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Design and Feasibility Analysis of a Self-Sustaining Biofiltration System for Removal of Low Concentration N_2O Emitted from Wastewater Treatment Plants

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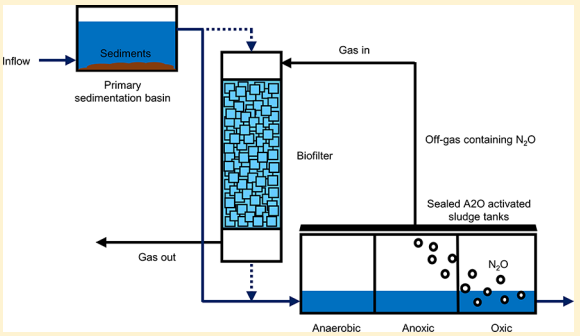
Supporting Information

ABSTRACT: N_2O is a potent greenhouse gas and ozone-depletion agent. In this study, a biofiltration system was designed for removal of N_2O emitted at low concentrations (<200 ppmv) from wastewater treatment plants. The proposed biofiltration system utilizes untreated wastewater from the primary sedimentation basin as the source of electron donor and nutrients and energy requirement is minimized by utilizing gravitational force and pressure differential to direct liquid medium and gas through the biofilter. The experiments performed with laboratory-scale biofilter in two different configurations confirmed the feasibility of the biofiltration system. The biofilter operated with cycling of raw wastewater exhibited up to 94% and 53% removal efficiency with 100 ppmv N_2O in N_2 and air, respectively, as the feed gas, corroborating that untreated wastewater can serve as a robust source of electron donor and nutrients. The laboratory-scale biofilter operated with a continuous flow-through of synthetic wastewater attained >99.9% removal of N_2O from N_2 background at the gas flow rate up to 2,000 mL·min^{-1} and >50% N_2O removal from air background at the gas flow rate of 200 mL·min^{-1}. *nosZ*-containing bacterial genera including *Flavobacterium* (5.92%), *Pseudomonas* (4.26%) and *Bosea* (2.39%) were identified in the biofilm samples collected from the oxic biofilter, indicating these organisms were responsible for N_2O removal.



INTRODUCTION

Nitrous oxide (N_2O), a potent greenhouse gas with the global warming potential 298 times that of CO_2, has an estimated net contribution of 5.9% to the total greenhouse gas emissions in terms of CO_2 equivalent (CO_2eq).[1,2] N_2O has also been the largest contributor to depletion of ozone in the stratosphere.[3,4] The Intergovernmental Panel on Climate Change (IPCC) estimated that approximately 40% of the total N_2O emissions (17.5 Tg N_2O−N·yr^{-1}) in the 1990s were of anthropogenic origin.[5] The increase in the anthropogenic N_2O emissions has led to a 1.2-fold increase in the atmospheric N_2O concentration over the past century. The major anthropogenic sources of N_2O include agriculture, animal feeding operations, fossil fuel combustion, and chemical industrial processes including production of nitric acid and adipic acid. Biological nitrogen removal (BNR) processes in modern wastewater treatment plants (WWTP) also have significant contribution to the global N_2O emissions, as up to 15% of total nitrogen load is released to the atmosphere as N_2O.[6] Although the contribution of WWTP to the total anthropogenic N_2O emissions appears to be relatively minor (1.5%),[1] WWTP facilities are concentrated and amenable to control compared to other major sources of N_2O. Thus, monitoring and modeling of N_2O emissions from WWTP and strategies for N_2O emission mitigation have been popular research topics that have recently drawn significant interests from engineering and scientific communities alike.[6,7]

N_2O released to the atmosphere from soil and aquatic environments, including WWTPs, is of predominantly biological origin.[9] The two biogeochemical reactions with the greatest contributions to N_2O emissions are nitrification and denitrification.[7] Nitrification consists of two reaction steps, ammonia oxidation (NH_4^+ oxidation to NO_2^- via NH_2OH), and nitrite oxidation (NO_2^- oxidation to NO_3^-).[10] These two reactions had been known to be carried out by separate groups of organisms, ammonia oxidizers and nitrite oxidizers, until the recent discovery of comammox, i.e., complete nitrification in a single organism, in specific *Nitrospira* strains.[11,12] Denitrification is a stepwise reduction of NO_3^-/NO_2^- to N_2O and N_2 via NO with each step mediated by a different enzyme.[13] In nitrification, N_2O is produced as a byproduct of NH_2OH oxidation in ammonia-oxidizing bacteria and archaea and also, from nitrifier denitrification, as ammonia oxidizers often possess the genes encoding for the enzymes in the denitrification cascade but lack N_2O reductase (N_2OR), the enzyme mediating the last step of denitrification from N_2O to N_2.[9,14] In denitrification, N_2O is produced as the final product or a stable intermediate and production of N_2O is greatly affected

