



Research article

Performance evaluation and microbial community of a sequencing batch biofilm reactor (SBBR) treating mariculture wastewater at different chlortetracycline concentrations



Dong Zheng^a, Qingbo Chang^a, Mengchun Gao^{a, b, *}, Zonglian She^a, Chunji Jin^a,
Liang Guo^a, Yangguo Zhao^a, Sen Wang^b, Xuejiao Wang^b

^a Key Lab of Marine Environment and Ecology, Ministry of Education, Ocean University of China, Qingdao 266100, China

^b College of Environmental Science and Engineering, Ocean University of China, Qingdao 266100, China

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ABSTRACT

The effects of chlortetracycline (CTC) on the performance, microbial activity, extracellular polymeric substances (EPS) and microbial community of a sequencing batch biofilm reactor (SBBR) were investigated in treating mariculture wastewater. Low CTC concentration (less than 6 mg/L) had no obvious effect on the SBBR performance, whereas high CTC concentration could inhibit the chemical oxygen demand (COD) and nitrogen removal of the SBBR. The microbial activity of the biofilm in the SBBR decreased with the increase of CTC concentration from 0 to 35 mg/L. The protein (PN) contents were always higher than the PS contents in both loosely bound EPS (LB-EPS) and tightly bound EPS (TB-EPS) at different CTC concentrations. The chemical compositions of LB-EPS and TB-EPS had obvious variations with the increase of CTC concentration from 0 to 35 mg/L. The high-throughput sequencing revealed the effects of CTC on the microbial communities of the biofilm at phylum, class and genus level. The relative abundances of some genera displayed a decreasing tendency with the increase of CTC concentration from 0 to 35 mg/L, such as *Nitrospira*, *Paracoccus*, *Hyphomicrobium*, *Azospirillum*. However, the relative abundances of the genera *Flavobacterium*, *Aequorivita*, *Buchnera*, *Azonexus* and *Thioalbus* increased with the increase of CTC concentration.

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1. Introduction

Mariculture has been rapidly developed in the past decades due to the continuing decrease of wild fishery resources and the increasing demand of consumption. Many mariculture facilities along coastal area have been widely used in the culture of shrimp, fish and sea cucumber, such as mariculture ponds and recirculating aquaculture systems, which can produce large volumes of mariculture wastewater from water exchange. Mariculture wastewater mainly contains suspended solids, nitrogenous compounds and organic matter from mariculture organism's excretion and uneaten food (Hagopian and Riley, 1998). When untreated mariculture wastewaters are discharged into the offshore and coastal zones, it

will be inevitably lead to algal blooms, oxygen depletion and eutrophication in the surrounding estuarine ecosystem (Gutierrez-Wing and Malone, 2006). Suspended solids are firstly removed by sand filter and mechanical filter, and then biological processes are used to remove organic matter, ammonia, nitrite and nitrate from mariculture wastewater. Some biological treatment processes have been proven to be very effective for the treatment of mariculture wastewater, including biological filter, sequencing batch reactor, membrane bioreactor and constructed wetland (Gregory et al., 2010; Fontenot et al., 2007; Matos et al., 2009; Webb et al., 2013).

Infectious disease is the main cause of the economic losses in mariculture, which has become a limiting factor for its development. The use of antibiotics has been essential to prevent the spread of pathogenic bacteria, whereas excessive use of these antibiotics will lead to high antibiotic concentration in mariculture wastewater and cause adverse effects on the environment. Previous researches have reported that the antibiotics in mariculture wastewater could affect on the ecological toxicity of aquatic

* Corresponding author. College of Environmental Science and Engineering, Ocean University of China, No. 238 Songling Road, Qingdao, Shandong Province 266100, China.

E-mail address: mengchungao@outlook.com (M. Gao).

organisms in the surrounding estuarine ecosystems (Hagenbuch and Pinckney, 2012; Zheng et al., 2012). Similarly, the antibiotics in mariculture wastewater could also inhibit the microbial activities of biological wastewater treatment systems and then decrease the removal efficiencies of organic matter and nitrogenous compounds. Some researches have reported that the presence of antibiotics could obviously affect the performance of wastewater biological treatment systems or livestock manure treatment systems (Álvarez et al., 2010; Shimada et al., 2008; Stone et al., 2011). In addition, the presence of antibiotics in the mariculture wastewater could affect the secretion of extracellular polymeric substances (EPS) from the activated sludge or the biofilm in biological treatment processes owing to protect the microorganisms against the toxicity of antibiotics. EPS exhibit a dynamic double-layered structure, which consists of loosely bound EPS (LB-EPS) and tightly bound EPS (TB-EPS). EPS in biological treatment processes are closely related to the flocculability, dewatering ability and stability of the activated sludge or the biofilm. However, little research has been performed to investigate the effect of antibiotics on the performance, EPS, microbial activity and microbial community of a sequencing batch biofilm reactor (SBBR) treating mariculture wastewater.

As chlortetracycline (CTC) is widely used in mariculture systems due to its broad-spectrum activity, it is chosen as a model compound for antibiotics in the present study. The objectives in the present study were (a) to evaluate the effects of CTC on the performance and microbial activity of a SBBR treating mariculture wastewater; (b) to analyze the effect of CTC on the compositions and functional groups of LB-EPS and TB-EPS in the biofilm; (c) to investigate the effect of CTC on the microbial community at phylum, class and genus level by the high-throughput sequencing.

