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Organic micropollutants in aerobic and anaerobic membrane bioreactors: Changes in microbial communities and gene expression

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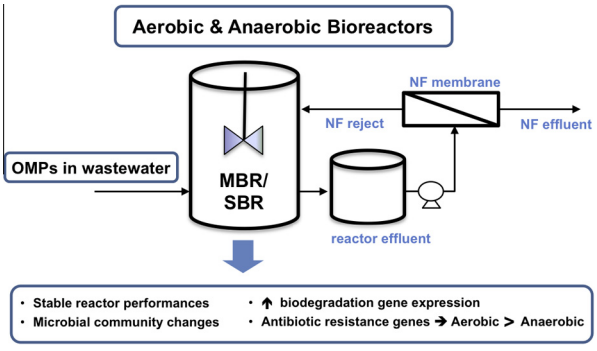
HIGHLIGHTS

- Aerobic and anaerobic MBR systems were fed with OMP-spiked wastewater.
- Key microbial communities in both systems exhibited significant shifts in abundance.
- BDG expression levels showed links with specific OMP presence in both MBRs.
- Antibiotic-type OMP removal efficiency was enhanced in the anaerobic system.
- ARGs generally exhibited higher abundances in the aerobic sludge.

GRAPHICAL ABSTRACT



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ABSTRACT

Organic micro-pollutants (OMPs) are contaminants of emerging concern in wastewater treatment due to the risk of their proliferation into the environment, but their impact on the biological treatment process is not well understood. The purpose of this study is to examine the effects of the presence of OMPs on the core microbial populations of wastewater treatment. Two nanofiltration-coupled membrane bioreactors (aerobic and anaerobic) were subjected to the same operating conditions while treating synthetic municipal wastewater spiked with OMPs. Microbial community dynamics, gene expression levels, and antibiotic resistance genes were analyzed using molecular-based approaches. Results showed that presence of OMPs in the wastewater feed had a clear effect on keystone bacterial populations in both the aerobic and anaerobic sludge while also significantly impacting biodegradation-associated gene expression levels. Finally, multiple antibiotic-type OMPs were found to have higher removal rates in the anaerobic MBR, while associated antibiotic resistance genes were lower.

Abbreviations: AOB, ammonia-oxidizing bacteria; ARG, antibiotic resistance gene; BDG, biodegradation gene; CAS, conventional activated sludge; COD, chemical oxygen demand; DEET, diethyltoluamide; HRT, hydraulic retention time; LC-MS/MS, liquid chromatography tandem mass spectrometry; LCFA, long chain fatty acid; MBR, membrane bioreactor; MG-RAST, metagenomic rapid annotation using subsystems technology; MLSS, mixed liquor suspended solids; mMDS, metric multidimensional scaling; MWCO, molecular weight cutoff; NF, nanofiltration; NOB, nitrite-oxidizing bacteria; OMP, organic micropollutants; OTU, operational taxonomic unit; PAC, power activated carbon; PBS, phosphate buffered saline; pKa, acid dissociation constant; PCA, principal component analysis; qPCR, quantitative polymerase chain reaction; RDP, ribosomal database project; SBR, sequencing batch reactor; TCEP, tris(2-chloroethyl)phosphate; TDCPP, tris(1,3-dichloroisopropyl)phosphate; UF, ultrafiltration.

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1. Introduction

Recent advances in aerobic membrane bioreactor (MBR) technology have led to the widespread implementation of full-scale MBR systems for municipal wastewater treatment. Anaerobic MBRs have not yet been successfully applied at a large scale for the treatment of low-strength (e.g., domestic) wastewaters, but research interest in the subject remains high (Smith et al., 2012). The advantages of MBRs when compared to conventional wastewater treatment are well established and include small treatment plant footprints and high quality effluent.

However, an emerging concern surrounding wastewater treatment is the issue of organic micropollutant (OMP) removal rates and their proliferation into the environment (Bolong et al., 2009). OMPs are present in municipal wastewater due to household use of pharmaceuticals, antibiotics, and other personal care products. Combined waste streams that include hospitals and other facilities producing high OMP concentration effluent amplify the potential impact of these compounds (Verlicchi et al., 2015). Many previous studies have addressed removal of OMPs in conventional activated sludge (CAS) treatment systems in comparison with aerobic MBRs (Cirja et al., 2008; De Wever et al., 2007; Radjenović et al., 2009; Zuehlke et al., 2006). Generally, these studies found that removal rates of OMPs were significantly affected by treatment plant operating conditions and that, overall, aerobic MBR systems were more efficient than CAS in the removal of OMPs. The issue of OMPs in anaerobic MBRs has only recently developed as a topic of interest and will become increasingly important as anaerobic MBR technology evolves as a suitable municipal wastewater treatment option (Monsalvo et al., 2014; Wijekoon et al., 2015).