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Environ. Sci. Technol. XXXX, XXX, XXX−XXX

by environmental parameters or composition of the denitrifier population. As N_2O reductase is more sensitive to acidic pH than the enzymes mediating upstream denitrification reactions, denitrification at acidic pH resulted in increased N_2O evolution in both axenic and mixed cultures.[15,16] Denitrifiers lacking *nosZ* are not uncommon and the abundance of such denitrifiers, with N_2O as the final product of denitrification, was also found to have significant correlation with N_2O production.[17,18] Kinetic imbalance or the lack of tight coupling between the denitrification enzymes may also be the cause of N_2O emissions, as transient N_2O production at trace concentration is often observed in denitrification carried out by typical denitrifiers with full suite of denitrification enzymes at the neutral pH.[19]

The question as to which of the two biological reactions, nitrification or denitrification, has larger contribution to N_2O emissions from the biological nitrogen removal (BNR) process is under controversy.[6,20] In a recent study that investigated N_2O generation from anoxic and oxic tanks in multiple full-scale WWTPs, consistent positive net N_2O generation was observed in the anoxic tanks, while net N_2O generation from oxic tanks varied from highly positive to negative values.[21] These results confirmed that both oxic and anoxic tanks contributed to N_2O production, but failed to elucidate the controversy regarding the relative contributions of the two reaction pathways. Based on the results from batch experiments, nitrification or nitrifier denitrification has often been regarded as the dominant N_2O source, and contribution of heterotrophic denitrification has been considered to be relatively minor;[22,23] however, no direct evidence supports this hypothesis. Regardless of the origin of N_2O, N_2O release to the atmosphere occurs predominantly from the oxic tanks, as aeration strips dissolved N_2O from wastewater, facilitating mass transfer from the aqueous phase into the atmosphere.[8,21]

To date, efforts to mitigate N_2O emissions from WWTPs have focused on source reduction through optimization of operating parameters, for example, recycle ratio, aeration scheme, and nitrogen load.[7] Active removal of produced N_2O from contaminated off-gases has not attracted due interest, as N_2O is rarely released in concentrations high enough to render use of chemical catalysis economically feasible.[24] Any continuous requirement of synthetic chemicals or electricity would compromise the benefits of active N_2O abatement, when life cycle CO_2 emission is considered. Recently, a bioscrubber was proposed for removal of environmentally relevant concentration (~100 ppmv) of N_2O;[25] however, the proposed system requires electricity to operate pumps for recycling of the liquid medium and additional input of CH_3OH and NH_4Cl. Neither life-cycle CO_2 emission associated with electricity generation and production of the chemicals nor CO_2 produced from CH_3OH oxidation was accounted for in the feasibility analysis performed with the laboratory-scale reactor.

Recent studies have demonstrated that certain denitrifiers and nondenitrifying N_2O reducers, in fact, exhibited high affinities toward N_2O, with Michaelis–Menten constants below 0.5 μM (equivalent to a headspace concentration of ~25 ppmv under equilibrium at 25 °C), and that these N_2O reducers may actually function as a significant sink of trace N_2O generated from soil.[26−28] Harnessing of these high-affinity N_2O reducers is a promising alternative for removal of trace N_2O from WWTP off-gases; however, several technological bottlenecks need to be addressed for its implementation. As dissimilatory N_2O reduction is the only identified biological mode of removal, N_2O reduction needs to be coupled with oxidation of organic electron donors, which results in CO_2 emission that partially offsets CO_2eq removed as N_2O.[9] Microbial N_2O reduction, in general, is known to be highly sensitive to O_2, although N_2O-containing WWTP off-gases consist of 15−20% O_2.[29] Here, a biofiltration system integrated into a WWTP is proposed as a strategy for N_2O removal from WWTP off-gases to overcome these dilemmas. In the proposed system, the gravitationally diverted wastewater stream from the primary sedimentation basin provides an unabated supply of organic carbon and nutrients to the biofilms in the bioreactor, wherein N_2O removal takes place at local anoxic or suboxic niches. In the proof-of-concept experiments performed with laboratory-scale biofilters, substantial N_2O removal was achieved both with and without O_2 in the gas stream.

