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Does the biological treatment or membrane separation reduce the antibiotic resistance genes from swine wastewater through a sequencing-batch membrane bioreactor treatment process

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ABSTRACT

Swine wastes are the reservoir of antibiotic resistance genes (ARGs), which can potentially spread from swine farms to the environment. This study establishes a sequencing-batch membrane bioreactor (SMBR) for ARG removal from swine wastewater, and analyzes the effect of biological treatment and membrane separation on the ARG removal at different solid retention times (SRTs). The SMBR removed 2.91 logs (copy number) of ARGs at a short SRT (12 days). Raising the SRT reduced the removal rates of the detected genes by the biological treatment. Under the relative long SRT (30 days), ARGs and mobile genetic elements (MGEs) were maximized within the reactor and were well removed by membrane separation, with the average genes removal rate of 2.95 (copy number) and 1.18 logs (abundance). At the relatively low SRT, the biological treatment showed the dominant ARG removal effect, while the membrane separation took the advantages of ARG removal especially at the relatively long SRT. The ARG profile was related to the shift of the microbial community structure. The ARGs coexisted with the functional bacteria (ammonia oxidizing bacteria, nitrite oxidizing bacteria and denitrifiers), suggesting they are hosted by the functional bacteria.

1. Introduction

According to a recent survey of antibiotics usage in China, animal production consumed 52% of China's total antibiotic consumption in 2013 (162,000 tons) (Zhang et al., 2015). Antibiotics are widely used in the livestock industry, as they not only prevent and cure disease, but also promote animal growth. However, antibiotic resistance is an emerging concern, and antibiotic resistance genes (ARGs) are recognized environmental pollutant (Pruden et al., 2006), originating from both hospitals and wider environments (Martínez, 2008).

Swine wastewater is an important reservoir of ARGs. The ARGs in swine wastewater are disseminated to the adjacent environment through discharge and land application of the swine wastewater.

The effluent quality can be improved by treating the swine wastewater in a membrane bioreactor (MBR). Previously, we established a sequencing-batch MBR (SMBR) with high TN, COD and total bacteria removal efficiencies (Sui et al., 2017). Munir et al. (2011) reported a 1–3 logs higher reduction of effluent ARGs in an MBR than in a conventional treatment process. Membrane separation is another effective technique for reducing bacteria number (Harb and Hong, 2017). A membrane module augmented with dense membrane foulants facilitated the reduction of ARGs (Zhu et al., 2018). But owing to the relatively high density of bacterial cells, biofilm, and ARGs within the MBR, a high frequency of horizontal gene transfer (HGT) is observed in the mixed liquor of MBR (Yang et al., 2013). To facilitate ARGs removed by biological process and/or membrane separation, we must comprehensively analyze the ARGs occurrence in the mixed liquor, foulants and effluent.

Regulating the solid retention time (SRT) is a common strategy to control the properties of mixed liquor and microbial community.

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Relatively short SRT (10–12 days) was applied to increase ammonia oxidizing bacteria (AOB) population while inhibiting nitrite oxidizing bacteria (NOB) population in order to enhance the TN removal rate in wastewater with low C/N ratio (Fitzgerald et al., 2015). Ma et al. (2011) reported that increasing SRT from 10 to 20 days significantly reduces the copy number of several genes (*sul*1, *sul*2, *tet*C, *tet*G and *tet*X) in mesophilic anaerobic digestion. Both the microbial community and environmental factors influence ARG occurrence (Zhang et al., 2015b). Nonetheless, how the SRT influences ARG occurrence in typical anoxic/aerobic treatments used in swine farms for biological nutrient removal has not (to our knowledge) been clarified.

In this study, a lab-scale SMBR was used to treat swine wastewater for the removal of ARGs. To separate the effects of ARG reduction by biological process and membrane separation, the ARG quantities in the mixed liquor, foulant and effluent were analyzed. The ARG occurrences in different regions of the SMBR were evaluated for various SRTs (12, 15 and 30 days). Especially, the impacts of the environmental factors, microbial community and HGT on the ARG occurrence were explored during the treatment of swine wastewater in the SMBR.

2. Materials and methods

2.1. Experimental design and sampling methods

The SMBR (length × width × height = 260 mm × 260 mm × 450 mm; effective volume 30 L) was operated at ambient temperature (20–25 °C). The framework of the reactor and its operational methods are described in the Supplementary Information and our previous study (Sui et al., 2017).

The raw swine wastewater was frequently taken from a confined swine farm (Beijing, China) with 5000 head capacity and stored in a cooler room at 4–6 °C. Each collected sample of raw swine wastewater could feed the reactor for 2 weeks to one month.

