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Effect of intermittent aeration cycle on nutrient removal and microbial community in a fluidized bed reactor-membrane bioreactor combo system

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HIGHLIGHTS

- Complete nitrification, high SND and phosphorus recovery were achieved in FBR–MBR.
- The pH in FBR and MBR were found working in harmony.
- The FBR–MBR combo system was a suitable niche for diverse microbial groups.
- Varying intermittent aeration cycle resulted in microbial community shift.
- *Proteobacteria*, *Firmicutes* and *Bacteroidetes* were the dominant phyla identified.

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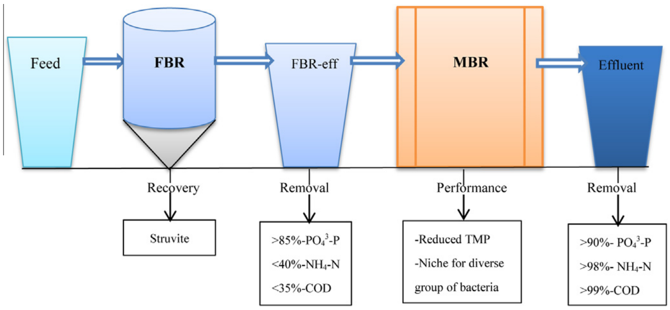
GRAPHICAL ABSTRACT



ABSTRACT

Effect of intermittent aeration cycle (IAC = 15/45–60/60 min) on nutrient removal and microbial community structure was investigated using a novel fluidized bed reactor–membrane bioreactor (FBR–MBR) combo system. FBR alone was found more efficient for removing PO_4-P (>85%) than NH_4-N (<40%) and chemical oxygen demand (COD < 35%). However, in the combo system, COD and NH_4-N removals were almost complete (>98%). Efficient nitrification, stable mixed liquor suspended solid and reduced trans-membrane pressure was also achieved. Quantitative real-time polymerase chain reaction results of total bacteria 16S rRNA gene copies per mL of mixed-liquor varied from $(2.48 \pm 0.42) \times 10^9$ initial to $(2.74 \pm 0.10) \times 10^8$, $(6.27 \pm 0.16) \times 10^9$ and $(9.17 \pm 1.78) \times 10^9$ for 15/45, 45/15 and 60/60 min of IACs, respectively. The results of clone library analysis revealed that *Proteobacteria* (59%), *Firmicutes* (12%) and *Bacteroidetes* (11%) were the dominant bacterial group in all samples. Overall, the combo system performs optimum nutrient removal and host stable microbial communities at 45/15 min of IAC.

Abbreviations: AOB, ammonia oxidizing bacteria; COD, chemical oxygen demand; DO, dissolved oxygen; EPS, extracellular polymerase substance; FBR, fluidized bed reactor; IAC, intermittent aeration cycle; MBR, membrane bioreactor; MLSS, mixed liquor suspended solid; MLVSS, mixed liquor volatile suspended solid; NE, nitrification efficiency; NOB, nitrite oxidizing bacteria; PCR, polymerase chain reaction; qPCR, quantitative PCR; SMP, soluble microbial product; SND, simultaneous nitrification denitrification activity; TMP, transmembrane pressure.

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1. Introduction

Enrichment of surface water with nutrients (phosphorus and nitrogen) from municipal wastewater treatment discharges is an important water quality concern due to eutrophication. As a result, regulations of the nitrogen and phosphorus contents from wastewater discharge are becoming increasingly more stringent (Ahmed, 2012). Currently, different technologies have been employed to remove these nutrients from wastewater.

Phosphate removal as struvite ($MgNH_4PO_4{\cdot}6H_2O$) using fluidized bed reactors (FBRs) has been widely reported in literatures (Adnan et al., 2003; Le Corre et al., 2007; Liu et al., 2008; Guadie et al., 2013a). Phosphorus recovery as struvite is not only solving the problem of eutrophication but also a sustainable option for replacing the stock phosphate that is being depleted. Phosphate reserve depletion has been estimated less than 100 years (Shu et al., 2006), which drew the attention of the scientific community to focus on alternative strategies to obtain phosphorus (Adnan et al., 2003; Shu et al., 2006; Le Corre et al., 2007). Guadie et al., 2013a designed a novel three cone-inserted FBR that could achieve an efficient phosphorus removal (>90%) and high quality struvite recovery at low and high phosphorus concentrations. Using an internal recycling reactor, Liu and his colleagues (Liu et al., 2008) also observed 78% phosphorus recovery at low phosphorus concentration. Although FBRs are efficient for phosphorus removal and recovery, parallel nitrogen removal as to the discharge limit was not reported. Using FBR for wastewater treatment, Adnan et al. (2003) and Le Corre et al. (2007) observed low nitrogen (<50%) removal than phosphorus (about 90%). Compared to FBR, membrane bioreactor (MBR) has been found a promising nitrogen removal technology as it can enhance growth of bacteria for nitrification and denitrification process (Duan et al., 2009; Guo et al., 2009; Kornboonraksa et al., 2009; Xia et al., 2010).

