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# Effects of fluoroquinolone antibiotics on reactor performance and microbial community structure of a membrane bioreactor

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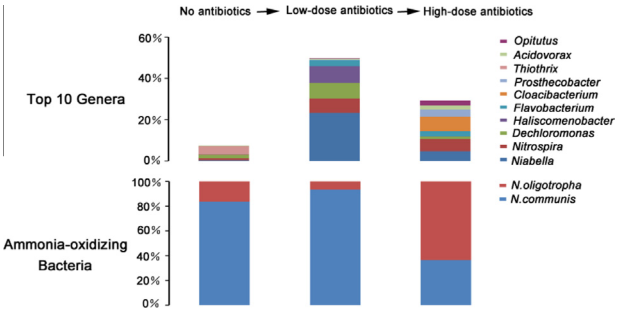
## HIGHLIGHTS

- The role of antibiotics in shaping the bacterial community was studied.
- The removal rates of N were 80–90% after long-term adaption to FQs.
- *Proteobacteria* and *Bacteroidetes* maintained higher abundance after FQs addition.
- The genera involved in N cycling were well-adapted to FQs after 4 weeks.

## GRAPHICAL ABSTRACT



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## ABSTRACT

The occurrence and fate of antibiotics in biological wastewater treatment facilities have given rise to significant concerns. This study assessed the effects of fluoroquinolones (FQs), one class of representative antibiotics in wastewater, on the performance and the bacterial community of an anoxic–aerobic membrane bioreactor operated for one year. Results show that addition of FQs to feed wastewater caused significant increases of chemical oxygen demand (COD) in the mixed liquor. Nitrogen species in permeate increased slightly after the addition of FQs but then decreased to steady levels after long-term adaptation. However, phosphorous removal was consistently poor after FQs addition. 16S rRNA gene-targeted 454-pyrosequencing revealed a sharp decrease in the microbial species richness after FQs addition and significant fluctuations of bacterial community structure at both phylum and genus levels. Members of the two dominant phyla (*Proteobacteria* and *Bacteroidetes*) appeared to be well-adapted to the FQs. Moreover, changes in relative abundance of genera (e.g., *Nitrospira*, *Dechloromonas*, *Acidovorax* and *Opitutus*) involved in nitrogen cycling were in agreement with the reactor performance of nitrification and denitrification. Clone library analysis of the *amoA* gene further revealed a drastic shift of dominant ammonia-oxidizing bacteria (AOB) from the *Nitrosomonas communis* lineage (before FQs addition) to the *Nitrosomonas oligotropha* lineage (after FQs addition). Batch tests on the nutrient removal and FQs biodegradation further supported the changes in microbial community structure. This study provides a much-needed look at the bacterial community dynamics in activated sludge bioreactors for the treatment of antibiotics-containing wastewater.

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## 1. Introduction

Occurrence of pharmaceuticals, which are mostly of anthropogenic origin, has been widely reported in water and wastewater [1,2]. Although pharmaceuticals are often present at trace concentrations, these compounds could be associated with specific environmental risks and impact on human health. Antibiotics are one of the most important and concerning pharmaceuticals that occur in different kinds of waters [3]. Growing production of antibiotics for clinical use and livestock has raised concerns, particularly because of their potentials to cause the yield of antimicrobial resistance in bacteria [4].

Because of the persistence of antibiotics, conventional wastewater treatment plants (WWTPs) usually fail to achieve successful performance on the removal of antibiotics in wastewater [4,5], making WWTP effluents main point discharges for antibiotics [5]. In last decades membrane bioreactors (MBRs) have been widely used to enhance the removal of antibiotics or pharmaceuticals in wastewater streams [4,6], although membrane fouling is still not well addressed [7–9]. The enhanced removals of these compounds by MBRs over conventional WWTPs are attributable to the membrane rejection of bacteria and colloids and the enrichment of specialized microorganisms responsible for the biodegradation of trace contaminants [10]. Thus, MBRs represent a promising alternative for the treatment of wastewater containing antibiotics.

To date, considerable efforts have been focused on how the trace contaminants are removed in MBRs and to what extent the MBRs are superior to conventional WWTPs [4,6,11]. It should be noted that some trace contaminants could be toxic to microorganisms [12] or can potentially shape the bacterial community in WWTPs [13]. For example, the erythromycin has been shown to inhibit selected pure cultures of Gram-negative and Gram-positive organisms depending on erythromycin concentrations [14] and inhibit the bacteria responsible for the conversion of butyric acids in anaerobic process [15]. More recently, antibiotics have been documented to inhibit the activity of nitrifying bacteria [16,17]. Overall, these reports suggest that the presence of antibiotics in wastewater streams could potentially impact the ecology of microbial community and thus its performance on nutrient removal. Nevertheless, such issues in MBRs treating antibiotics-containing wastewater have been rarely explored. It is of high interest to understand what kinds of functional bacteria are susceptible to the exposure to antibiotics and how the reactor performance including nutrient removal and membrane permeability will be impacted by the antibiotics. In addition, such knowledge has great implications for the operation of MBRs treating wastewater streams from the effluent of pharmaceutical industry and hospitals [18].

