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Pyrosequencing analysis of microbial communities in hollow fiber-membrane biofilm reactors system for treating high-strength nitrogen wastewater

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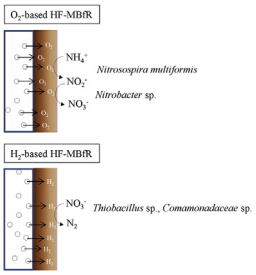
HIGHLIGHTS

- The nitrogen removal from wastewater was evaluated in autotrophic Hf-MBfR system.
- Only gaseous substrates such as O_2 and H_2 were supplied through a hollow fiber membrane.
- The pyrosequencing was performed for evaluation to establish autotrophic microbial community.
- The autotrophic microorganism was enriched within a relatively short period in Hf-MBfR.

GRAPHICAL ABSTRACT



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ABSTRACT

Wastewaters from swine farms, nitrogen-dealing industries or side-stream processes of a wastewater treatment plant (e.g., anaerobic digesters, sludge thickening processes, etc.) are characterized by low C/N ratios and not easily treatable. In this study, a hollow fiber-membrane biofilm reactors (HF-MBfR) system consisting of an O_2-based HF-MBfR and an H_2-based HF-MBfR was applied for treating high-strength wastewater. The reactors were continuously operated with low supply of O_2 and H_2 and without any supply of organic carbon for 250 d. Gradual increase of ammonium and nitrate concentration in the influent showed stable and high nitrogen removal efficiency, and the maximum ammonium and nitrate removal rates were 0.48 kg NH_4^+-N m^{-3} d^{-1} and 0.55 kg NO_3^--N m^{-3} d^{-1}, respectively. The analysis of the microbial communities using pyrosequencing analysis indicated that *Nitrosospira multiformis*, ammonium-oxidizing bacteria, and *Nitrobacter winogradskyi* and *Nitrobacter vulgaris*, nitrite-oxidizing bacteria were highly enriched in the O_2-based HF-MBfR. In the H_2-based HF-MBfR, hydrogenotrophic denitrifying bacteria belonging to the family of *Thiobacillus* and *Comamonadaceae* were initially dominant, but were replaced to heterotrophic denitrifiers belonging to *Rhodocyclaceae* and *Rhodobacteraceae* utilizing by-products induced from autotrophic denitrifying bacteria. The pyrosequencing analysis of microbial communities indicates that the autotrophic HF-MBfRs system well developed autotrophic nitrifying and denitrifying bacteria within a relatively short period to accomplish almost complete nitrogen removal.

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1. Introduction

High-strength ammonia wastewater, e.g., effluents from sludge thickening and digestion processes, landfill leachate, and wastewater from fertilizer producers is not easily treatable by a conventional biological process, since it can exert potential ammonia toxicity and does not contain enough carbon to support complete denitrification (Pollice et al., 2002; Guo et al., 2009; Sun and Nemati, 2012). In general, a ratio of carbon: nitrogen is 2.7–3 for complete nitrate reduction. Low C/N ratio waste such as landfill leachate and fertilizers need additional carbon source for denitrification microbial process. Therefore, application of autotrophic denitrification is one of alternatives for high nitrogen content waste.

Recently, researches have been carried out to improve the O_2 transfer or utilization rate and to apply autotrophic denitrifiers to reduce NO_2^- and NO_3^- to N_2, for purifying nitrogen-rich wastewater (Sun et al., 2010). Recently, hollow fiber-membrane biofilm reactor (HF-MBfR) systems have been investigated to treat NH_4^+-rich wastewater (Lee and Rittmann, 2000, 2002; Martin and Nerenberg, 2012). The unique feature of this HF-MBfR system is that gas is supplied from the inside of membrane fibers to the liquid phase. Therefore, gas transfer flux to liquid phase can be better controlled to induce higher gas utilization, and achieve a higher nitrogen removal efficiency (Lee and Rittmann, 2000, 2002; Martin and Nerenberg, 2012). Absence of a liquid diffusion layer between the biofilm and the membrane induces higher gas transfer efficiency (Tang et al., 2010; Martin and Nerenberg, 2012). The gas is diffused via gas-permeable membrane from inner side of biofilm but ionic substrates such as NH_4^+ and NO_3^-, are supplied from the bulk liquid phase (counter-diffusion). Compared with a conventional biofilm system (co-diffusion), in the membrane-aerated biofilm reactor, the microbial composition of the biofilm is important for reactor performance (Lackner et al., 2010). Specially, the autotrophic nitrification and denitrification induce less biomass than heterotrophic treatment and improve nitrogen removal efficiencies by increasing substrate transport in the stratified biofilm (Beyenal and Lewandowski, 2000; Celmer et al., 2008). Previous studies reported energy savings as 40–75% comparing to conventional activated sludge (Suzuki et al., 1993; Semmens, 2005). However, coexistence of autotrophic and heterotrophic bacteria has been found in autotrophic nitrifying biofilms (Rittmann et al., 1994; Lebuhn et al., 2003). The microbial products by autotrophic nitrifying bacteria support the growth of heterotrophic nitrification.