MATERIALS AND METHODS

Schematic Design of the Full-Scale N_2O Biofiltration System. A biofiltration system was designed for N_2O removal from off-gas streams of typical activated sludge WWTPs (Figure 1). Installation of the designed biofiltration system

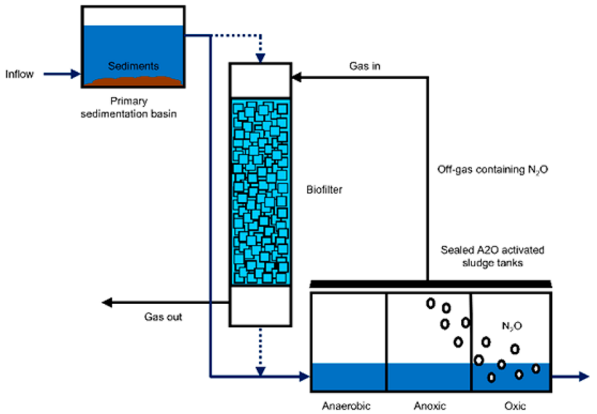

Figure 1. Schematic design of the proposed biofiltration system and modifications to the activated sludge WWTP where the system is to be installed.

will involve construction and installation of a biofilter, as well as design modifications to the configuration of the target WWTP. The necessary modifications include enclosure of the activated sludge tanks of WWTP and channeling of the off-gas streams to a gas outlet where a biofilter is to be installed. An enclosed activated sludge system would be fed with continuous air supply for aeration of the oxic tank. Due to pressure build-up, off-gas will be channeled through the biofilter without aid of blowers. Positioning of the biofilter is crucial for zero-carbon operation of the N_2O biofiltration system. To utilize wastewater in the primary sedimentation basin as the source of organic compounds and nutrients, the biofilter is to be positioned between the basin and the activated sludge tanks. Primary sedimentary basins are, in general, placed at higher elevations than activated sludge tanks to allow for gravitational flow of wastewater. By partially diverting this gravitational flow to the biofilter, raw wastewater, rich in organic carbon and nutrients, can be supplied to the biofilter without external energy. N_2O will be removed via two separate mechanisms: (1) the main mode of N_2O removal is the biological N_2O reduction by *nosZ*-possessing N_2O reducers in the biofilm, and (2) some N_2O will be dissolved into the flow-through wastewater and introduced

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Environ. Sci. Technol. XXXX, XXX, XXX−XXX

to the activated sludge for removal via N_2O reduction in the anaerobic and anoxic tanks.

WWTP Site Description and Wastewater Sampling. The wastewater used in the laboratory-scale experiments was collected from Daejeon municipal WWTP located in Daejeon, Korea (36°23′5″ N, 127°24′28″ E). The WWTP is a typical anaerobic-anoxic-oxic (A2O) system with a treatment capacity of 20,741 $m^3 \cdot day^{-1}$ (Supporting Information (SI) Figure S1). The primary sedimentation basin with the capacity of 76,074 m^3 is located upstream of the anaerobic tank at an elevated elevation. The design hydraulic retention time (HRT) of the primary sedimentation basin is 1.98−3.03 h, and the HRT and the sludge retention time (SRT) of the activated sludge are 5.92−8.51 h and 1.29−1.31 days, respectively. For evaluation of untreated wastewater as the source of electron donor and acceptor, wastewater in the primary sedimentation basin was grab sampled on a weekday each month between July and November, 2016 and its physicochemical properties were analyzed. The collected wastewater was transported in an ice-filled cooler to the laboratory immediately after sampling and stored at 4 °C until further use. For characterization of the wastewater samples, 50 mL of each stored sample was centrifuged at 10 000*g* for 10 min and the supernatant was analyzed for dissolved organic carbon contents (DOC), chemical oxygen demand (COD), NH_4^+, NO_3^-, NO_2^-, total phosphorus (TP), sulfate, sulfide, chloride, and pH (SI Table S1).

