Effect of Oxygen Content on NO Removal and Microbial Communities of a Hybrid Catalytic Membrane Biofilm Reactor

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Research Article

ABSTRACT

The effect of oxygen content on NO removal and microbial communities of a hybrid catalytic membrane biofilm reactor (HCMBR) has been investigated. NO removal efficiency reached 84.8%, 85.1%, 94% and 94.5% at oxygen content of 2%, 6%, 10% and 17% in flue gas (H_2, H_6, H_{10} and H_{17}), respectively. NO removal increased with increasing oxygen content.

Denitrification was dominant in H_2, H_6; simultaneous nitrification and denitrification occurred in H_{10}, H_{17}. Oxygen content influenced the microbial community in HCMBR as shown by 16S rDNA, metagenomics sequencing method. The dominant phylum was *Fluviicola*, *Arcobacter*, *Brachymonas* in H_2, H_6, H_{10}, while *Brachymonas*, denitrificans, *vadinCA02* in H_{17}. *Fluviicola*, *Acrobacter*, *Brachymonas* and *Brachymonas* were dominant denitrifiers in H_2, H_6, H_{10} and H_{17}, respectively. Primary coordinate analysis (PcoA) indicated phylogenic structures in H_{10} and H_{17} were highly alike but dissimilar to those in H_2 and H_6. Canonical correlation analysis (CCA) classified visualized oxygen content dependence distinction of bacterial genera.

INTRODUCTION

Nitrogen oxides (NO_x) are an important precursor of haze particles, acid rain, photochemical smog and tropospheric ozone depletion, which generated from fossil fuel combustion [1]. China NO_x emissions can reach 18.5 million tons in 2015 [2]. Nitrogen oxides reduction through denitrification measures, such as selective catalytic reduction (SCR), selective non-catalytic reduction (SNCR), ozone oxidation and absorption [3]. The drawbacks of SCR including high cost, high energy assumption, leakage of ammonia and catalysts poisoning had posed unprecedented challenges [4]. Biotechnology is an effective technology for flue gas denitrification featured by cost-effectiveness, low consumption, no secondary pollution and simplicity in configuration. Bioprocesses for NO_x removal can be classified into bio-scrubber, bio-trickling filter, membrane biofilm reactor and hybrid membrane catalytic biofilm reactor, the flue gas denitration has nitrification and denitrification [5].

Membrane biofilm reactor offers large specific area for efficient mass transfer and biofilm colonization which is especially favorable for hydrophobic nitrogen oxides [6]. A hollow-fiber membrane bioreactor was used for NO removal by nitrification/denitrification at temperatures between 20°C and 55°C [7]. A hollow-fiber membrane bioreactor was stability and high efficiency for NO removal, NO removal efficiency and elimination capacity were inversely proportional to the inlet oxygen concentration [8]. The denitrification was inhibited by the increase of oxygen concentration, nitrification/denitrification and the dynamics of key bacterial communities were highly influenced by the dissolved oxygen concentration concentration [9]. NO removal efficiency in a bio-trickling filter significantly increased as oxygen content

increased from 4% to 20% [19]. Hybrid catalytic membrane bioreactor (HCMBR), intimate coupling of membrane catalysis and nitrification /denitrification, can significantly improve NO removal in flue gas [5]. The oxygen content of flue gas in medium and small sized boiler, industries furnaces, power station boiler are different and changes. However, influence of oxygen content in flue gas on NO removal performance and microbial communities in HCMBR may be poorly understood.

The objective of this work is to study the effect of oxygen content on nitric oxide removal performance and microbial community of a hybrid catalytic membrane biofilm reactor (HCMBR). The study evaluates the effect of oxygen content on nitrification/denitrification and membrane catalysis, and analyzes microbial diversity, bacterial community in HCMBR assessed by 16S rDNA and metagenomics sequencing method, which is believed to promote the application of the HCMBR.

MATERIALS AND METHODS

Experimental Procedure

The experimental flow loop of $N-TiO_2/PSF$ hybrid catalytic membrane biofilm reactor used in the study was shown in our previous study [5]. The influence of oxygen on nitrification/denitrification and microbial community of a hybrid catalytic membrane biofilm reactor (HCMBfR) were evaluated during the 120-d continuous running test under oxygen concentration of 2%, 6%, 10% and 17% (four distinct phases featured by H_2, H_6, H_{10} and H_{17}) a waste gas mixture (NO, O_2 and Ar) system.

