Effect of UV on De-NO\textsubscript{x} performance and microbial community of a hybrid catalytic membrane biofilm reactor

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Abstract. The hybrid membrane catalytic biofilm reactor provides a new way of flue gas denitrification. However, the effects of UV on denitrification performance, microbial community and microbial nitrogen metabolism are still unknown. In this study, the effects of UV on de-NO\textsubscript{x} performance, nitrification and denitrification, microbial community and microbial nitrogen metabolism of a bench scale N-TiO\textsubscript{2}/PSF hybrid catalytic membrane biofilm reactor (HCMBR) were evaluated. The change from nature light to UV in the HCMBR leads to the fall of NO removal efficiency of HCMBR from 92.8\% to 81.8\%. UV affected the microbial community structure, but did not change microbial nitrogen metabolism, as shown by metagenomics sequencing method. Some dominant phyla, such as Gammaproteobacteria, Bacteroidetes, Firmicutes, Actinobacteria, and Alphaproteobacteria, increased in abundance, whereas others, such as Proteobacteria and Betaproteobacteria, decreased. There were nitrification, denitrification, nitrogen fixation, and organic nitrogen metabolism in the HCMBR.

1. Introduction

Biological technology is an attractive alternative technology for flue gas denitrification due to their low cost of maintenance. A biotrickling filter (BTF) established with aerobic denitrifying bacterium \textit{Pseudomonas aeruginosa} PCN-2 [1]. Biofilter of nitric oxide has been undertaken using different packing materials such as compost, perlite, biofoam, carbon foam, lava, and soil [2]. A biotrickling filter was designed and operated to remove NO from an air stream using \textit{Pseudomonas} [3]. The effect of glucose added into biofilter would significantly enhance the NO removal efficiencies for both anaerobic and aerobic conditions, respectively [4]. Nitrate accumulation of too much would cause N\textsubscript{2}O formation, affect the NO removal [5]. A rotating drum biofilter was applied to removal of NO denitrification in comparison with traditional bioreactors [6]. A hollow-fiber membrane bio reactor was used for NO removal at temperatures between 20\degree C and 55\degree C due to concurrent nitrification/denitrification using an endogenous carbon source [7]. Oxygen concentration was a key factor of denitrifying[8]. The suspended biofilter was constructed for NO removal under thermophilic conditions[9].

UV pretreatment could enhance performance of biofilter and metabolic activities [10]. Presence of UV photodegradation had better effects on the subsequent biotrickling filter performance[11]. With the addition of UV, an overall efficiency of more than 85\% was re-achieved in BTF since excessive
biomass was removed. Microbial community structure in BTF became more complicated with the addition of UV photodegradation [12]. Dichloromethane removal performance of the combined UV – BTF was much better than the single BTF. Ozone remaining from UV controlled the secretion of EPS and thus maintained normally. The microbial diversity was higher and biomass distributed evenly in combined BTF [13]. A Fe-TiO2/PSF hybrid catalytic membrane biofilm reactor offered potential for flue gas denitrification [14]. However, the effect of UV on denitrification performance, microbial community and microbial nitrogen metabolism are still unknown.

The objective of this work is to study the effect of UV on de-NOx performance, microbial community and microbial nitrogen metabolism of a bench scale N-TiO2/PSF hybrid catalytic membrane biofilm reactor (HCMBR). The study analysis bacterial community composition assessed by High-throughput sequencing, and analysis the nitrification and denitrification process, as well as microbial nitrogen metabolism pathway, which is believed to promote the application of the hybrid catalytic membrane biofilm reactor.

2. Materials and methods

2.1. Experimental procedure

The experimental flow loop of hybrid catalytic membrane biofilm reactor (HCMBR) used in the study was shown schematically in Figure 1. The HCMBR included an N-TiO2/PSF hybrid catalytic membrane biofilm reactor, a waste gas mixture (NO and clean air) generation system, and a nutrient supply system. Burkholderia sp., NO-biodegrading bacteria adhered to the surface of the polysulfone hollow fiber membrane to form the biofilm. The NO supplied from the gas cylinders, was first diluted with the compressed air, passed through an air mixture bottle, then flowed upwards the bottom of the hybrid catalytic membrane bioreactor.

