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# Activated sludge filterability improvement by nitrifying bacteria abundance regulation in an adsorption membrane bioreactor (Ad-MBR)

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## HIGHLIGHTS

- Nitrifying bacteria abundance regulation in Ad-MBR.
- Sludge filterability related closely with the sludge microbial community structure.
- Abundance of nitrifier in MBR impacts sludge and liquid properties.
- Nitrifying bacteria enrichment in MBR improves sludge filterability significantly.

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## ABSTRACT

Autotrophic nitrifying bacteria have its intrinsic properties including low EPS production, dense colonial structure and slow-growth rate, favoring the sludge filterability improvement. An adsorption-MBR (Ad-MBR) was developed to enrich nitrifier abundance in the MBR chamber by inlet C/N regulation, and its possible positive effect on sludge filterability and underlying mechanisms were investigated. By DNA extraction, PCR amplification and Illumina high-throughput pyrosequencing, the abundance of nitrifying bacteria was accurately quantified. More than 8.29% nitrifier abundance was achieved in Ad-MBR sludge, which was above three times of that in conventional MBR. Regulated C/N ratio and thereafter nitrifier abundance enrichment improved sludge filterability by altering sludge mixture and its supernatant properties, reflected by a good sludge settleability, a low supernatant viscosity and turbidity, a low supernatant organic substances concentration, and a small amount of strong hydrophobic fractional components, thus to profoundly improve sludge filterability and decelerate membrane fouling.



## 1. Introduction

Membrane bioreactor (MBR) that combines an activated sludge process with a membrane filtration for sludge-liquid separation gives rise to numerous merits for its application in the field of biological wastewater treatment and reclamation, including an exceptional excellent effluent quality, a complete decoupling of the sludge retention time (SRT) and hydraulic retention time (HRT), and a high biomass content to enhance shocking tolerance (Meng et al., 2009; Lin et al., 2014). However, membrane fouling and accompanying flux decline that constrain MBR application are deemed as inevitably, and are still far from being ultimately recognized, in spite of many research and engineering efforts conducted (Meng et al., 2009; Kumar et al., 2012). From the viewpoint of filtration definition, MBR is operated indeed as a physical process among which the interception efficiency and fouling behavior depends on mutual interaction between membrane material and sludge biomass. As for given membrane materials, sludge properties, especially its filterability, is eventually crucial for MBR permeability and flux maintenance.

It is reported that sludge filterability has to be dictated by MBR operational conditions, including influent wastewater composition (Khan et al., 2013), volumetric or biomass organic loading rate (Johir et al., 2012), sludge retention time (SRT) (Sabia et al., 2013), and aeration intensity or dissolved oxygen (DO) concentration in bulk (Jin et al., 2006), and is often improved by additional dosage or presence of polyelectrolyte (Dizge et al., 2011), ozone (Huang and Wu, 2008), and inorganic coagulants (Wu et al., 2006). Moreover, sludge properties and co-existed bulk supernatant characteristics (Huang and Wu, 2008) also play an important role in sludge filterability and membrane fouling. It has been revealed that almost all of sludge properties display influential correlation with membrane fouling by affecting sludge filterability,

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such as biomass concentration (Yigit et al., 2008), viscosity (Sun et al., 2013), floc size (Zhang et al., 2006), bound and/or free extracellular polymeric substances (EPS) (Lin et al., 2014), although their interdependency is unclear, up to now. Recently, researchers suggest that organic substance polymeric transformation in the MBR liquid phase is primarily responsible for sludge filterability (Wang and Li, 2008; Sun et al., 2013; Johir et al., 2012). Notwithstanding, the sludge properties and bulk supernatant characteristics, in turn, are also affected in a certain level by microbial population and activity in the activated sludge (Lee et al., 2010; Wu et al., 2012; Ma et al., 2013). However, these relationships complicate the membrane fouling and often educe to controversial results and conflict conclusion, making the importance of sludge microbial community structure always being overlooked.

Sludge components and filterability, which are determined fundamentally by the intrinsic properties of biomass microbial population and community, are very important to understand fouling mechanisms and control (Van den Broeck et al., 2011; Lin et al., 2014). Activated sludge is a rather complex ecological system, in which different types of microbes co-exist, and mutually interact and compete. As for municipal wastewater treatment process, activated sludge is usually relative stably comprised of heterotrophic microbes and autotrophic nitrifier (Yu et al., 2010; Kumar et al., 2012; Gao et al., 2014), by former one the organic pollutants are biodegraded and generate large fraction of EPS fragment and soluble microbial product (SMP) in treated effluent, while by later one nutrients are removed biologically and produce less organic polymeric substances. Autotrophic nitrifiers are known to form rather dense and solid floc or compact micro-colonial structure (Yu et al., 2010), and hence it is reasonable to expect that enrichment of nitrifying bacteria in MBR sludge would improve the sludge filterability. The relative abundances of heterotrophic microbes and autotrophic nitrifiers in activated sludge are often influenced by influent wastewater composition in terms of C/N ratio or carbon and nitrogen loading (Wu et al., 2012; Gao et al., 2014; Gómez-Silván et al., 2014). Kumar et al. (2012) found that ammonia oxidizing bacteria to total bacteria under low C/N ratio condition is 10 times higher than that observed in a high C/N ratio condition, despite of that accurate nitrifying bacteria abundance estimation was needed to unveil its impact to fouling behavior. Lee et al. (2010) employed a single MBR for nitrification inorganic influent, and observed that its membrane fouling was quite moderate than that in a conventional MBR for domestic wastewater treatment. Moreover, according to the experimental results of Johir et al. (2012), a low C/N ratio influent for MBR resulted in a small organic loading rate, which could decrease the hydrophilic substances fraction in MBR bulk organic matters that was beneficial to sludge filterability. However, to the best of author's knowledge, most of studies mainly concerned about the MBR system treatment performances, membrane fouling and related microbial community shift that evaluated by using traditional molecular approaches, and there were few reports about the effect onto sludge filterability by nitrifying bacteria abundance in an MBR treating real municipal wastewater, with assist of accurate bacteria quantification technique.

