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Metagenomic insights into the influence of salinity and cytostatic drugs on the composition and functional genes of microbial community in forward osmosis anaerobic membrane bioreactors

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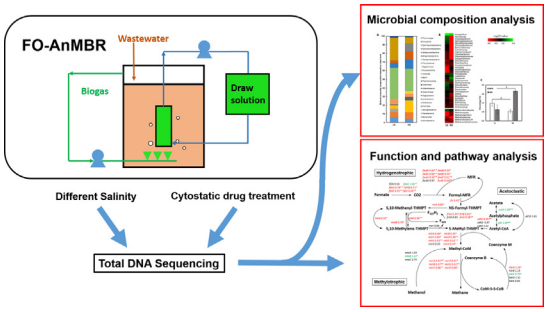
HIGHLIGHTS

- Elevated salinity drives transition in microbial composition and function.
- Salt-tolerant microorganisms were enriched in the FO-AnMBR operation.
- Methane production yield was lower under high salinity (HS) condition.
- Abundance of hydrogenotrophic and acetoclastic methanogens decreased at HS.
- Cytostatic drugs increased abundance of biofilm and siderophore synthesis genes.

GRAPHICAL ABSTRACT



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ABSTRACT

Wastewater treatment using forward osmosis anaerobic membrane bioreactors (FO-AnMBR) has a number of advantages over traditional wastewater treatment approaches. Previous studies have investigated the overall performance of lab-scale FO-AnMBR in treating synthetic wastewater. Due to enhanced physical rejection of osmosis membrane, salinity gradually increased and emerging contaminants such as cytostatic drugs in wastewater would also be accumulated during operation. The objective of this study was to investigate the influence of increased salinity and the accumulation of cytostatic drugs on the composition and function of the microbial community in FO-AnMBR through a metagenomics approach. We found that salinity increase in FO-AnMBR was the key factor driving compositional and functional transitions in microbial community. At high salinity condition, methane-producing archaea (MPA) became less competitive than sulphate-reducing bacteria (SRB), resulting in a reduced methane yield rate. The reduced methane production could be restored in the next successive cycle and salt-tolerant MPA was continuously enriched in the entire operation. Relative abundance of genes involved in nitrogen metabolism, cell attachment and biofilm related signalling increased from low salinity to high salinity condition, while those for methanogenesis and iron metabolism were found to decrease. The presence of cytostatic drugs caused the inhibition of microbial metabolism and increased extracellular polymeric

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substances (EPS) concentration. Due to their differential influences on community members, the evenness of community decreased after drug treatment. After the drug treatment, biofilm-forming and siderophore-producing bacteria were found to be more dominant in the microbial community.
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1. Introduction

Anaerobic membrane bioreactor (AnMBR) for wastewater treatment is attracting increasing attention because it has a low sludge yield, reduced energy consumption, small footprint and the capability to recover biogas. With these advantages, AnMBR has been applied to treat high-strength wastewater [1,2]. Conventional AnMBR also suffers from some drawbacks, including low efficiency in treating wastewater with low COD, severe biofouling and relatively poor rejection of soluble organic matters [3]. To enhance the capability of AnMBR, osmotic membrane bioreactor (OMBR) has been developed, in which the flux was driven by osmotic pressure difference between the mixed liquor and draw solution, resulting in a lower fouling tendency, less energy consumption and higher effluent quality than in conventional membrane bioreactors with porous microfiltration or ultrafiltration membranes [4]. For example, recent studies on forward osmotic anaerobic membrane bioreactors (FO-AnMBR) have demonstrated a high performance in treating different types of wastewater [5,6].

Differing from conventional AnMBRs, salt rejection in OMBR often causes an increase in salinity in the feed solution, which may then affect the composition and function of the wastewater microbial community. For example, in an aerobic OMBR, the transition of microbial communities was reported by using PCR-DGGE [7,8]. In a FO-AnMBR system, Ding et al. [9] identified the main microorganisms involved in membrane fouling. However, the transition of microbial community in FO-AnMBR, especially at the functional level, remains largely unexplored. In addition, the occurrence of emerging organic contaminant is another important environmental issue in conventional wastewater reclamation [10]. These contaminants are not easily treated by the indigenous microbial community in wastewater due to their low concentration and inherent resistance to biodegradation. For example, cytostatic drugs were found to enter wastewater treatment plant by cancer patient excretion and discharges from hospitals [11]. The exposure to cytostatic drugs was found to change physicochemical properties of the sludge in MBR and induce endogenous respiration [12,13]. Although cytostatic drugs may accumulate in OMBR due to the physical separation, the effect of these drugs on the microbial community in FO-AnMBR remains elusive.