![Figure 1. Schematic diagram of the N-TiO2/PSF hybrid catalytic membrane biofilm reactor (HCMBR).](image)

2.2. Analytical methods

Bacterial community compositions and the gene function of bacterial in the hybrid catalytic membrane bioreactor were assessed by 16S rDNA and metagenomics method. Nitric oxide concentration was measured by a Testo350 flue gas analysis device (Testo AG, Germany). Gas flow rates were measured using Model LZB-1 flow meters with units of 0.1 Lmin⁻¹. Liquid flow rates were measured using Model LZB-1 flow meters with units of 0.1 mLmin⁻¹. Illumination intensity was measured using SIGMA AR823 Separation of illuminance analysis device (SIGMA, Hong Kong). The pH values were measured by a Model pHB-3 pH Tester (Sanxin Instrument Company, Shanghai, China).

3. Results and discussion

3.1. Effect of UV on performance of the HCMBR system

Figure 2 showed the performance of the HCMBR for NO removal during the 270-d continuous running test under the conditions of pH of 7.2~7.4, sprinkling amount of 50~100 mL·min⁻¹·m⁻²·min⁻¹, NO inlet load of 25~200 g·m⁻³·h⁻¹, illumination intensity (620-700 lux) and residence time (RT) of 4.1~16.3 s, during which in three phases at different light source were tested. Three distinct phases
were observed: phase I inlet load of 25 g·m⁻³·h⁻¹, lasted from days 1 to 50, corresponds to the acclimation of microbial communities; in phase II load change from 25 to 200 g·m⁻³·h⁻¹, from days 51 to 270 under nature light irradiate, effect of inlet load, pH, spray flow (SF) on total performance of the HCMBR was investigated; in phase III high load from 200 g·m⁻³·h⁻¹, from days 271 to 296 under UV irradiate. In each phase, performance of the HCMBR was investigated.

In phase I from days 1 to 50 under solar irradiate (figure 2(a)), NO was removed from the start of the injection pollutants highlighting a short acclimation phase of PSF, NO inlet load was set to 25 g·m⁻³·h⁻¹ and residence time (RT) of 16.3 s from days 1 to 20. NO removal efficiency (RE) changed from 46.8 to 55% before 7 d, then increased from 67.3% at 8th d to 84% at 20th d. NO removal efficiency attained 90% from days 21 to 30. Nitrification and denitrification bacteria were attached to the hollow fiber membrane at the beginning, but also easy to be washed down by circulation fluid because it didn’t form a stable ecosystem. The possible reason for the small increase in NO removal efficiency before 30 d could be that the membrane catalysis, absorption yield to membrane biodegradation. NO removal efficiency increased from 69.8% to 97% when NO inlet load was set to 50 g·m⁻³·h⁻¹, and residence time (RT) of 8.2 s, HCMBR under nature light from days 31 to 50, showing good NO degradation effect.

In phase II from days 51 to 270 (figure 2(b)), NO RE rose after falling, and changed from 85.3% at the 51st d to 89.7% at the 54th d, when inlet load increased from 50 to 75 g·m⁻³·h⁻¹, RE kept about 91.5% from days 57 to 60. NO removal efficiency changed small from 88% at 71th d to 86% at 75th d when inlet load was set to 100 g·m⁻³·h⁻¹. RE also rose after falling, kept at 78% from days 108 to 170 with increasing of inlet load from 100 to 200 g·m⁻³·h⁻¹. NO removal efficiency decreased from 76% to 65% when pH value was decreased from 6.6 at 171~180 days to 5.3 at 191~200 days. RE decreased from 78% to 64% when pH value increased from 7.0 at 201-215 days to 8.6 at 226-235 days by adding lye. So, NO RE was increased to 78% when pH may be adjusted to neutral from days 236 to 245. NO removal efficiency changed small, HCMBR stability becomes worse when spray flow was changed from 50 to
200 mL·min⁻¹ from days 246 to 270. NO removal efficiency was up to 92.8%, and the maximum elimination capacity was 242 g-NO·m⁻³·h⁻¹ during the nature light time of 220 days, respectively.