Construction of the Laboratory-Scale Biofilter. A laboratory-scale biofilter was constructed to examine the feasibility of N_2O removal from gas streams with untreated wastewater as the source of organic carbon and nutrients (Figure 2). A cylindrical biofilter with an inner diameter of 160 mm and a height of 1,000 mm was constructed with acryl plastic with a thickness of 5 mm. The packed bed height of the biofilter was 800 mm, as the space within 100 mm of each end was kept void of packing materials. Thus, the total packed volume of the biofilter was 16.1 L. Seven evenly spaced sampling ports were installed along the height of the biofilter and additional pair of sampling ports were installed at the gas inlet and outlet. Tube connections that served as the inlet and outlet for circulating medium were installed at the two ends of the biofilter, separate from the inlet and outlet of the gas stream. Liquid medium was sprayed from the cap of the biofilter through a fog nozzle to ensure even distribution of the medium. Polyurethane foam cubes (JLO, Nürnberg, Germany) with a surface-area-to-volume ratio of 8.2 cm^2/cm^3 were used as the packing materials, as they have been successfully used previously for biofilters targeting various organic and inorganic contaminants.[30,31] To ensure sufficient available surface area for attached growth, the polyurethane foams were cut to 2 × 2 × 2 cm cubes. The biofilter, with a packing density of 0.92, provided a total reactive surface area of 12.1 m^2.

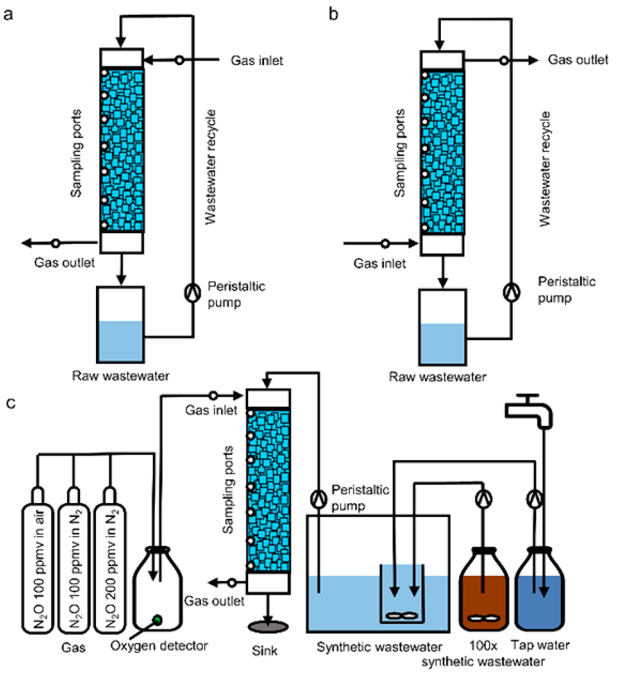

Figure 2. Schematic representations of the setups of the laboratory-scale biofilter experiments: the biofilters operated in (a) a cocurrent configuration and (b) a counter-current configuration with internally recycled wastewater and (c) the biofilter operated with continuous flow-through of automatically generated synthetic wastewater.