High-throughput Sequencing Data Analysis

Bacterial community compositions and the gene function of bacterial in the hybrid catalytic membrane bioreactor were assessed by 16S rDNA and High-throughput sequencing method, and identify the colonies of the predominant microorganisms by the procedures of total DNA extraction, polymerase chain reaction (PCR) amplification of 16S rDNA, cloning and sequencing, calculation of similarity and diversity indexes, analysis of the successional route of the community. Sequencing libraries were generated by NEB Next® Ultra™ DNA Library Prep Kit for Illumina (NEB, USA) following manufacturer's recommendations and index codes were added. Nonmetric multidimensional scaling (NMDS) analysis is a commonly used tool to compare the similarity and dissimilarity between two complex systems. Chimera was detected by UCHIME, and the resulting high quality sequences were processed to generate operational taxonomic units (OTUs) by CD-HIT at the 97% sequence similarity threshold. The taxonomic assignment was performed with the RDP classifier with a confidence cutoff of 0.5. Hierarchical clustering analysis was performed using CLUSTER and visualized using TREEVIEW, and other statistical analyses were performed with the IEG pipeline (http://ieg.ou.edu).

Analytical Methods

The Testo350 flue gas analyzer (Testo AG, Germany) analysis device with a measurement accuracy of 1ppm was used for the analysis of nitric oxide concentration. Gas flow rates were measured using Model LZB-1 flow meters with units of 0.1 L min^{-1}. Liquid flow rates were measured using Model LZB-1 flow meters with units of 0.1 mL min^{-1}. The pH values were measured by a Model pHB-3 pH Tester with units of 0.1 (Sanxin Instrument Company, Shanghai, China). The dissolved oxygen concentration was measured by the YSI 550A Handheld Dissolved Oxygen Instrument with a field-replaceable YSI dissolved oxygen probe(YSI Environmental company, The United States).

RESULTS AND DISCUSSION

The Effect of Oxygen Content on NO Removal Performance of HCMBR

Figure 1 showed the effect of oxygen content on nitric oxide removal performance of the HCMBR during 120-d continuous running test under the conditions of sprinkling amount of 45 mL min^{-1}, NO inlet concentration of 133.9 mg m^{-3}, gas residence time (GRT) of 8.3 sec at normal temperature. Four distinct phases were observed by oxygen concentration of 2%, 6%, 10% and 17% (designated as H_2, H_6, H_{10} and H_{17}). In low oxygen conditions (H_2), NO removal efficiency (RE) gradually increased from 64.5% to 69.1% from 1st day to 3rd day. Afterwards, from 4th day to 9th day, RE maintained at 67.6%-69.4%. During the first 9 days, RE fluctuation was about 5.0% and average RE was 67.9%. After 3 days' sharp increase, RE became more stable in the following 6 days suggesting far better microbial acclimatization. RE increased from 71.9% to 75.4% from days 10 to 21, further from 80.6% to 85.6% from days 22 to 30. This indicated biofilm formation upon hollow fiber membrane surface was progressively underway and a stable microbial community structure formed gradually. But the newly-formed biofilm might block visible light and weakened NO catalytic removal performance. Thus, as NO biological removal performance enhanced, NO catalytic removal performance might decline. High NO removal performance might still be attributed to joint contribution of catalysis and biodegradation. At low O_2 content, denitrification might be the metabolic pathway for NO removal. When O_2 content in flue gas was increased to

6%, NO removal efficiency suddenly declined to 80.2% at 31st day. RE gradually increased from 80.2 % to 84.5 % from 31st day to 36th day. RE changed from 78.4% to 85.3% from 37th day to 52nd day. RE fluctuated in the range of 77.7%-82.1% from 53rd day to 60th day with an average of 80.2%. Despite frequent fluctuation of RE, the average RE was relatively high (81.0%).
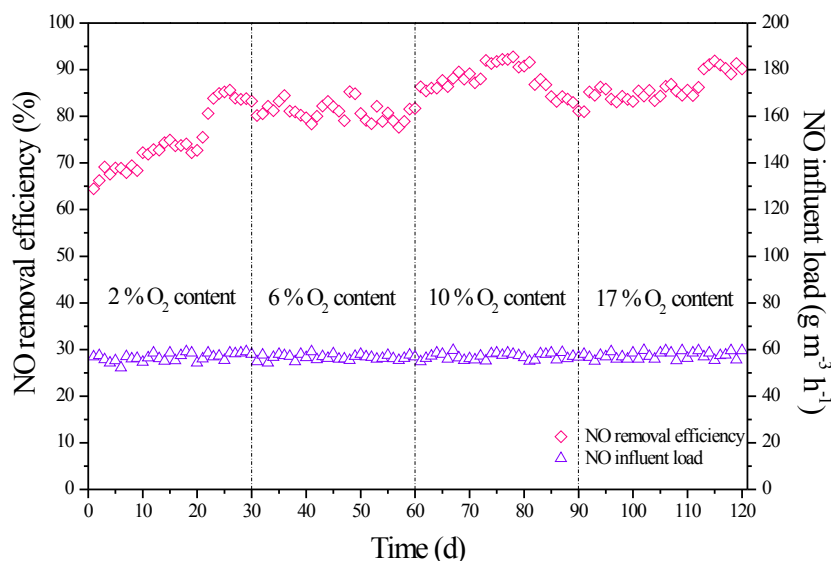

