Bioresource Technology 241 (2017) 849–856

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

# Pollutant removal and microorganism evolution of activated sludge under ofloxacin selection pressure

Qiang Kong [a,b,c,*,1], Xiao He [a,b,1], Yu Feng [a,c], Ming-sheng Miao [b], Qian Wang [a], Yuan-da Du [a], Fei Xu [a]

[a] College of Geography and Environment, Shandong Normal University, 88 Wenhua Donglu, Jinan 250014, Shandong, PR China
[b] College of Life Science, Shandong Normal University, 88 Wenhua Donglu, Jinan 250014, Shandong, PR China
[c] Institute of Environment and Ecology, Shandong Normal University, 88 Wenhua Donglu, Jinan 250014, Shandong, PR China

## HIGHLIGHTS

- More than 80% of COD, $NH_4^+$-N and TP were removed under OFL pressure.
- OFL promoted the secretion of EPS and changed the EPS chemical composition.
- OFL decreased the diversity and richness of the microbial community in the reactor.
- OFL affected the read numbers of the functional bacteria community in the reactor.

## ARTICLE INFO

*Article history:*
Received 24 April 2017
Received in revised form 2 June 2017
Accepted 3 June 2017

*Keywords:*
Activated sludge
Ofloxacin
Extracellular polymeric substances (EPS)
Microbial community

## ABSTRACT

An activated sludge sequencing batch reactor (SBR) was fed with synthetic wastewater containing ofloxacin (OFL) for 52 days to study the overall performance of the SBR, the characteristics of extracellular polymeric substances (EPS) and the bacterial community shift. Removal efficiencies of chemical oxygen demand, ammonia nitrogen, phosphorus and OFL were maintained at 90%, 96%, 80% and 65%, respectively. The EPS contents increased with increasing OFL concentration because more EPS was secreted to protect cells from OFL damage. Moreover, the EPS compositions shifted. For denitrifying bacteria, the read number of *Pseudomonas* and *Bacillus* sharply decreased initially ($p < 0.05$) and increased from Day 25 to Day 50, which agreed with the tendency of *Nitrosomonadaceae* (ammonia oxidizer), while *Paracoccus* significantly decreased ($p < 0.05$). The read number of *Rhodocyclaceae*, a phosphorus-accumulating bacterium, increased. Other functional microbes such as *Nitrospirales* (nitrite oxidizer) and *Planctomyces* (anammox) sharply decreased under OFL pressure ($p < 0.05$).



## 1. Introduction

Overuse of antibiotics in animal feeding and disease treatment has caused increasing concern because of the associated potential environmental risks and perturbances to microbial ecology (Renew and Huang, 2004). Recent studies have shown the presence of various antibiotics in hospital effluent, municipal sewage, surface water and groundwater (Kummerer, 2009). The antibiotics can reach wastewater treatment plants (WWTPs) by various transmission routes, but most wastewater treatment procedures cannot completely remove antibiotic contaminants (Zhang and Li, 2011). Although effluents discharged from WWTPs were detected at low concentrations ranging from μg/L to ng/L, antibiotic residues still threaten the environment and human health (Golet et al., 2002).

Fluoroquinolones (FQs) are broad-spectrum antibacterial agents and are widely used in human antimicrobial therapies and livestock operations. One example is ofloxacin (OFL), which is one of the most frequently used antibiotics among FQs (Sukul and Spiteller, 2007). Consumption of OFL can be high; for example, OFL consumption in Finland was estimated at around 411 kg/year (Vieno et al., 2007). Excessive use of OFL can result in serious side effects because of its potential genotoxicity and ecotoxicity (Hartmann et al., 1999). The OFL used to combat human and animals diseases is not completely metabolized by organisms and is usually excreted into the environment (Peng et al., 2012). Significant concentrations of OFL were found in tertiary treated effluents

* Corresponding author at: College of Geography and Environment, Shandong Normal University, 88 Wenhua Donglu, Jinan 250014, Shandong, PR China.
*E-mail address:* kongqiang0531@hotmail.com (Q. Kong).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.biortech.2017.06.019
0960-8524/

and untreated municipal waste, which contaminate the water environment (Dong et al., 2011).