2. Materials and methods

2.1. Experimental set-up

A lab-scale SBBR filled with fibrous carrier was used to treat mariculture wastewater in the present study, which had an internal diameter of 14 cm and a total height of 55 cm. The synthetic mariculture wastewater was fed into the SBBR by a peristaltic pump. The effluent was drawn at a height of 15 cm from the bottom by a solenoid valve, and the volume exchange rate for every cycle was 50%. The synthetic mariculture wastewater was mixed at the anoxic stage by a magnetic stirrer, and air was introduced at the aerobic stage by two air diffusers at the bottom of the reactor. One cycle of the SBBR consisted of 0.25 h of influent addition, 7 h of anoxic stage, 3 h of aerobic stage, 1.5 h of settling and 0.25 h of effluent withdrawal. The dissolved oxygen (DO) concentration at the aerobic stage and anoxic stage was over 2.0 mg/L and less than 0.5 mg/L, respectively.

2.2. Seed sludge and composition of synthetic mariculture wastewater

The seed sludge was collected from a parent SBR treating synthetic mariculture wastewater in our laboratory. The compositions of synthetic mariculture wastewater were as follows (mg/L): CH₃COONa, 112; NH₄Cl, 17.5; KH₂PO₄, 15; NaNO₂, 2.5; NaNO₃, 30; and seawater crystal, 3 × 10⁴ (corresponding to 3‰ salinity). The main components of the seawater crystal solution at 3‰ salinity were as follows (mg/L): Na⁺, 9880; Cl⁻, 18,025; Mg²⁺, 950; SO₄²⁻, 2500; K⁺, 360; Ca²⁺, 300; Zn²⁺, 0.015; Mn²⁺, 0.013; Fe²⁺, 0.13; Co²⁺, 3 × 10⁻⁴; Mo⁶⁺, 3 × 10⁻³; I⁻, 0.07; Sr⁺, 7.5 × 10⁻³; I⁻, 70; Se⁶⁺, 3.5 × 10⁻⁴. The CTC concentrations were 0, 2, 4, 6, 10, 15, 25, 35 mg/L at different operational periods, respectively.

2.3. Analytical methods

COD, NH₄⁺-N, NO₂⁻-N, NO₃⁻-N, mixed liquor suspended solids (MLSS), and mixed liquor volatile suspended solids (MLVSS) were measured according to Chinese NEPA standard methods (Chinese NEPA, 2002). LB-EPS and TB-EPS were extracted by a heat extraction method (Wang et al., 2013). The PN and PS contents in the EPS were measured by the Lowry method and the anthrone-sulfuric acid method, respectively (Lowry et al., 1951; Dubois et al., 1956). Three-dimensional excitation-emission matrix (3D-EEM) fluorescence spectra of LB-EPS and TB-EPS were recorded according to Wang et al. (2013). The specific oxygen utilization rate (SOUR), specific ammonium oxidation rate (SAOR), specific nitrite oxidation rate (SNOR) and specific nitrate reduction rate (SNRR) were determined according to Wang et al. (2015).

2.4. Microbial community analysis

The biofilm samples in the SBBR under steady states were collected on day 78, 190, 235 and 256, corresponding to 0, 10, 25 and 35 mg/L CTC in the influent. The genomic DNA was extracted directly from the biofilm sample by using the PowerSoil DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA, USA) according to the manufacturer's instructions. Partial 16S rDNA based high-throughput sequencing was used to determine the diversity and composition of the microbial communities in each sample. PCR amplifications were conducted with the primer set 515F (5'-GTGCCAGCAGCCGCGTAA-3') and 806R (5'-GGACTACAGGG-TATCTAAT-3') that amplified the V4 region of the 16S rDNA. Sequencing was subsequently determined on an Illumina MiSeq platform of Novogene (Beijing, China).

Paired-end reads from the original DNA fragments were merged using FLASH (Magoč and Salzberg, 2011), and assigned to each sample according to the unique barcode of each sample. QIIME software package (<http://qiime.org/>) and UPARSE pipeline (<http://drive5.com/uparse/>) were used to analyze the reads and pick operational taxonomic units (OTUs). Sequences with ≥97% similarity were assigned to the same OTUs. A representative sequence was selected for each OTU, and the RDP classifier was used to assign taxonomic data to each representative sequence. In order to reveal Alpha diversity, rarefaction curves were generated based on the count of unique OTUs in the sample and Shannon index.

3. Results and discussion

3.1. Effect of CTC on performance of SBBR treating mariculture wastewater

The COD and nitrogen removals of SBBR treating mariculture wastewater were evaluated at different CTC concentrations in the influent. As shown in Table 1, the CTC concentration at 0–6 mg/L in the influent had no obvious effect on the performance of the SBBR. Matos et al. (2014) pointed out that the influent tetracycline concentration at 49.8 µg/L did not affect the COD and nitrogen removals of a SBBR. However, the COD and nitrogen removal efficiency sharply decreased from 90.87% to 93.05%–76.91% and 79.18% with the increase of CTC concentration from 6 to 35 mg/L, respectively. Zheng et al. (2016) reported that the removal efficiencies of COD and nitrogen in the SBBR had no obvious changes at 0–4 mg/L oxytetracycline (OTC) concentration and then showed a distinct decrease at 4–12 mg/L OTC. Cetecioglu et al. (2013) illustrated that the tetracycline concentration at 8.5 mg/L could inhibit the substrate/COD utilization and biogas generation in an anaerobic sequencing batch reactor. In addition, the batch experiments also demonstrated that some antibiotics (e.g. erythromycin and

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