Figure1: The effect of oxygen on performance of HCMBR in different oxygen content during 120-d continuous running test

When O_2 content was further elevated to 10%, RE suddenly increased to 86.3% on 61st day. RE fluctuated in the range of 87.3% ± 2.0% from 61st day to 72nd day. NO removal performance achieved further improvement with O2 content increasing. RE suddenly increased to 92.0% on 73rd day and henceforth RE slightly fluctuated within the range of 91.7% ± 1.0% until 81st day. RE underwent an undulant decrease from 86.8% to 81.1% from 82nd day to 90th day. NO removal performance decline was possibly due to catalyst deactivation after long-term operation. RE in H_{10} was much higher than that in H_6. When O_2 content was further increased to 17%, RE increased from 81.0% to 85.2% from 91st day to 92nd day. RE slightly fluctuated within in the range of 84.9% ± 1.8% from 92nd day to 122nd day. This was probably attributed to oxygen and nitrate co-respiration thereby aerobic denitrifiers sustainably grew in aerobic environment [10]. HCMBR at 17% O_2 content exhibited far more remarkable NO removal performance.

NO removal performance improved and achieved stability gradually in different O_2 content. NO removal efficiency reached 84.8%, 85.1%, 94% and 94.5% in H_2, H_6, H_{10} and H_{17}, respectively; and elimination capabilities (EC) were up to 50.1, 50.3, 55.5, 55.8 g m^{-3} h^{-1}, respectively. O_2 content elevation led to higher NO removal, which was attributed to aerobic denitrification, heterotrophic nitrification and catalytic oxidation. Catalytic of nitric oxide could further improve NO removal performance and alleviate membrane fouling [11]. Continuous running strengthened microbial acclimatization and adaptability leading to further improvement of NO removal.

The Effect of Oxygen Content on Catalysis and Biodegradation

The effect of oxygen content on catalysis and biodegradation was shown in **Figure 2** under the conditions of sprinkling amount of 45 mL min^{-1}, NO inlet load of 133.9 mg m^{-3}, gas residence time (GRT) of 8.3 sec, oxygen content of 2%, 6%, 10%, 17% respectively. The catalytic reaction rate was 0.62, 1.19, 0.17, and 0.46 mg m^{-2} h^{-1}, the biochemical degradation rate was 1.08, 1.36, 1.89 and 2.86 mg m^{-2} h^{-1} with different oxygen contents of 2%, 6%, 10%, 17% respectively. The amount of membrane catalytic denitrification was 0.53, 1.0, 0.15 and 0.4 mg h^{-1}; the amount of biochemical denitrification was 1.02, 1.17, 1.63 and 2.46 mg h^{-1} at oxygen content of 2%, 6% ,10%, 17% in the HCMBR, separately. For comparison, higher oxygen content, the faster the biochemical degradation rate, the greater the amount of biochemical denitration, biochemical denitrification capability of HCMBR in H_{17}, H_{10}, H_6 were 2.41, 1.6, 1.15 times of that in H_2, respectively. It can be concluded that nitrification and denitrification was accelerated with increasing oxygen content in flue gas. The catalytic denitrification capability was generally in the order: $H_{10}<H_{17}<H2<H_6$. The possible reason for this was that the biofilm coverage increased at the catalytic layer with increasing oxygen content, resulting in decline of photocatalytic efficiency.
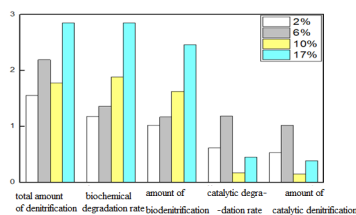
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Figure 2: The effect of oxygen content on catalysis and biodegradation