Various methods such as sonophotocatalytic degradation (Hapeshi et al., 2013), ozonation (Carbajo et al., 2015), the solar Fenton method (Michael et al., 2013a) and adsorption (Crespo-Alonso et al., 2013) have been recently used for the removal of OFL. Although there are many methods of OFL degradation, the removal of antibiotics at WWTPs relies primarily on biological methods (Kasprzyk-Hordern et al., 2009), and activated sludge is the most widespread approach. Previous studies have investigated the ability of activated sludge inoculum to degrade OFL and levofloxacin under light or dark conditions, with or without acetate as an additional carbon source (Maia et al., 2016). However, the variations in extracellular polymeric substance (EPS) contents and components of activated sludge under OFL pressure have not been previously reported. The secretion of EPS by bacteria under antibiotic pressure can delay or prevent toxicants reaching microbes (Sheng et al., 2010). However, it remains uncertain how the microorganisms shift when exposed to OFL. Therefore, it is essential to further investigate how the EPS and microbial communities change in response to OFL.

In this study, the main goal was to investigate the effect of OFL on chemical oxygen demand (COD), ammonia nitrogen ($NH_4^+$-N), total phosphorus (TP) and OFL removal efficiency. Changes in EPS and shifts in the bacterial community in a sequencing batch reactor (SBR) system under increasing OFL concentration were also determined using three-dimensional excitation emission matrix (3D-EEM) and high-throughput sequencing techniques, respectively.

## 2. Materials and methods

### 2.1. SBR set up and operation

A SBR with an effective volume of 6 L (60 cm high, 12 cm internal diameter) was used to cultivate the OFL activated sludge. The SBR was continuously operated in a 12 h successive working cycle, which consisted of 30 min feeding, 600 min aeration, 60 min settling and 30 min discharging. The reactor was kept at room temperature (20 ± 3 °C) and was protected from light to avoid the photodegradation of OFL. The hydraulic retention time was 12 h with a volumetric exchange ratio of 50% and the sludge retention time was set at 10 days by discharging an appropriate amount (500 mL) of suspended sludge every day.

Activated sludge taken from the aerobic basin of the municipal WWTP in Jinan was used as the inoculum. The concentration of mixed liquor suspended solids (MLSS) was about 5000 mg/L. The activated sludge was fed with artificial wastewater containing the following components (per liter): 0.26 g glucose, 0.415 g sodium acetate, 0.24 g $NH_4Cl$, 0.058 g $K_2HPO_4$, 0.024 g $KH_2PO_4$, 0.067 g $CaCl_2$, 0.042 g $MgSO_4{\cdot}7H_2O$, 0.042 g ethylenediaminetetraacetic acid (EDTA), 0.25 g $NaHCO_3$ and 1 mL trace element solution. The composition of the micronutrient solution (per liter) was as follows: 1.5 g $FeCl_3{\cdot}6H_2O$, 0.15 g $H_3BO_3$, 0.03 g $CuSO_4{\cdot}5H_2O$, 0.03 g KI, 0.12 g $MnCl{\cdot}4H_2O$, 0.06 g $NaMoO_4{\cdot}2H_2O$, 0.12 g $ZnSO_4{\cdot}7H_2O$ and 0.15 g $CoCl_2{\cdot}6H2O$. The pH was kept at 6.8–7.0 by dosing with 1M $NaHCO_3$. Initially, 1 mg/L OFL was spiked into the reactor, and the concentration of OFL was gradually increased every 7 days from 1, 3, 5, 8, 10, 12 and 15 mg/L to evaluate the possible effect of OFL on the microbial community.

