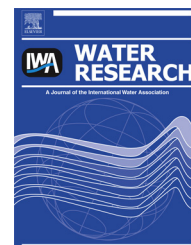


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# Linkages between microbial functional potential and wastewater constituents in large-scale membrane bioreactors for municipal wastewater treatment

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## ABSTRACT

Large-scale membrane bioreactors (MBRs) have been widely used for the municipal wastewater treatment, whose performance relies on microbial communities of activated sludge. Nevertheless, microbial functional structures in MBRs remain little understood. To gain insight into functional genes and their steering environmental factors, we adopted GeoChip, a high-throughput microarray-based tool, to examine microbial genes in four large-scale, in-operation MBRs located in Beijing, China. The results revealed substantial microbial gene heterogeneity (43.7–85.1% overlaps) among different MBRs. Mantel tests indicated that microbial nutrient cycling genes were significantly ( $P < 0.05$ ) correlated to influent COD,  $\text{NH}_4^+ - \text{N}$ , TP or sulfate, which signified the importance of microbial mediation of wastewater constituent removal. In addition, functional genes shared by all four MBRs contained a large number of genes involved in antibiotics resistance, metal resistance and organic remediation, suggesting that they were required for degradation or resistance to toxic compounds in wastewater. The linkages between microbial functional structures and environmental variables were also unveiled by the finding of hydraulic retention time, influent COD,  $\text{NH}_4^+ - \text{N}$ , mixed liquid temperature and humic substances as major factors shaping microbial communities. Together, the results presented demonstrate the utility of GeoChip-based microarray approach in examining microbial communities of wastewater treatment plants and provide insights into the forces driving important processes of element cycling.

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## 1. Introduction

Membrane bioreactor (MBR) has been widely applied to treat municipal wastewater (Huang et al., 2010). With a combination of biological treatment process and membrane technology, MBR has remarkable advantages in producing high-quality reclaimed water over conventional activated sludge process. The high biomass retained in the systems by membrane rejection and the long sludge retention time (SRT) were thought to be important for the stable performance of MBRs (Drews et al., 2005). However, it remains unclear whether and how microbial community in activated sludge is linked to the MBR performance. Most of the current studies focused on the understanding of the influence of specific influent components, single operational conditions or the reactor configuration on microbial communities. Furthermore, most of them were conducted in lab-scale or pilot-scale plants (Xia et al., 2012), which did not reflect actual conditions in large-scale municipal MBRs due to substantial differences in design, scale, operational time and parameters, as well as influent components and the fluctuations (Table S1) (Shen et al., 2012). In addition to differences in influent alkalinity, biochemical oxygen demand (BOD), chemical oxygen demand (COD), BOD/COD and C/N/P ratios, real wastewater often contains antibiotics, heavy metals and other organic pollutants from domestic and industrial discharges, which seldom appears in

the synthetic wastewater treated by lab-scale MBRs. To date, few studies were done with large-scale, operational MBR plants except for two recent studies (Wan et al., 2011; Hu et al., 2012). These two studies targeted bacterial phylogenetic or ammonia-oxidizing community composition. Thus, microbial functional structure and metabolic potentials remained elusive.

High-throughput functional gene array (e.g., GeoChip) technology has been proven to be powerful in examining microbial functional potentials, since it targets a wide range of functional genes involved in C, N, P, S cycling, metal resistance and organic contaminant degradation (such as aromatics, herbicides and pesticides related compounds) and so on. To date, it has been used to profile microbial communities in various habitats, including soil, marine sediments, contaminated groundwater and lab-scale bioreactors (Van Nostrand et al., 2009; Yang et al., 2013; Zhong et al., 2012). However, its utility in examining microbial communities of wastewater treatment plants is yet to be demonstrated.

In this study, we applied GeoChip to examine four large-scale, in-operation MBRs located in Beijing, China. These plants, each with a capacity over 10,000 m<sup>3</sup>/d, were combined with a common nutrient-removal anaerobic-anoxic-oxic process (Table 1). Considering that different tanks might have similar microbial profiles due to activated sludge circulated between each tanks by return sludge pumping system, and on the other hand membrane tank has unique features enduring

**Table 1 – Process and environmental variables of MBRs.**

Factors		B1	B2	B3	B4
Processes and membrane information	Process <sup>a</sup>	A <sub>1</sub> -A <sub>2</sub> -O-MBR	A <sub>1</sub> -A <sub>2</sub> -O-MBR	A <sub>1</sub> -A <sub>2</sub> -O-MBR	A <sub>1</sub> -A <sub>2</sub> -O-MBR
	Capacity (m <sup>3</sup> /d)	60,000	35,000 <sup>b</sup>	40,000 <sup>b</sup>	30,000 <sup>b</sup>
	Commissioned date	2007.11	2007.11	2010.05	2009.11
	Membrane type	PVDF, 0.04 μm	PVDF, 0.1 μm	PVDF, 0.4 μm	PVDF, 0.4 μm
	Wastewater type	Domestic	80% domestic + 20% industrial	80% domestic + 20% industrial	Domestic
Operation conditions	HRT (h)	17.3	40.0	30.0	16.5
	SRT (d)	20.0	27.0	28.3	26.0
	MLSS (g/L)	10.1	2.2	5.9	4.0
	F/M <sup>c</sup>	54.6	239.3	11.6	69.8
Influent characters	COD (mg/L)	550	520	68	276
	NH <sub>4</sub> <sup>+</sup> -N (mg/L)	26.1	20.1	21.5	39.6
	TN (mg/L)	52.6	43.5	42.1	45.0
	TP (mg/L)	4.8	10.7	2.0	4.5
Mixed liquid properties	Temperature (°C)	16.7 ± 1.4	15.3 ± 0.6	8.5 ± 0.2	13.7 ± 0.2
	pH	7.9 ± 0.2	7.7 ± 0.1	7.2 ± 0.1	7.4 ± 0.1
	DO (mg/L)	6.4 ± 0.5	7.6 ± 0.9	10.9 ± 0.5	7.9 ± 1.4
	Viscosity (mPa·s)	5.2 ± 0.5	1.3 ± 0.0	2.3 ± 0.1	2.1 ± 0.2
	Conductivity (μS/cm)	941.0 ± 10.2	1307.0 ± 4.3	1193.3 ± 5.5	1025.0 ± 21.9
	SVI	9.4 ± 0.5	9.9 ± 0.6	16.0 ± 0.7	20.8 ± 2.2
	MLVSS/MLSS	0.6 ± 0.0	0.6 ± 0.0	0.4 ± 0.1	0.7 ± 0.1
	TOC (mg/L)	9.2 ± 0.4	13.3 ± 0.5	22.3 ± 1.7	11.5 ± 1.8
	Polysaccharides (mg/L)	7.7 ± 0.3	7.3 ± 0.1	17.0 ± 0.5	7.8 ± 0.8
	Proteins (mg/L)	1.6 ± 0.3	2.2 ± 0.5	2.8 ± 1.1	3.3 ± 0.6
	Humics (mg/L)	4.8 ± 0.6	5.6 ± 0.4	8.4 ± 0.3	3.6 ± 0.6

PVDF: polyvinylidene fluoride.

<sup>a</sup> A<sub>1</sub>: anaerobic; A<sub>2</sub>: anoxic; O: oxic; MBR: membrane bioreactor.

<sup>b</sup> These plants' actual capacity is around 40–80% of the design. B1 plant reached full-capacity operation.

<sup>c</sup> Sludge load.

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