The Effect of Oxygen on Microbial Diversity

The microbial diversity in the HCMBR in different oxygen content (H_2, H_6, H_{10} and H_{17}) was assessed by 16S rDNA sequencing test. Sequences number in H_2 was the highest (208199), followed by that in H_{17} (110571). Sequences numbers in H_6 and H_{10} were 22105 and 29570, respectively, far lower than those in H_{17} and H_2. Operational taxonomic unit (OTUs) number in H_{17} turned out to be the highest (765), followed by that in H_6 (545). OTUs numbers in H_{10} and H_2 were 488 and 385, respectively. Thus, microbial diversity in H_{17} was the highest, microbial diversity in H_2 was the lowest. Chao 1 index was the theoretical estimate of OTUs number. Numerical order of Chao 1 indices conformed to that of OTUs numbers. Thus Chao 1 indices further confirmed degree of microbial diversity in each sample. Shannon index quantified evenness of microbial community. Shannon index in H_{17} was the highest (4.70) indicating highest evenness in sequences assignment, in another word, sequences number per OTU was the highest in H_{17}. Shannon index of H_2 was the lowest (4.24), thus microbial evenness in H_2 was the lowest. Simpson index in H_6 was the highest (0.938), while that in H_{17} was the lowest (0.865).Thus, microbial community in H_6 showed both better evenness and richness. Especially, number of observed species in H_6 (545) was equivalent to its OTU number (545). But number of observed species in the other samples deviated from their OTUs numbers. PD whole tree indices in 4 samples also followed accordant numerical trend to their OTUs numbers.

Similarity of each sample was visualized by means of principal coordinates analysis (PcoA) (**Figure 3a**). Approximately 95.16% systematic informational variation was explained by the first principal coordinate (PC_1: 59.58%) and the second principal coordinate (PC_2: 35.58%). According to distances between scatters, 4 samples can be classified into 3 groups: (1) H_2; (2) H_6; (3) H_{10} and H_{17}. H_2 deviated from the other 2 groups suggesting phylogenetic structure at 2% O_2 content was hugely different from those in the other 2 groups. H_6 also showed considerable spatial deviation from the other 2 groups suggesting phylogenetic structure at 6% O_2 content was also greatly dissimilar to those in the other 2 groups. H_{10} and H_{17} were closely clustered but deviated from the other 2 groups suggesting phylogenetic structures at 10% and 17% O_2 content were similar but different from those in the other 2 groups. This grouping indicated phylogenetic structure underwent 3 levels of microbial structural shift in response to oxygen content variation.
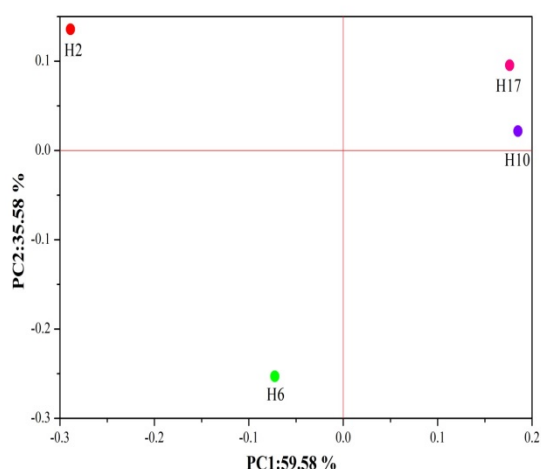

Figure 3a: Principal coordinates analysis of the four different communities

The influence of oxygen content on microbial community was shown by canonical correlation analysis (CCA) (**Figure 3b**). The result was visualized in a two-dimensional graph explaining 71.6% systematic information (Axis 1: 34.1%; Axis 2:

37.5%).The distances between the genera points in the diagram represented their similarity in relative abundance distribution across 4 samples. More precisely, shorter distance between 2 genera points indicated their relative abundance distribution underwent similar trend in response to O_2 content variation and they frequently occurred together. Based on these principles, the bacterial genera were classified into 6 groups. O_2 content arrow pointed in the direction of sharpest numerical increase. The specie points could be projected perpendicularly onto the line overlaying the O_2 content arrow. These projections can be used to approximate the optimal O_2 content for each genus. O_2 content arrow also pointed the increasing direction of optima for projection point of each genus. Optimal O_2 content for *vadinCA02* was the highest while that for *Xanthobacter* was the lowest. Genus with higher optimal O_2 content suggested it was more O_2 dependent. Thus, top 5 O_2 dependent genera were *vadinCA02*, *Brachymonas*, *HA73*, *Comamonadaceae(f)* and *BA008(f)* whereas the last 5 O_2 dependent genera were *Xanthobacter*, *Brevundimonas*, *Fluviicola*, *Citrobacter* and *Arcobacter*. In summary, PcoA indicated O_2 content variation caused 3 levels of phylogenetic shift to the microbial community. Clustering result revealed genera undergoing similar relative abundance variationacross 4 samples. CCA implied the phylogenetic shift was due to languish and flourish of different O_2 dependent genera. When O_2 content approximated optimum for a specific genus, this genus thrived and vice versa.
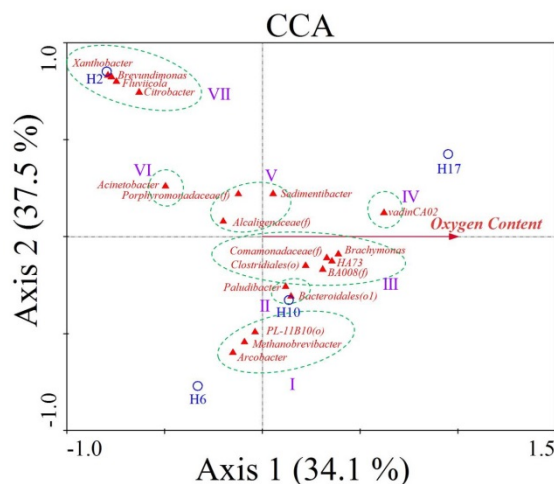

Figure 3b: Canonical correlation analysis of the four different communities

The clustering of 19 dominant bacteria genera $\geq$ 1% in different oxygen content was shown in **Figure 4**. Clustering of 4 samples was consistent with grouping in PcoA result. H_{17} and H_{10} were clustered together due to similar phylogenetic structure and further grouped with H_6, whereas phylogenetic structure in H_2 was distinct from the other 2 groups. Bacterial genera of similar relative abundance in 4 samples were clustered together, suggesting O_2 content variation wielded similar influence on their bacterial growth. The clustering result was consistent with analysis by16S rDNA sequencing. PcoA indicated O_2 content variation caused 3 levels of phylogenetic shift to the microbial community. Clustering result revealed genera undergoing similar relative abundance variation across 4 samples. CCA implied the phylogenetic shift was due to languish and flourish of different O_2 dependent genera.
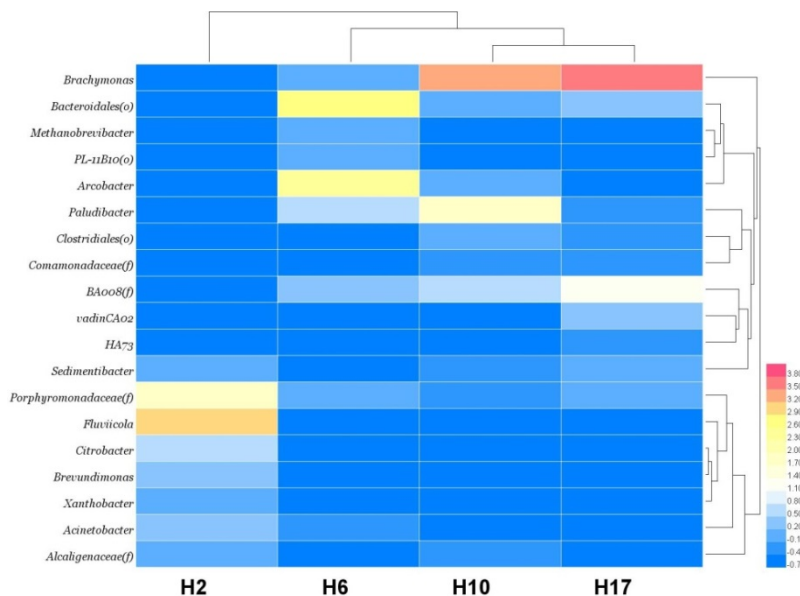
Figure 4: Clustering of 19 bacterial genera (≥ 1%) in different oxygen content