The SMBR was implemented at three SRTs (30, 15 and 12 days) by daily wasting sludge from the reactor. The operational parameters, water quality of the influent and effluent are listed in Table 1, and antibiotic concentrations are shown in Table S1. The whole experiment continued for 262 days, in which the period of these three SRT treatments was operated for 123, 82 and 57 days, respectively. During each treatment period, triplicate samples were collected on days 44, 83, 110, 148, 175, 203, 223, 241 and 259. Samples were taken from the influent (Inf), the mixed liquor suspended solid (ML), the supernatant (Supn), the foulant attached on the membrane (Mem), and the effluent (Eff) of the SMBR. The seed sludge from a municipal wastewater treatment plant was named as S_0. After the end of oxic phase, the mixed liquor sample was collected. The Supn was the supernatant after the mixed liquor sample settled for 30 min. The Mem sample was obtained by washing the membrane surface with deionized water, and then centrifuged at 10000 rpm for 5 min.

2.2. DNA extraction

The liquid samples of influent (8 mL), supernatant (20 mL) and effluent (200 mL) were first filtered through 0.22-μm filters, and the remaining sludge samples (ca. 0.04 g-dry weight) were subjected to DNA extraction using the FAST DNA extraction Kit (MP Biomedicals, USA) according to the manufacturer's instructions. The extracted genomic DNA was detected and quantified by 1% agarose gel electrophoresis and NanoDrop 2000 (Thermo Scientific, USA), respectively, and then stored at −80 °C until further analysis.

2.3. Quantitative PCR (qPCR)

ARGs of *tet*M, *tet*G, *tet*X (tetracycline resistance), *erm*B, *erm*F, *mef*A, *ere*A (macrolide resistance), *sul*1 and *sul*2 (sulfonamide resistance) and bla_{TEM} (β-lactam resistance), as well as mobile genetic elements (MGEs) of *int*I1 (class 1 integron gene) Tn*916/1545* (conjugative transposon Tn*916/1545*), *ISCR1* (insertion sequence common region I gene), and total bacteria (16S rRNA) were quantified by qPCR. The primers and annealing temperature of the determined genes are listed in Table S2. The plasmids containing these specific genes were manufactured by Zhejiang Tianke Biotechnology Company (Zhejiang, China). The standard samples were diluted to yield a series of 10-fold concentrations and subsequently used for qPCR standard curves. The 25 μL PCR reaction mixtures contained 12.5 μL SYBR Green qPCR Super-Mix-UDG with Rox (Invitrogen, USA), 0.5 μL each of 10 μM forward and reverse primers, 10.5 μL DNA-free water, and 1.0 μL standard plasmid or DNA extract. The thermo cycling steps for qPCR amplification were as follows: (Ahmed et al., 2007) 50 °C, 2 min; (Berendonk et al., 2015) 95 °C, 5 min; (Bonfante & Anca, 2009) 95 °C, 20 s; (Caporaso et al., 2011) annealing temperature, 30 s; (Edgar et al., 2011) 72 °C, 31 s; (Fitzgerald et al., 2015) plate read, go to (Bonfante & Anca, 2009)~(Edgar et al., 2011), 39 more times; (Fu et al., 2017) Melt-curve analysis: 60 °C to 95 °C, 0.2 °C/read. The reaction was conducted using an ABI Real-time PCR system 7500 (ABI, USA). The specificity was assured by the melting curves and gel electrophoresis. Each gene was quantified in triplicate with a standard curve and negative control.

2.4. High-throughput sequencing and bioinformation analysis

The V4 region of the bacterial 16S ribosomal RNA gene was amplified by PCR (95 °C for 2 min, followed by 25 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s and a final extension at 72 °C for 5 min) using the primers 515F/806R (Caporaso et al., 2011). Barcode at the reverse primer is an eight-base sequence unique to each sample. PCR reactions were performed in triplicate in 20 μL containing 4 μL of

Table 1
The operational parameters of the SMBR at three SRTs.

Item	Parameter	SRT 30	SRT 15	SRT 12
Operational parameters	HRT (d)	5–6	5–6	4–5
	SRT (d)	30	15	12
	Duration time (d)	123	82	57
	MLSS (mg/L)	11,745 ± 690	12,508 ± 753	10,592 ± 1438
	COD load (kgCOD/kgVSS d)	0.15 ± 0.01	0.13 ± 0.01	0.14 ± 0.02
	TN load (kgTN/kgVSS d)	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00
Influent	COD (mg/L)	7737 ± 899	7118 ± 313	5453 ± 682
	TN (mg/L)	1116 ± 91	1027 ± 110	874 ± 125
	NH_4^+-N (mg/L)	822.3 ± 38.1	831.9 ± 99.0	760.7 ± 102.4
Effluent	COD (mg/L)	392 ± 102	317 ± 42	209 ± 49
	TN (mg/L)	88 ± 25	95.6 ± 26	45 ± 9
	NH_4^+-N (mg/L)	13.7 ± 6.3	22.9 ± 10.3	3.5 ± 0.4
	NO_2^--N (mg/L)	16.9 ± 0.6	12.4 ± 5.7	13.4 ± 1.6
	NO_3^--N (mg/L)	3.5 ± 0.6	12.2 ± 11.25	2.3 ± 0.7