Despite the essential role that microbes play in both aerobic and anaerobic wastewater treatment, studies characterizing the relationship between OMPs and the microbial communities of those systems have been limited (Fang et al., 2013). This is mainly due to the fact that, at trace levels, these compounds exhibit negligible toxicity or antibacterial effect to change the viability and performance of biological treatment systems (Radjenović et al., 2009). Both aerobic and anaerobic wastewater treatment technologies are generally very robust once acclimatized, therefore a lack of impact by OMPs on overall performance does not necessarily imply a lack of effect on microbial dynamics and their associated gene expressions. Given the inherent differences between the core microbial communities of aerobic and anaerobic reactors, it is likely that those OMP compounds would have unique influences on the microbial community structures and gene expression in each system. Considering the recent increase in aerobic MBRs used for municipal wastewater treatment and a growing interest in anaerobic MBRs for similar applications, there is a need for further understanding of the effect of OMPs on each of the two systems.

Specifically, among the OMPs that are commonly found in untreated wastewater, antibiotics are of interest due to their potential to facilitate antibiotic resistance gene (ARG) propagation and transfer (Hong et al., 2013). The influence of these OMPs on ARG abundance is especially critical in high concentration microbial environments such as biological wastewater treatment systems and their effluents. Although several recent studies have focused on the fate and persistence of ARGs in CAS and anaerobic digestion systems (Burch et al., 2015; Christgen et al., 2015; Yang et al., 2014a; Zhang et al., 2015), no research to date has compared the effect of specific antibiotic-type OMPs on associated ARGs in aerobic versus anaerobic MBR systems. Furthermore, no studies have been conducted that assess the impact of OMPs on microbial communities and their gene expression in MBR systems. As a result, the present study was designed to examine the effects of the presence and accumulation of various OMPs on the microbial communities in lab scale aerobic and anaerobic MBRs by using high-throughput 16S rRNA gene sequencing, metatranscriptomics, and quantitative PCR to analyze the core microbial communities, gene expression profiles, and ARG abundance, respectively.

2. Material and methods

2.1. Description of treatment systems and operational conditions

This study compared two different lab-scale wastewater treatment systems; an aerobic sequential batch reactor (SBR) and an anaerobic MBR operated with a side-stream ultrafiltration (UF) membrane. Schematic diagrams of each system are shown in Figs. S1 and S2 of the Supplementary data and have been detailed previously (Wei et al., 2015a,b). Reactors were operated and sampled in 2 primary phases: (1) without any additional membrane separation and (2) with a nanofiltration (NF) membrane downstream of the reactor effluent (flat-sheet DOW NF90, 200–400 Da MWCO). These 2 phases are subsequently referred to as Phase 1 and Phase 2. Phase 1 was 55–60 days in duration while Phase 2 was 25–30 days. At the end of Phase 2, a single dose (100 mg/L) of powder activated carbon (PAC) was added to the sludge of each reactor to assess its effect on OMP removal. Operational conditions were maintained based on Phase 2 parameters for 10 days after the addition of PAC. Reactors had working volumes of 2 L and were maintained at pH 7. pH was monitored and controlled continuously by a built-in pH controller using 1 M NaOH and 1 M HCl. The aerobic and anaerobic reactors were maintained at 20 °C and 35 °C, respectively to represent typical operating conditions for each system. Hydraulic retention times (HRTs) of both systems were set at 12 h during Phase 1. Upon the addition of the NF membrane to both systems in Phase 2, HRTs were increased to 24 h due to *trans*-membrane flux limitations. To maintain reactor organic loading rates of 0.8 g/L/d throughout operation, influent chemical oxygen demand (COD) was set at 400 mg/L and 800 mg/L for Phases 1 and 2, respectively. The synthetic wastewater was made up of a mix of organic and inorganic compounds and trace metals, as summarized in Table S1. A cocktail of OMP compounds was spiked into the feed synthetic wastewater at individual compound concentrations of 10–20 μg/L for Phase 1 (12 h HRT) and 20–40 μg/L for Phase 2 (24 h HRT) to maintain consistent OMP loading rates to each system. Samples were also taken from reactor sludges before the commencement of OMP spiking in Phase 1 and after the addition of PAC at the end of Phase 2. The cocktail of spiked OMPs consisted of a mixture of pharmaceutical compounds, antibiotics, personal care products, and pesticides that are commonly detected in raw wastewater (Teerlink et al., 2012). Those compounds included acetaminophen, amitriptyline, atenolol, atrazine, bezafibrate, bisphenol A, caffeine, carbamazepine, clofibric acid, dilantin, diclofenac, diethyltoluamide (DEET), diphenhydramine, fluoxetine, gemfibrozil, ibuprofen, iopromide, methylparaben, naproxen, oxybenzone, primidone, propylparaben, sucralose, sulfamethoxazole, trimethoprim, tris(2-chloroethyl)phosphate (TCEP), and tris(1,3-dichloroisopropyl)phosphate (TDCPP). The chemical properties of these OMPs and their skeletal structures are presented in Table S2 and Fig. S3, respectively.

2.2. Liquid chromatography–mass spectrometry

OMP compound concentrations were determined by liquid chromatography coupled tandem mass spectrometry (LC-MS/MS). Samples were analyzed using an Agilent Technology 1260 Infinity Liquid Chromatography unit with AB SCIEX QTRAP 5500 mass spectrometer (Applied Biosystems) as previously described (Wei et al., 2015a). Isotopically labeled standards of each OMP