The Effect of Oxygen on Bacterial Community

At phylum level, the relative abundances of dominant bacteria *Bacteroidetes* and *Proteobacteria* of H_2, H_6, H_{10} and H_{17} were in the change of 53.78%, 44.52%, 37.12%, to 34.08%; and 36.22%,31.93%, 4.24%, to 40.03% respectively (**Figure 5a**).
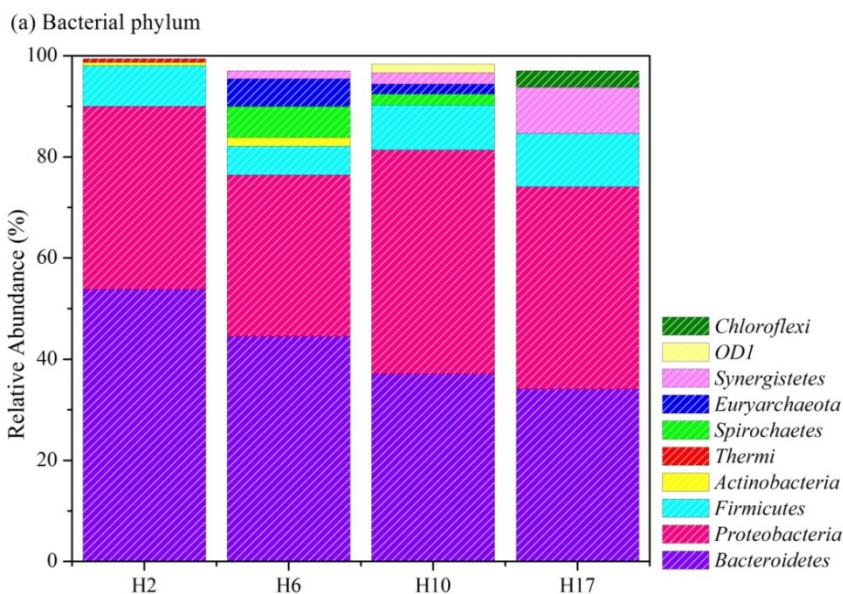

Figure 5a: Relative abundance of tax annotation at phylum (≥ 1%)

Bacteroidetes gradually decreased as O_2 content increased, this suggested it's active and competitive were decreasing in aerobic environment. *Bacteroidetes* intervened in proteolysis and performed amino acids fermentation to acetate and *Bacteroidetes* were denitrifiers [32]. *Bacteroidetes* was relevant to nitrogen removal and also dominated bacterial phyla in a simultaneous denitrification and sludge fermentation reactor [13]. Many nitrifiers and denitrifiers were affiliated to *Proteobacteria* [12]. The increase in abundance of *Proteobacteria* with O_2 content increased showed *proteobacteria* was not strictly anaerobic or aerobic genera. The relative abundances of subdominant phylum *Firmicutes* of H_2, H_6, H_{10} and H_{17} were in the change of 8.00%, 5.66%, 8.90%, 10.48%. *Firmicutes* was facultative anaerobic and possibly not sensitive to O_2 content variation, which was frequently found subdominant in nitrogen removal system [12,14]. In addition, subdominant phyla in H_2 included *Euryarchaeota* (5.49%) and *Spirochaetes* (6.13%). *Spirochaetes* only existed

Dominance in H_2, was chemoheterotrophic anaerobic digester and fermented glucose to acetate, ethanol and lactate. Besides, *Synergistetes* (9.15%) was another subdominant phylum in H_{17}. *Synergistetes* increased in abundance as O_2 content increased. *Synergistetes* usually dominated microbial phylum in anaerobic sludge and was capable of amino acid degradation [15]. *Euryarchaeota* was only subdominant phylum in H_6. Hydrogenotrophic and acetoclastic methanogens in anaerobic digester belonged to *Euryarchaeota* [16].

The relative abundances of *Fluviicola*, *Porphyromonadaceae(f)*, and *Acinetobacter* decreased with the increase of oxygen content at genus level. *Fluviicola* was the dominant genus in H_2, its relative abundance was 27.82%, decreased to less than 0.1% in H_6, H_{10} and H_{17} (**Figure 5b**). *Fluviicola* was aerobic denitrifying species [17]. The relative abundance of *Porphyromonadaceae(f)* was 18.36% in H_2, decreased to 4.10%, 2.32% and 5.60% in H_6, H_{10} and H_{17}, respectively. Genus within *Porphyromonadaceae* family was strictly anaerobic and related to butyric acid production [18]. The relative abundance of *Acinetobacter* was 7.59% in H_2, decreased to 1.96%, 0.38% and 0.20% in H_6, H_{10} and H_{17} respectively. *Acinetobacter* was also aerobic denitrifier [19].
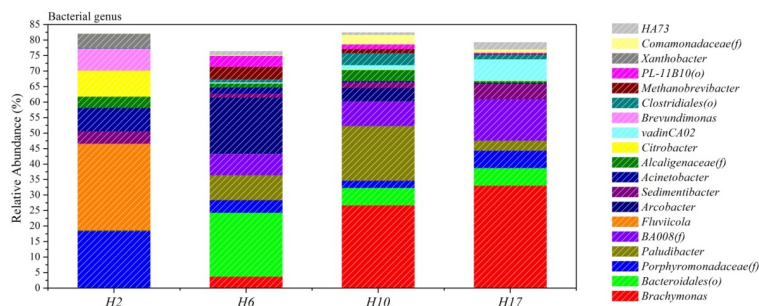