Thereafter, the goal of this research is to investigate the effect of nitrifying bacteria abundance onto sludge filterability, and to alleviate membrane fouling from viewpoint of sludge microbial community regulation in an MBR treating real domestic wastewater. Abundance of nitrifying bacteria was estimated by using DNA extraction, PCR amplification and Illumina High-throughput pyrosequencing methods. An adsorption-MBR was developed to regulate the C/N ratio in MBR inlet for nitrifier enrichment. More attentions were paid to the sludge mixture solid- and liquid-phase properties, both of which were important for membrane fouling. The obtained results will be very important to understand sludge filterability improvement and to mitigate membrane fouling with practical significance.

## 2. Methods

### 2.1. MBR system and experimental design

A submerged membrane bioreactor (marked as $R_1$) with a working volume of 180 L was used as a blank for comparison. A 0.4 μm polyethylene hollow-fiber membrane module (surface area = 3 $m^2$, Mitsubishi Rayon) was immersed. A suction pump (Master FLEX, Cole-Parmer) was used to withdraw the effluent through the membrane at a filtration-to-idle cleaning ratio of 8 min:2 min. Aeration was provided at the bottom of the reactor for continuous membrane cleaning. While the $R_1$ was operated in constant flux mode kept of 0.45 $m^3/(m^2\text{-d})$, the trans-membrane pressure (TMP) was monitored with a manometer in mmHg to indicate membrane fouling evolution.

An adsorption MBR system (hereafter termed as $R_2$) for nitrifying bacteria regulation comprised of an organic degradation tank, an adsorption tank, an intermediate sedimentation tank and a MBR chamber (Fig. 1). The total HRT of $R_1$ and $R_2$ was kept identical at 4 h, the former which was consisted by the HRT of in every tank was about 0.27, 0.65, 1.14 and 2 h, respectively. The raw wastewater flowed firstly into bio-adsorption tank for a quick adsorption of the organic substance. Afterwards, the sludge mixture was subjected to a quick solid–liquid separation in the intermediate sedimentation tank, and then flowed to MBR chamber for residue organic degradation and nitrification, while the sludge concentrate was recirculated to the oxidation chamber for further COD removal. As that nitrogen compounds could not be absorbed by activated sludge, the C/N ratio in the MBR inlet would be decreased, by which the abundance of the nitrifying bacteria was well controlled.

The influent for $R_1$ and $R_2$ was real domestic wastewater collected from University Town Campus (Shenzhen, China). Besides, more than 10–20% glucose-based synthetic wastewater, which was prepared according to the basic recipe given in the Environmental Engineering Process Laboratory Manual of the AEESP (2001), was added to maintain the volumetric organic and/or nitrogen loading rates relatively stable. With a SRT and HRT kept around 20 d and 4 h, respectively, the biomass concentration in terms of the mixed liquor suspended sludge (MLSS) was maintained at a level of around 5 g/L throughout the 10 months experiment by regular sludge discharge.

### 2.2. Sludge samples and characterization

The sludge mixture was sampled from $R_1$ and $R_2$ twice a week. Activated sludge (AS) in MBR was analyzed in the terms of its MLSS, SMP, EPS and biopolymer cluster (BPC) according to the previous procedure (Sun and Li, 2011; Sun et al., 2011). For a sludge suspension sample of 50 mL, the biomass and the liquid phase were separated by sedimentation at 4 °C for 2 h. The centrate of the sludge, or the AS supernatant, was collected to analyze its organic content, including its TOC and the concentrations of proteins and polysaccharides. The organic content of the MBR effluent was regarded as SMP. The difference in TOC concentration between the AS supernatant and the MBR effluent was assigned to the BPC. The biomass of the MBR sludge was also analyzed for its EPS according to previous reports (Sun et al., 2011; Wang and Li, 2008).

The SMP, one major precursor of BPC, were characterized in terms of hydrophilicity fractionation and molecular weight distribution. The former one was determined according to the method of resin fractionation (Xiao et al., 2009). XAD-8, XAD-4 and IRA