### 2.2. Analytical methods

Water samples were filtered on filter paper to remove biomass. The COD, $NH_4^+$-N and TP were measured regularly using standard methods (APHA, 2005). The MLSS was determined by a gravimetric method. The pH was determined with pH probes (HQ30d53LDO, Hatch, USA). To determine the concentration of OFL, water samples collected from the influent and effluent were centrifuged in centrifuge tubes, and then the supernatants were filtered through a 0.45 μm filter membrane. Next, 100 mL solutions were taken out into 250 mL conical flask. EDTA-2Na (0.2 g) was added to the solution and HCl was used to adjust the pH to 3 before extraction. The OFL was extracted using a solid-phase extraction method (Peng et al., 2006). The final extract was put in an amber high-performance liquid chromatography (HPLC) vial and stored at 0 °C. The samples were analyzed using HPLC (Agilent 1100 HPLC, USA) (Peng et al., 2006).

### 2.3. EPS extraction and analysis

Activated sludge samples were collected from the SBR on Days 1, 25 and 50 in triplicate, and EPS was extracted. The cation-exchange resin (CER) method was used to extract EPS. First, the 30 mL samples were centrifuged in 50 mL tubes at 2000*g* (Sigma3k15, Germany) for 15 min at room temperature. Next, buffer solution (2 mM $Na_3PO_4$, 4 mM $NaH_2PO_4$, 9 mM NaCl and 1 mM KCl) was added to re-suspend the sludge pellets to their primary volume (30 mL). The 30 mL sludge samples were then transferred to beakers, and the CER (a dosage of 80 g per g suspended solids) was added. The mixtures were stirred for 1 h at room temperature. The mixtures were then centrifuged at 7000*g* for 1 h to remove CER and filtered through 0.45 μm acetate cellulose membranes to harvest EPS (Kong et al., 2016). The EPS mainly contained proteins (PN) and polysaccharides (PS), the PN content was determined using the Coomassie brilliant blue G-250 dye-binding method with bovine serum albumin as the standard (Pierce and Suelter, 1977), and the PS content was determined using the phenol–sulfuric acid method with glucose as the standard (M et al., 1956).

A fluorescence spectrophotometer (F-4600, Hitachi, Japan) was used to record three-dimensional EEM fluorescence spectra. The emission wavelength ranged from 220 to 550 nm, with 10 nm increments, and the excitation wavelength was varied from 200 to 450 nm, with 10 nm increments. Excitation and emission slits were determined to 5 nm, and the scanning speed was maintained at 1200 nm/min. The spectrum of deionized water was recorded as the blank. The 3D-EEM data were managed using Origin 9.0 (OriginLab, USA).

### 2.4. Sample collection, DNA extraction, polymerase chain reaction (PCR) amplification, sequencing and data analysis

Activated sludge samples were taken from the SBR reactor on Days 1, 25 and 50 in triplicate for analysis of the microbial community. Total genomic DNA was extracted with an Omega D5625-01 Soil DNA Kit (50), according to the manufacturer's instructions. The qualities of the extracted DNA were detected using 0.8% (w/v) agarose gel electrophoresis, and an ultraviolet spectrophotometer (Nanodrop NC2000, USA) was used to determine the DNA concentrations.

The forward primer 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and reverse primer 907R (5′-CCGTCAATTCMTTTRAGTTT-3′) were selected to amplify the hypervariable V4–V5 region of the bacterial 16S rRNA gene. The PCR reactions were performed in triplicate per sample and the products were mixed into one single pool before analysis. Amplification was conducted using the following cycling conditions according to the manufacturer's instructions: initial denaturation at 98 °C for 2 min, then 25–30 cycles of 15 s at 98 °C for denaturing, annealing for 30 s at 55 °C, extension at 72 °C for 30 s, followed by a final extension for 5 min at 72 °C and holding at 10 °C. The PCR-amplified products were electrophoresed using 2% (w/v) agarose gel and recovered using an