Figure 5b: Relative abundance of tax annotation at class (≥ 1%)

The dominant bacteria genus in H_2 such as *Xanthobacter*, *Citrobacter*, *Brevundimonas*, *Sedimentibacter*, *Sphingobacterium*, *Dysgonomonas* and *Devosia*, theirs relative abundance was the highest in four oxygen levels. The relative abundance of *Xanthobacter* was 4.64% in H_2, but 0.02% in H_6 and even zero in H_{10} and H_{17}. *Xanthobacter* was microaerophilic and conducted dissimilatory denitrification [20]. The relative abundance of *Citrobacter* was 8.42% in H_2, but less than 0.2% in H_6, H_{10} and H_{17}. *Citrobacter* performed citrate fermentation and aerobic denitrification [21]. The relative abundance of *Brevundimonas* was 7.07% in H_2, but less than 0.1% in the other samples. *Brevundimonas* was antibiotic resistant and denitrifying genus [22]. The abundance of *Sedimentibacter* was 3.97% in H_2, 1.27% in H_6 and 1.89% in H_{10}, slightly increased to 4.98% in H_{17}. *Sedimentibacter* was related to anaerobic hydrolysis of amino acids [16]. The relative abundance of *Sphingobacterium* was 3.21% in H_2, but zero in H_6 and less than 0.01% in H_{10} and H_{17}. *Sphingobacterium* was able to reduce nitrate to N_2 [23]. The relative abundance of *Dysgonomonas* was 3.13% in H_2 but less than 0.01% in the other samples. This genus was correlated with anaerobic saccharide fermentation [24]. The relative abundance of *Devosia* was 1.57% in H_2 but less than 0.1% average in the other samples. *Devosia* could reduce nitrate and carried *nirK* gene [25].

The dominant bacteria genus in H_6 such as *Bacteroidales*, *Arcobacter* and *Paludibacter*, theirs relative abundance was the highest in four oxygen levels. *Bacteroidales* was the dominant genus in H_6, its relative abundances was 20.57% in H_6, 5.65% in H_{10} and 5.70% and H_{17}, but only 0.045% in H_2. The relative abundance of *Arcobacter* was 18.10% in H_6, its abundance downshifted to 4.32% in H_{10}, 0.05% in H_{17} and even zero in H_2, suggesting it was also microaerophilic, growth properly in 6% O_2 content. *Arcobacter* could carry out both nitrification and denitrification [26,27]. The relative abundances of *Methanobrevibacter* are zero, 4.06%, 1.34%, and 0.21% in H_6, H_{10}, H_2 and H_{17}. This genus was hydrogenotrophic methanogen and related to nitrogen fixation [28]. Other genera of 1.0%-4.0% relative abundance in H_6 included *PL-11B10(o)* (3.48%), *Blvii28* (2.23%), *Sphaerochaeta* (2.02%) , *Beijerinckiaceae(f)* (1.95%), *Corynebacterium* (1.42%), *Alcaligenaceae(f)* (1.33%), *HA73* (1.32%) and *vadinCA11* (1.02%). *Sphaerochaeta* and *VadinCA11* were associated with anaerobic fermentation [29]. *Beijerinckiaceae* was a distinct family consisted of proteobacterial aerobic methanotrophs [30]. *Corynebacterium* was facultative anaerobe and could perform nitrate reduction [31]. Clones of *nosZ* and *nirK* genes had beenaffiliated to *Alcaligenaceae* suggesting denitrifying ability of this family, *HA73* was able to hydrolyze amino acid and produce acetic acid [32].

The dominant bacteria genus in H_{10} such as *Paludibacter*, *Methanobrevibacter* and *Brachymonas*, theirs relative abundance was the highest in four oxygen levels.