Lab-Scale Biofilter with Untreated Wastewater As the Nutrient Medium. After the biofilter was constructed and packed with the polyurethane foam cubes, an abiotic control experiment was performed before initiation of bacterial growth in the biofilter. Double distilled water (>18 MΩ·cm) was circulated through the biofilter with a peristaltic pump at a flow rate of 286 $mL \cdot min^{-1}$ and ∼100 ppmv N_2O prepared in 99.999% N_2 gas (Deokyang, Ulsan, Korea) was blown through the biofilter at a flow rate of 37.5 $mL \cdot min^{-1}$ (i.e., an empty bed retention time (EBRT) of 8.89 h). The biofilter was initially operated in a cocurrent configuration, in which the flow directions of gas and liquid were the same (Figure 2a). After inactivity of the uninoculated biofilter was confirmed for 4 days (i.e., at $t = 0$), the raw wastewater collected from the primary sedimentary basin replaced double distilled water as the nutrient medium. The wastewater sample also served as the inoculum. The biofilter was operated with constant gas and aqueous flow rates and N_2O removal by the biofilter was monitored. The removal efficiencies ($RE = \frac{C_{in} - C_{out}}{C_{in}} \times 100$, where C_{in} and C_{out} are N_2O concentrations at the inlet and the outlet, respectively) and elimination capacities ($EC = \frac{C_{in} - C_{out}}{V} \times Q_{gas}$, where V is the bed volume and Q_{gas} is the gas flow rate) of the biofilter was calculated from the N_2O mixing ratios at the gas inlet and outlet. As a precipitous drop in the removal efficiency was observed upon depletion of labile organic carbon in the wastewater, the batch of wastewater was replaced every 6 h to fresh wastewater stored at 4 °C. After a brief oxic operation with N_2O prepared in air background (∼21% O_2), the biofilter was preconditioned under anoxic condition (with ∼100 ppmv N_2O in N_2 at the flow rate of 500 $mL \cdot min^{-1}$) for 98 h before switching back to oxic operation. Over 105 h of operation, the biofilter was operated with varying gas flow rates (37.5−300 $mL \cdot min^{-1}$). During operation, the biofilter and the recycling tank were covered with aluminum foil to prevent algal growth which may hinder N_2O removal by exposing local environments to O_2. Upon termination of the biofiltration experiment (at $t = 217$ h), the biofilter was disassembled. Seven polyurethane foams covered with biofilms were collected in 50 mL Falcon tubes and were immediately frozen and stored at −20 °C until DNA extraction procedure was performed. The experiment was repeated with a counter-current configuration to examine whether shift in the

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Environ. Sci. Technol. XXXX, XXX, XXX−XXX

configuration of the reactor had any significant effect on the performance (Figure 2b).

Operation of the Laboratory-Scale Biofilter with Continuous Flow of Synthetic Wastewater. An experimental setup with continuous flow-through of synthetic wastewater was constructed with the same biofilter, to better emulate the actual proposed biofiltration system at laboratory scale (Figure 2c). Synthetic wastewater was used for this experiment, as use of actual raw wastewater would have required 2.85 m^3 of wastewater transported to the laboratory. The 100× concentrate of model synthetic wastewater contained per liter of tap water: 16.0 g peptone, 11.0 g meat extract, 3.0 g urea, 2.8 g K_2HPO_4, 0.7 g NaCl, 0.4 g $CaCl_2{\cdot}2H_2O$, 0.2 g $MgSO_4{\cdot}7H_2O$, and 5 mg $CuCl_2$.[32] All chemicals for preparation of the synthetic wastewater concentrate were purchased from Sigma-Aldrich (St. Louis, MO), except for peptone and meat extract, which were purchased from Oxoid (Hampshire, England). Five liters of the synthetic wastewater concentrate was prepared in a 5 L Duran glass bottle and autoclaved. Into an empty 5 L Duran bottle placed in a 120-L acryl tank, tap water was pumped at 88.9 mL·min^{-1} and the synthetic wastewater concentrate was pumped at 0.9 mL·min^{-1} using peristaltic pumps. A homogenizer was operated at 200 rpm to ensure uniform dilution of the concentrate into tap water. The diluted synthetic wastewater overflowed into the acryl tank and eventually, the tank was filled with 1× synthetic wastewater, mimicking the primary sedimentation tank of a typical WWTP. After the basin was at least half-filled, the synthetic wastewater was pumped into the biofilter through the aqueous phase inlet at a flow rate of 88.9 mL·min^{-1} using the remaining channel of the two-channel peristaltic pump. This experimental setup allowed for automated supply of fresh synthetic wastewater (i.e., source of electron donors and nutrients for microorganisms in the biofilter) through the biofilter without recycling of the used medium.