During the 26-day phase III, investigating the effect of UV light on performance of HCMBR (figure 2(c)). When the light source was changed from nature light to UV light in the HCMBR at 271th d, NO removal efficiency increased from 77.8% at 271th d to 81.8% at 271th d, but decreased from 75.9% at 273th d to 74.5% at 274th d; increased from 76.9% at 275th d to 80% at 276th d, and then stayed about 80% from days 277 to 285 under illumination intensity of UV (80 lux). Possible reasons for the increase could be the N-TiO₂ photocatalysis promoting NO gas membrane mass transfer, NO oxidation and biochemical degradation, long-term UV irradiation inevitably produces a certain negative influence on the microbial community in a biofilm. When illumination intensity of UV was further increased from 80 to 180 lux at 286th d, NO removal efficiency fell after rising, attained 77.1% at 290th d. This suggests that the illumination intensity too strong, resulting in a decline in removal efficiency. Therefore illumination intensity was transferred to 80 lux at 291th d, NO removal efficiency rose, and then stayed about 80%. NO removal efficiency was up to 81.8%, and the maximum elimination capacity was 252 g-NO·m⁻³·h⁻¹, respectively. For comparison, in the presence of HCMBR, the changes from nature light to UV leads to the falling of NO removal efficiency of HCMBR from 92.8% to 81.8%, and the enhance of maximum elimination capacity up from 242 to 252 g-NO·m⁻³·h⁻¹, respectively.

3.2. The changes of nitrogen from nature light to UV

As demonstrated in figure 3(a), nitrate (NO₃⁻N) average concentration was 270 mg·L⁻¹, nitrite (NO₂⁻N) average concentrations were 12 mg·L⁻¹, ammonia nitrogen (NH₄⁻N) average concentration was 21 mg·L⁻¹ in the HCMBR under nature light. The results revealed that nitrification was the main one and denitrification existed in the nature light illumination process. While NO₃⁻N concentrations were 132 mg·L⁻¹, NO₂⁻N concentrations were 37 mg·L⁻¹, ammonia nitrogen (NH₄⁻N) average concentration was 32 mg·L⁻¹ in the HCMBR under UV light (Figure 3(b)). In this stage, although denitrification was strengthened, nitrification was still the main one in the UV illumination process. For comparison, nitrate (NO₃⁻N) concentrations of circulation in the HCMBR under UV light were significantly lower than that in the HCMBR under nature light, while NO₂⁻N, NH₄⁻N concentrations of circulation in the HCMBR under UV light were higher than that in the HCMBR under nature light. So it could reasonably be speculated that there must be nitrification and denitrification in the HCMBR reactor.

3.3. Effect of UV on microbial community

The microbial community structure and functional genes in N-TiO₂/PSF hybrid catalytic membrane biofilm reactor (HCMBR) were investigated for the samples of 210th day under nature light (S1) and 300th day under UV light (S2) by means of high-throughput sequencing. Results show that the microbial community structure was affected due to illuminant from nature light to UV light.

**Figure 3(a).** The change of nitrogen components of the recycled liquid under natural light.

**Figure 3(b).** The change of nitrogen components of the recycled liquid under natural light.
At phylum level (Figure 4), 2 communities were dominated by the *Proteobacteria* with the relative abundances of 82.6% and 64.9%, respectively. The relative abundances of dominant bacteria *Gammaproteobacteria* and *Betaproteobacteria* of S1 and S2 changed from 44.6 to 46.1% and 16.9 to 11.1%, respectively. Some phyla, such as *Gammaproteobacteria*, *Bacteroidetes*, *Firmicutes*, *Actinobacteria*, and *Alphaproteobacteria* increased in abundance, whereas others, such as *Proteobacteria* and *Betaproteobacteria* decreased. Some bacterial phyla including *Spirochaetes*, *Euryarchaeota*, *Thermotogae*, *Basidiomycota* and *Epsilonproteobacteria* appeared when light source was shifted to UV light. Photosynthetic bacteria were found such as *Cyanobacteria*, *Chloroflexi* and *Chlorobi*. *Bacteroidetes* used nitrate or nitrite as the ultimate electron acceptor at hypoxia or anaerobic condition [15]. *Proteobacteria* functioned in nitrification and denitrification [16-17].

![Figure 4](image1.png)  
**Figure 4.** Relative abundance of bacterial community at class levels in HCMBR under natural light (S1) or UV light (S2).

At order level, S1 and S2 were over 55% of tags sequence on the classification, 15 kinds of main composition as shown in figure 5. The relative abundances of dominant bacteria *Enterobacteriales* and *Burkholderiales* of S1 and S2 were in the change of 42.1 to 44.8% and 12.1 to 11%, respectively. Some orders, such as *Flavobacteriales*, *Rhizobiales*, *Sphingomonadales*, *Caulobacteriales*, *Sphingobacteriales*, *Cytophagales*, *Bacteroidales*, *Bacillales*, and *Actinomycetales*, increased in abundance, whereas others, such as *Xanthomonadales*, decreased. *Enterobacteriales* could fix nitrogen using N₂ from denitrification for microbes absorbing ammonia nitrogen in the HCMBfR reactor [18]. *Burkholderiales* has the functions of nitrification, denitrification [19-21]. *Xanthomonadales* has nirS for cytochromed c nitrite reductase [22-23].