Paludibacter was strictly anaerobic and related to propionate and acetate production [33]. *Brachymonas* was an aerobic denitrifiers. The genera of relative abundance over 1.0% in H_{10} included *BA008(f)* (7.90%), *Bacteroidales(o1)* (5.65%), *Alcaligenaceae(f)* (3.57%), *Arcobacter* (4.32%), *Comamonadaceae(f)* (3.09%), *Porphyromonadaceae(f)* (2.32%), *Sedimentibacter* (1.82%), *PL-11B10(o)* (1.47%) and *Methanobrevibacter* (1.34%). The unmentioned genera of relative

abundance over 1.0% in H_{10} included *Clostridiales(o)* (3.83%), *ZB2(c)* (1.72%), *GZKB119(f)* (1.32%), *Rhodocyclaceae(f)* (3.06%), *vadinCA02* (1.42%) and *Anaerovorax* (1.21%). *Clostridiales* was mesophilic anaerobic digester and able to degrade cellulose [34]. ZB2 was often detected in aerobic biofilm samples and capable of complex carbon degradation [35]. *Rhodocyclaceae* was denitrifiers [36], could degrade polycaprolactone [22]. *VadinCA02* degraded protein and produced acetic acid [37]. *Anaerovorax* was obligate anaerobic chemoorganotrophic fermentative genus [27].

The relative abundances of dominant bacteria *Brachymonas*, *BA008(f)*, *vadinCA02*, *Bacteroidales(o1)* and *Porphyromonadaceae(f)* in H_{17} were 33.02%, 13.31%, 6.87%, 5.7% and 5.60% respectively. The relative abundances of *Brachymonas* were 3.71%, 26.67%, 33.02% in H_6, H_{10} and H_{17} separately. *Brachymonas* boomed as O_2 content increased. *Brachymonas* was a chemoorganotrophic denitrifier [22,27], suggesting denitrification dominated nitrogen metabolism in H_{17}. NO removal at 17% O_2 content could be largely attributed to aerobic denitrification. The other genera of relative abundance over 1.0% in H_{17} included *Sedimentibacter* (4.98%), *Paludibacter* (3.29%), *HA73* (2.28%), *T78* (2.21%), *Bacteroidales(o2)* (1.94%), *Clostridiales(o)* (1.53%), *GZKB119* (1.33%) and *Comamonadaceae(f)* (1.09%).

Oxygen content greatly affected microbial community and shifted phylogenetic structure dramatically as O_2 content increasing from 2% to 17%. Some *genera*, such as *Brachymonas*, *BA008(f)*, *VadinCA02*, *Bacteroidales(o2)* and *GZKB119*, increased in abundance, whereas others, such as *Acinetobacter* and *Devosia*, decreased with oxygen content increased from 2% to 17%. For comparison, the dominant microbial community structure changed, ten dominant *genera* of H_2 included *Porphyromonadaceae(f)*, *Alcaligenaceae(f)*, *Acinetobacter*, *Citrobacter*, *Fluviicola*, *Brevundimonas*, *Sphingobacterium*, *Devosia*, *Dysgonomonas* and *Xanthobacter*; ten dominant *genera* of H6 included *Bacteroidales(o)*, *HA73*, *PL-11B10(o)*, *Blvii28*, *vadinCA11*, *Methanobrevibacter*, *Sphaerochaeta*, *Beijerinckiaceae(f)*, *Corynebacterium* and *Arcobacter*; ten dominant *genera* of H_{10} included *Paludibacter*, *Clostridiales(o)*, *Comamonadaceae(f)*, *Anaerovorax*, *ZB2(c)* and *Rhodocyclaceae(f)*; ten dominant *genera* of H_{17} included *Brachymonas*, *BA008(f)*, *VadinCA02*, *Sedimentibacter*, *HA73*, *T78*, *Bacteroidales(o)* and *GZKB119*.

CONCLUSION

The paper revealed that NO removal efficiency increased with increasing oxygen concentration. NO removal efficiency reached 84.8%, 85.1%, 94% and 94.5% under oxygen content of 2%, 6%, 10% and 17% in HCMBR, respectively. Denitrification was dominant in H_2, H_6; simultaneous nitrification and denitrification occurred in H_{10}, H_{17}. Oxygen affected the microbial community. The dominant phylum was *Fluviicola*, *Arcobacter*, *Brachymonas* in H_2, H_6, H_{10}, while *Brachymonas*, *denitrificans*, *vadinCA02* in H_{17}. *Fluviicola*, *Acrobacter*, *Brachymonas* and *Brachymonas* were dominant denitrifiers in H_2, H_6, H_{10} and H_{17}, respectively. Primary coordinate analysis (PcoA) indicated phylogenic structures in H_{10} and H_{17} were highly alike but dissimilar to those in H_2 and H_6. Canonical correlation analysis (CCA) classified visualized O_2 dependence distinction of bacterial genera.

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