Nitrification is completed in two steps: ammonium (NH_4) oxidized into nitrite (NO_2) by ammonia-oxidizing bacteria (AOB), and NO_2 oxidized into nitrate (NO_3) by nitrite-oxidizing bacteria (NOB) (Ahmed, 2012). Although the stoichiometric showed the requirement of 2 mol of oxygen (75% for AOB and 25% for NOB) per mol of nitrogen to be nitrified (Ruiz et al., 2003), there has been several reports that also showed complete nitrification below 2.0 mg/L dissolved oxygen (DO) concentration (Bellucci et al., 2011; Wang et al., 2012). Current research efforts on intermittent aeration cycles (IAC) are leading to reduce the level of DO required for nitrification process. During IAC (on/off), the main advantage has been reported the coexistence of nitrifier and denitrifier microbial communities that can perform simultaneous nitrification-denitrification (SND) activities in a single reactor (Third et al., 2003; Rong et al., 2007; Wang et al., 2012). This is due to DO level fluctuation which creates anoxic/oxic microenvironment during "on" and "off" operations (Huang and Ju, 2007; Guadie et al., 2013b). Depending on DO level in the environment, some microbes were reported to shift their electron accepter from oxygen to other preferential sources which in turn leads metabolic activity shift (Feng et al., 2007; Sadaie et al., 2007). Using an online nicotinamide adenine dinucleotide (NAD) fluorescence profiles, Huang and Ju (2007) confirmed that the change of metabolic state observed in the reactor was associated with the shift of electron-accepting mechanisms in the sludge's microbial populations. Sadaie et al. (2007) detected *Burkholderiales* which could survive under aerobic and anaerobic conditions, based on electron acceptor shift. Ammonia oxidizers (like *Nitrosomonas europaea* and *Nitrosomonas eutropha*) were also identified a versatile group of microorganisms found in many natural and engineered ecosystems due to their metabolism flexibility (Sofia et al., 2004; Feng et al., 2007).

Apart from oxygen saving and avoiding two reactor configuration, the advantages of SND such as reduced organic substrate requirements for heterotrophic denitrification, lower biomass production, and self-balanced pH were also reported (Rong et al., 2007; Wang et al., 2012). During nitrification process, hydrogen ion (H^+) released causes a pH drop in the reactor. However, this drop counter balanced during denitrification process that produces hydroxyl ion (OH^-) (Rong et al., 2007).

Traditionally, microorganisms in wastewater has been analyzed using microscopic observation and culture dependent techniques, although these methods only estimate 1–15% of the total activated sludge community (Amann et al., 1995). To overcome the limitation of culture dependent methods, recently molecular techniques such as polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE), fluorescence in situ hybridization, quantitative PCR (qPCR) and 16S rRNA clone library methods have been applied to the detection of microbial community abundance and diversity at various environmental samples (Duan et al., 2009; Wittebolle et al., 2009; Xia et al., 2010; Zhang et al., 2012).

Although a novel combination of MBR with other systems have been evaluated at laboratory and pilot scale for efficient nutrient and organic matter removal (Guo et al., 2009; Kornboonraksa et al., 2009; Guadie et al., 2013b), parallel microbial community analysis data have not been available yet. For instance, Guadie and his colleagues (Guadie et al., 2013b) has been designed a novel FBR–MBR combo system and found a promising reactor performance, however; microbial community information has not been provided. As a result, this study was carried out to support the biochemical data with microbial community analysis. Nutrient removal efficiency (targeting struvite recovery in FBR and nitrogen removal in MBR) and microbial community structures has been investigated by considering IACs as a major factor that has been previously showed significant difference in the reactor performance. The abundance and diversity of the microbial community in the MBR system was investigated using qPCR and clone library construction for total bacteria 16S rRNA gene.

2. Methods

2.1. Wastewater characteristics

The synthetic wastewater used in this study was prepared according to Xia et al. (2010) with some modifications. It contains mg/L: starch (175), glucose (200), peptone (28), urea (64), ammonium chloride (150), potassium dihydrogen phosphate (52.5), magnesium chloride hexahydrate (150) and calcium sulfate dihydrate (50). Trace mineral solution containing (mg/L): $MnCl_2{\cdot}4H_2O$ (30), H_3BO_3 (300), $CoCl_2{\cdot}6H_2O$ (10), $CuCl_2{\cdot}2H_2O$ (10), $NiCl_2{\cdot}6H_2O$ (10), $ZnCl_2$ (100) and $FeSO_4{\cdot}7H_2O$ (300) were mixed together and 1 mL/L was used. Sodium hydroxide (NaOH = 12.5 mM) was also separately prepared for pH adjustment in the FBR (Guadie et al., 2013a).

2.2. Sludge source

The reactor was inoculated with mixed liquor that was obtained from a secondary clarifier from Quyang wastewater treatment plant (Shanghai, China). The pH, DO, mixed liquor suspended solid (MLSS) and mixed liquor volatile suspended solid (MLVSS) were measured and their average values were found 6.93 ± 0.32, 0.64 ± 0.41 mg/L, 3.62 ± 0.40 g/L and 2.72 ± 0.23 g/L, respectively. In order to make the source uniform, the sample was stored at 4 °C using 20% glycerol. When the new running conditions were needed, the stored inoculum was acclimatized before feeding to the MBR.