At the level of genus, 46 species of bacteria genera were identified in S1, and bacteria genera in the S2 was over 200. 50 species were the dominant bacteria genera, as shown in figure 6. 24 dominant genera in S1 were not dominant in S2. 23 genera appeared. 19 genera didn’t change. *Delftia* (1.1%) dominated in S1while *Burkholderia* (9.75%) dominated in S2. *Delftia* converted organic nitrogen and *Burkholderia* was a nitrifying/denitrifying bacterium [24-25]. *Klebsiella*, *Comamonas* and *Escherichia* were nitrifiers and denitrifiers [26-28].

3.4. Effect of UV on metabolism genes and function gene

Figure 6 showed basic metabolism genes in 23 categories, dominated by membrane, amino acid metabolism, carbohydrate metabolism, cell process and signal, energy metabolism, replication and
repair, cofactors and vitamins (> 4%); followed by xenobiotics biodegradation metabolism, enzyme families, nucleotide metabolism, metabolism of other amino acids, motility, chemotaxis and photosynthesis (relative abundance: 2~3%); nitrogen metabolism and sulfur metabolism were below 1.0%. The relative abundances of membrane transport protein gene and motility and chemotaxis of S1 and S2 changed from 13.93 to 10.56%, 2.46% to 1.88%, respectively. Possibly UV photocatalytic activity was higher than that of visible light, but UV light had a certain inhibitory effect on microorganism. The relative abundances of replication & repair gene and nucleotide metabolism gene of S1 and S2 changed from 4.36 to 5.2%, 2.55% to 3.4%, respectively. Cells possibly accelerated destruction under UV irradiation. The relative abundances of nitrogen metabolism in S1 and S2 were 0.78% and 0.75% respectively. UV light had effect on microbial activity but little on denitrification.

Numbers of nitrification genes (Nitric oxide dioxygenase) in S1 and S2 were the highest (63 to 58), followed by denitrification (Periplasmic nitrate reductase, 12 to 5), Nitrate reductase (5 to 2), Nitrite reductase (8 to 7), Nitrous oxide reductase (14 to 16), Nitric oxide reductase (1 to 11), and nitrogen fixation (Nitrogenase (4 to 7), Glutamine synthetase(15 to 26)) (Figure 7). Aerobic nitrification first oxidized NO to nitrate, nitrogen fixation and ammonium assimilation enhanced and NO was photolysis as nitrous oxide under UV irradiation. Organic nitrogen metabolism, heterotrophic nitrifying bacteria existed in the HCMBR due to urease (6 to 7).

**Figure 6.** Category and distribution of functional genes in S1, S2.

**Figure 7.** The number of matched genes of functional enzyme in nitrogen metabolism.

### 4. Results and discussion

The paper revealed that UV affected de-NOx performance, microbial community structure, but not changed microbial nitrogen metabolism in the N-TiO2/PSF hybrid catalytic membrane biofilm reactor. In 220 days of nature light irradiation, NO removal efficiency was up to 92.8%, and NO elimination capacity at 242 g m⁻³ h⁻¹. In 26 days of UV light irradiation, NO removal efficiency was up to 81.8%, and NO elimination capacity at 252 g m⁻³ h⁻¹. The changes from nature light to UV to HCMBR leads to the falling of NO removal efficiency of HCMBR from 92.8% to 81.8%, denitrification was strengthened, the process of nitrification was still the main one. Some dominant phyla, such as *Gammaproteobacteria, Bacteroidetes, Firmicutes, Actinobacteria,* and *Alphaproteobacteria,* increased in abundance, whereas others, such as *Proteobacteria* and *Betaproteobacteria,* decreased. There were a microbial nitrification and denitrification process, nitrogen fixation process and organic nitrogen metabolism process in the N-TiO2/PSF hybrid catalytic membrane biofilm reactor.
Acknowledgment
The authors gratefully acknowledge the financial support from the Nation Nature Scientific Research Foundation of China (21377171).

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