wastewater.

Identification of the intermediates formed because of the microalgal degradation of organic contaminants is important to confirm the mode of biodegradation. It also indicates that whether the existing processes can be recommended for large scale applications. The metabolites formed after microbial degradation can be used for an interpretation of the various corresponding enzymes related to their respective degradation pathways. Therefore, in this study, the ecotoxicological effects of LEV on *Scenedesmus obliquus* and the removal kinetics of LEV (1 mg $L^{-1}$) by *S. obliquus* were evaluated. An extensive investigation with regard to the enhancement of *S. obliquus* mediated LEV removal by the addition of sodium chloride (NaCl) in a microalgal culture was performed. The mechanism of LEV removal (abiotic removal, bioadsorption, bioaccumulation, and biodegradation) by *S. obliquus* was thoroughly studied and a metabolic pathway was proposed using gas chromatography–mass spectrometry (GC–MS) analysis of the products retrieved after the biodegradation of LEV.

## 2. Materials and method

### 2.1. Preparation of S. obliquus inoculum

A method by which to prepare an inoculum of *S. obliquus* GU732426 is described in our previous studies [14,15]. In summary, the cultivation of *S. obliquus* was performed in 250 mL Erlenmeyer flasks containing 150 mL of Bold's Basal Medium (BBM) at a 10% concentration ($V_{inoculum}/V_{media}$) in an illumination incubator for 7 days under the following cultivating conditions: 45–50 μmol photon $m^{-2}$ $s^{-1}$ light intensity, 16:8 light/dark cycle, 27 °C temperature, and a 150 rpm shaking speed. The microalgal suspension was diluted with sterilized BBM to achieve the desired optical density (OD-1.0) at 680 nm using a visible spectrophotometer (DR/3900, Hach, USA) for further experiments.

### 2.2. Batch experiments

Levofloxacin (98% purity) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Batch studies were performed in 250 mL Erlenmeyer flasks containing 150 mL sterilized synthetic saline wastewater with different salinities inoculated with 1.0% of a microalgal suspension with an $OD_{680}$-1.0. All flasks were cultivated in a shaking incubator (150 rpm and 27 °C) under white fluorescent light illumination (alternating light/dark periods of 16/8 h) of 45–50 μmol photon $m^{-2}$ $s^{-1}$ for 11 days. All experiments were likewise performed in triplicate.

#### 2.2.1. Toxicological effects of LEV on S. obliquus

The toxicological effects of LEV (0, 1, 20, 50, and 100 mg $L^{-1}$) on *S. obliquus* were studied according to the changes of its dry cell weight (DCW). The dry cell weight of *S. obliquus* in the presence of different LEV concentrations was measured at regular cultivation intervals (day 0, 2, 4, 7, and 11). The cultivation conditions are described at the beginning of Section 2.2.

#### 2.2.2. Removal kinetics and mechanism of LEV by S. obliquus

The removal efficiency of 1 mg LEV $L^{-1}$ by *S. obliquus* in synthetic saline wastewater with different NaCl concentrations (51, 171, 513, 856 mM) were studied in a shaking incubator. The abiotic removal of 1 mg LEV $L^{-1}$ was also investigated by amending the same NaCl concentration in the absence of *S. obliquus*. The cultivation conditions are described at the beginning of Section 2.2. Aliquots (2 mL) of the microalgal suspension were withdrawn at regular cultivation intervals (day 2, 4, 7, and 11) and microalgal cells were separated via centrifugation at 15,000 rpm for 10 min. The supernatant was filtered through a 0.20 μm membrane filter (Pall Life Sciences, USA) and was used to analyze the residual concentration of LEV in the aqueous medium. An aliquot of 50 mL was recovered via centrifugation at day 11 for the evaluation of the bioadsorption, bioaccumulation, and biodegradation of LEV by *S. obliquus*. The harvested microalgae cell pellets were suspended in distilled water (5 mL). The suspension was centrifuged again and the supernatant was recovered for analysis of the LEV concentration adsorbed onto the cell surface. The leftover cell pellets were resuspended in 5 mL of dichloromethane:methanol (1:2 v/v) via sonicating for 1 h (40 kHz, 2.2 kW), followed by centrifugation at 15,000 rpm for 10 min to lyse the microalgal cells. The supernatant recovered via centrifugation was used to determine the concentration of the bioaccumulated LEV. The biodegradation ($P_b$) of LEV by *S. obliquus* was determined using a previously reported equation as follows:

$$P_b(\%) = (A_t - A_r - A_d - A_a - A_c)\frac{100}{A_t}$$

where $A_t$ is the initial amount of LEV added to the aqueous medium, $A_r$ is the residual amount of LEV in the medium, $A_d$ is the amount of LEV adsorbed by the microalgal cells, $A_a$ is the amount of LEV removed via abiotic factors, and $A_c$ is the amount of bioaccumulated LEV within the microalgal cells.

The LEV removal kinetics of *S. obliquus* were analyzed by fitting the data to a first order model as follows:

$$\ln C_t = -kt + \ln C_0$$

where $C_0$ is the initial concentration of LEV at time zero, $C_t$ is the concentration of LEV at time $t$, and $k$ and $t$ are the degradation rate constant ($d^{-1}$) and degradation period in days, respectively.

### 2.3. Measurement of cell density

Dry cell weight (DCW) and specific growth rate ($\mu$) measurements are described in detail in our previous studies [14,15]. In a brief, an aliquot of 10 mL suspension of microalgal species was withdrawn at regular cultivation intervals (0, 2, 4, 7 and 11 days) and then filtered through a pre-weighted Whatman filter paper (GF-52) and dried at 105 °C for 24 h. After cooling to room temperature, the filter papers with algal cells were weighed again and the dry cell weight (DCW) was calculated.

### 2.4. Analysis of LEV and its metabolites

LEV was monitored via high-pressure liquid chromatography (Alliance 2695 system, Waters, USA) equipped with a Waters 2487 UV-VIS detector. A 20 μL sample was injected into a C18 column (250 × 4.6 mm, 5 μm) while maintaining a column temperature of 35 °C. Acetonitrile and water solution (20:80 v/v) was used as the