The overall goal of this study was to investigate the influence of selected antibiotics on the microbial community structure and activity in an MBR for biological nutrient removal (BNR) and whether these changes in microbial community impact reactor performance. Fluoroquinolones (FQs), a mixture of ciprofloxacin, enrofloxacin and difloxacin, was studied for the following reasons: (i) FQs represent a large class of antibiotics commonly present in wastewater streams [19]; (ii) FQs are broad-spectrum antibiotics, which can kill both Gram-positive and Gram-negative bacteria by means of inhibiting DNA synthesis [12]; and (iii) because of their strong adsorption to sludge and their low biodegradability in activated sludge processes [20], FQs are expected to be persistent in biological treatment processes. To explore the temporal variations in microbial populations upon FQs addition, we profiled total and ammonia-oxidizing microbial communities using high-throughput 454-prosequencing and clone library analyses targeting the 16S rRNA and ammonia monooxygenase (*amoA*) genes. Additionally, bacterial activities associated with nutrient removal were assessed using batch tests.

## 2. Materials and methods

### 2.1. MBR setup and operation

An anoxic–aerobic MBR with an effective volume of 10 L (5 L for anoxic tank and 5 L for aerobic tank, see Fig. S1 In Supplementary materials) was operated at a solids retention time (SRT) of 20 d and a hydraulic retention time (HRT) of 10 h, respectively. A hollow fiber membrane module (0.1 $m^2$, 0.01 μm, PVDF, Litree Co., Suzhou, China) was submerged in the aerobic tank where aeration rate was set at *ca.* 100 L/h to maintain a dissolved oxygen (DO) concentration of 1.5–2.0 mg/L. Seeding sludge was collected from a local wastewater treatment plant and incubated for 2 weeks using the synthetic wastewater mentioned below before startup of the MBRs. Sludge recirculation from the aerobic tank to the anoxic tank was set at 200% of the influent flow. Synthetic wastewater with average concentrations of chemical oxygen demand (COD), total nitrogen (TN) and phosphorous (P) of about 350 mg/L, 60 mg/L and 9 mg/L was prepared (Table 1) and used as nutrients for the MBR biomass. The organic carbon of synthetic wastewater was composed of Na-acetate (35 mg/L), starch (162 mg/L), milk powder (200 mg/L), sucrose (141 mg/L), peptone (33 mg/L), yeast extract (77 mg/L) and beef extract (80 mg/L). When the MBR achieved steady and satisfactory performance on nutrient removal (day 0–129), mixtures of FQs (1:1:1 of enrofloxacin, norfloxacin and ciprofloxacin obtained from Zhejiang Guobang Pharmaceutical Co., Ltd. with >98% purity) were added to the synthetic wastewater at a final concentration of 0.9 mg/L (day 130–249) and 9 mg/L (day 250–360). Thus, the entire MBR operation can be divided into three runs: Phase I (without FQs addition, day 0 – day 129), Phase II (with low dosage of FQs, day 130 – day 249) and Phase III (with high dosage of FQs, day 250 – day 360). The operating days of each phase were at least 5 times of the SRT, which can warrant the reactor reach steady performance. The permeate was sucked continuously from the membrane by a peristaltic pump without any physical backwashing or relaxation over the entire operation. When the transmembrane pressure (TMP) increased to *ca.* 0.025 MPa, the membrane module was flushed with high pressure water and then soaked in 0.1 M NaOH solution for 12 h to recover the permeability.

### 2.2. Batch tests for measurements of FQs biodegradation and bacterial activities

FQs biodegradation was examined with fresh sludge and adapted sludge collected on days 100 and 316, respectively. The

**Table 1**
Compositions of the feed wastewater.

| Main nutrients | Concen. (mg/L) | Trace elements | Concen. (mg/L) |
|---|---|---|---|
| Na-acetate | 35 | $FeSO_4 \cdot 7H_2O$ | 2.50 |
| $KH_2PO_4$ | 23 | $ZnCl_2$ | 0.06 |
| $K_2HPO_4$ | 21 | $MnCl_2 \cdot 4H_2O$ | 0.06 |
| $NH_4Cl$ | 40 | $NaMoO_4 \cdot 2H_2O$ | 0.19 |
| Starch | 162 | $CoCl_2 \cdot 6H_2O$ | 0.13 |
| Milk powder | 200 | $NiCl_2 \cdot 6H_2O$ | 0.04 |
| Sucrose | 141 | $CuSO_4$ | 0.06 |
| Urea | 49 | $CaCl_2$ | 0.44 |
| Peptone | 33 | $H_3BO_3$ | 0.06 |
| Yeast extract | 77 | $MgCl_2$ | 0.19 |
| Beef extract | 80 | | |
| $NaHCO_3$ | 30 | | |