An oxygen-based HF-MBfR (Eq. (1)) was used for the nitrification (Shin et al., 2005, 2008) and a hydrogen-based HF-MBfR (Eq. (2)), for removing oxidized nitrogen, e.g., nitrate through denitrification (Lee and Rittmann, 2000, 2003, 2002; Shin et al., 2005, 2008; Tang et al., 2010).

$$NH_4^+ + 1.863\ O_2 + 0.098\ CO_2 \rightarrow 0.0196\ C_5H_7NO_2 + 0.98\ NO_3^- + 0.941\ H_2O + 1.98\ H^+ \quad (1)$$

$$NO_3^- + H^+ + 2.86\ H_2 + 0.15\ CO_2 \rightarrow 0.0286\ C_5H_7NO_2 + 0.49\ N_2 + 3.14\ H_2O \quad (2)$$

According to Eqs. (1) and (2) (Hwang et al., 2010), stoichiometric oxygen and hydrogen requirements for nitrification and denitrification are 4.26 g O_2/g N and 0.41 g H_2/g N, respectively. In terms of alkalinity, autotrophic nitrification of 1 mg N L^{-1} NH_4^+ consumes 0.588 mg L^{-1} $NaHCO_3$ while 0.9 mg L^{-1} $NaHCO_3$ is required for autotrophic denitrification. Autotrophic hollow fiber-membrane biofilm reactors system could be optimized by preventing gas overconsumption without biofouling. Additionally, the study of microbial community structure and the identification of autotrophic bacteria in a real autotrophic HF-MBfR could help understanding the system performance in nitrogen removal. However, none of the papers has analyzed how the microbial community would develop in an autotrophic HF-MBfR.

Nitrogen is removed by two sequential microbial processes; the first process is nitrification in which NH_4^+ is oxidized (=nitrified) to NO_2^- and NO_3^- by autotrophs using O_2 as a terminal electron acceptor, and the second is denitrification in which NO_3^- or NO_2^- is reduced (=denitrified) to N_2 by heterotrophs using organic carbon as an electron donor. In previous studies on microbial communities in biofilms for nitrogen removal, ammonia-oxidizing bacteria (AOB) were dominant inside the biofilm (Hibiya et al., 2003) or only near the membrane (Cole et al., 2004). However, our previous work showed there was no local variation of microbial community in nitrification while a special variation in denitrification (Shin et al., 2015). In an aerobic fluidized bed reactor, *Nitrosomonas*-like bacteria was dominant in the granules after 300 d operation (Tsuneda et al., 2003). The analysis of microbial community in membrane-aerated biofilm reactors showed *Nitrosomonas* sp. was dominant (Gong et al., 2008). In a study where the impact of COD/N ratio on the microbial community structure of a membrane aerated biofilm reactor was investigated, only *Nitrosospira* could be detected at COD/N ratio of 5, while they were co-existed with *Nitrosomonas* at COD/N of 0 (Cydzik-Kwiatkowska and Wojnowska-Baryła, 2011). The structure of the ammonia oxidizer community did not change even at increased ammonium concentration (Mendum et al., 1999; Avrahami et al., 2002). *Nitrosomonas oligotropha*, which was the most abundant in the inoculated sludge, was often found in a biofilter (Wahman et al., 2011) or in a full-scale activated sludge process for sewage treatment (Dionisi et al., 2002; Limpiyakorn et al., 2006). This species well adapts to a low DO environment (Park and Noguera, 2004) and to a high N load condition (Cabrol et al., 2016). In a study on the stratification of AOB in a biofilm system, *Nitrosomonas* was found more abundant in the oxic-zone whereas *Nitrosospira* was found in the interface between the oxic and the anoxic zones (Schramm et al., 2000). *Nitrosospira multiformis*, autotrophic AOB relatively well adapt to an environment of high ammonium concentration (Jordan et al., 2005). For the analysis of nitrite-oxidizing bacteria (NOB), *Nitrobacter* outcompeted *Nitrospira* among NOB in a membrane-aerated biofilm reactor (Terada et al., 2010). *Nitrobacter* showed a higher specific activity than *Nitrospira* and it was dominant at a relatively high nitrite concentration (Kim and Kim, 2006; Haseborg et al., 2010) or in biofilm regions of 2 mg L^{-1} DO or greater (Downing and Nerenberg, 2008).

Denitrification biofilm showed a more complex microbial community structure. Autotrophic denitrifying bacteria (*Thiobacillus denitrificans*) constituted only 32% of all clones (Koenig et al., 2005) because microbial decay products could support the growth of heterotrophic denitrifying bacteria (Martin and Nerenberg, 2012). In an H_2-based MBfR for nitrate and perchlorate reduction, *Marinobacter hydrocarbonoclasticus* represented 53% of all clones in the MBfR biofilm (Van Ginkel et al., 2010). In a glass bead biofilm reactor using H_2 as an electron donor for denitrification, *Hydrogenophaga* sp. was a dominant species (Park et al., 2005). Previous studies show that the co-existence of autotrophs and heterotrophs in a hydrogenotrophic denitrification system is unavoidable, but it results in a higher denitrification efficiency (Lee et al., 2008; Zhao et al., 2012).

The pyrosequencing is a high-throughput DNA sequencing method to investigate the microbial community in a complex system such as wastewater treatment plants (Wang et al., 2012),