The objective of this study was to investigate the effects of the increased salinity and accumulated cytostatic drugs on the composition and function of the microbial community during the operation of FO-AnMBR for wastewater treatment. Illumina HiSeq sequencing for the total DNA of the activated sludge was performed. The key wastewater features driving community development were determined and the taxonomic features under different conditions were characterized. The transition of the microbial community at the functional level was further explored to explain the changes in reactor performance and sludge properties.

2. Materials and methods

2.1. FO-AnMBR configuration and operating conditions

A laboratory-scale FO-AnMBR was set up and operated according to a previous study [5] (see Supporting Information for more details). The sludge taken from Ulu Pandan Water Reclamation Plant was firstly cultivated for 30 days under anaerobic conditions and then used as the seed inoculum for the FO-AnMBR. The initial mixed liquor suspended solids (MLSS) in the FO-AnMBR was controlled at about 5 g/L. NaCl at a concentration of 0.5 M was used as the draw solution. The conductivity of the draw solution was maintained between 45.0 and 45.5 mS/cm. The liquid in the reactor was maintained at a constant volume by feeding synthetic wastewater. During the entire operation, the sludge retention time (SRT) was kept at 60 days, and the hydraulic retention time (HRT) varied at 15–40 h, depending on the permeate membrane flux. In each cycle of operation, the salinity increased from 2.0 to 21.0 mS/cm within 22–26 days. When salinity reached 3.3 mS/cm (low salinity) and 21.0 mS/cm (high salinity), sludge samples were taken and divided into three technical replicates. After sampling, sludge was mixed with 100% ethanol (1:1, v/v) and stored at −20 °C for DNA extraction.

2.2. Treatment with cytostatic drugs

Eight cytostatic drugs including azathioprine, cyclophosphamide, doxorubicin, epirubicin, flutamide, methotrexate, mitotane and tamoxifen (purity ≥ 98%, Sigma–Aldrich) were chosen based on their relatively high predicted environmental concentrations [14]. The stock solution of drugs was directly dosed into the FO-AnMBR on day 84 to a final concentration of 100 μg/L for each of them. Sludge samples before and after drug treatment were collected when the salinity was between 7.8 mS/cm (0.5%) and 15.6 mS/cm (1.0%) in which the performance and microbial community were relatively stable [15,16].

2.3. DNA extraction and sequencing

Total DNA was extracted from sludge samples by using the FastDNA Spin Kit for Soil (MP biomedical) following the manufacturer's instructions. Extraction was performed three times for each set of sludge samples (technical replicates). DNA was sequenced using a HiSeq 2500 platform with 250-bp paired-end sequences (Illimina, USA). The run yielded 189.9 Gbp of data, 361.9 million reads in total, with an average read length of 251 bp. The raw sequencing data was deposited in the NCBI Short Reads Archive (SRA) Database with accession numbers of SRX1676955, SRX1677267, SRX1677268 and SRX1677270.

2.4. Analysis of microbial community composition and diversity

All the reads were quality-trimmed and filtered using cutadapt 1.2.1 to remove adapters [17]. Sequences corresponding to 16S rRNA genes were extracted from the trimmed sequences using RiboTagger with the built-in dictionary compiled from Silva and Greengenes databases (80% identity level). The resulting BIOM-format OTU table was then converted to taxon descriptions using MEGAN 5.11.3 [18]. To ensure the same sampling depth for further analyses, OTU abundance was rarefied to the lowest number of sequences for each sample. The α-diversity indices were determined by "alpha_diversity" script in QIIME. Canonical correspondence analysis (CCA) was performed based on the Bray-Curtis distance to visualize the differences in microbial community (rarefied OTU table) and their relationships with wastewater proper-

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