The biofilter was initially operated with ∼100 ppmv N_2O prepared in 99.999% N_2 gas blown at a flow rate of 200 mL·min^{-1}. A cocurrent configuration was used to maximize the time of contact between the gas and liquid phases and dissolution of N_2O into the wastewater. Before inoculation, tap water was passed through the biofilter at a flow rate of 88.9 mL·min^{-1} for 24 h to determine the rate of N_2O removal from the gas stream through mass transfer into the liquid phase. After steady state was attained in the uninoculated system, 20 L of raw wastewater from Daejeon municipal WWTP was passed through the biofilter at a flow rate of 88.9 mL·min^{-1} for inoculation of the reactor. The biofilter was then operated with a continuous flow of the synthethic wastewater for 100 h and N_2O removal was monitored. The extended period of anoxic incubation ensured that N_2O-reducing biofilm developed on the polyurethane foams. Gas concentration and flow rate were eventually increased up to 200 ppmv and 2000 mL·min^{-1}, respectively, to examine the removal efficiency at the increased gas feed rate. After 25 h of pseudo-steady state operation (i.e., at t = 301 h), the anoxic gas was replaced with mixtures of N_2 gas and air containing ∼100 ppmv N_2O. In order to investigate the effect of O_2 concentration on the performance of the biofilter, varying concentrations of O_2 (5%, 10%, 15%, and 21%) were prepared by mixing streams of N_2O-containing N_2 gas and N_2O-containing air in a sealed 5 L glass bottle before passing the mixed gas through the biofilter at a net flow rate of 200 mL·min^{-1}. After the system was operated for 70 h after pseudosteady state was attained with N_2O-containing air, the performance of the biofilter was examined with varied flow rates of N_2O-containing gas streams (200−2000 mL·min^{-1}). At the end of the experiment, the gas flow rate was changed back to 200 mL·min^{-1} and the biofilter was operated for additional 11 h to confirm that the N_2O removal efficiency remained intact. The operating conditions are summarized in Table 1. Upon termination of the biofiltration experiment (at t = 532 h), the biofilter was disassembled. Seven polyurethane foams covered with biofilms were collected in 50 mL Falcon tubes and were immediately frozen and stored at −20 °C until DNA extraction procedure was performed.

Analytical Procedure. The gas samples for N_2O concentration measurements were extracted with a 1700-series gastight syringe (Hamilton Company, Reno, NV) from the sampling ports on the biofilter and at the inlet and the outlet. The syringe was flushed at least twice with pressurized pure (>99.999%) N_2 gas immediately before each sampling event to remove residual N_2O and O_2. 100 μL samples (500 μL for N_2O mixing ratio below 10 ppmv) were manually injected into a HP6890 Series gas chromatograph equipped with an HP-PLOT/Q column (20.00 μm film thickness, 30 m × 0.320 mm inner diameter) and an electron capture detector (Agilent, Palo Alto, CA).[33] The injector, oven, and detector temperatures were set to 200 °C, 85 °C, and 250 °C, respectively. He (>99.999%; Deokyang Gas Co.) and 95% Ar/5% CH_4 mixture (Deokyang Gas Co.) were used as the carrier gas and the makeup gas, respectively. For calculations of removal efficiencies and elimination capacities, three measurements were made with intervals of 3× EBRT after the pseudosteady state was attained, and the average and the standard deviation were calculated from the three measurements.

The chemical properties of wastewater samples were analyzed using various analytical methods. The dissolved organic carbon (DOC) concentrations were measured with TOC-L TOC Analyzer (Shimadzu, Kyoto, Japan). Frozen stock (−20 °C) of the samples were thawed and diluted 5-fold with double distilled water. The DOC concentrations of the diluted samples were measured against the standards prepared with varying concentrations of $C_6H_4(COOK)(COOK)$ (Nacalai Tesque, Inc., Kyoto, Japan) in double distilled water. Concentrations of aqueous NO_3^-, NO_2^-, NH_3, and TP were measured colorimetrically with HS-NO_3 (N)-CA, HS-NO_2 (N)-L, HS-NH_3 (N)-L, and HS-TP-L kits (Humas, Daejeon, Korea), respectively, according to the protocols provided by the manufacturer. O_2 mixing ratios were measured with a FireSting-O_2 oxygen meter and fiber-optic oxygen sensor spots (Pyroscience, Aachen, Germany).

Microbial Community Analyses. The falcon tubes were thawed in ice and 30 mL sterile double-distilled water (>18 MΩ·cm) was pipetted into the tubes. Tubes were gently vortexed for 5 min and sonicated for 10 min. Two milliliter aliquots of the cell suspension were pipetted into autoclaved nuclease-free 2 mL Eppendorf tubes. The tubes were centrifuged at 10 000g and the supernatants were removed. Three identically prepared cell pellets were combined for DNA extraction using Mo Bio PowerSoil DNA Isolation kit (Carlsbad, CA), which was performed according to the protocol provided by the manufacturer. Amplicon sequencing targeting the hypervariable V6−8 region of 16S rRNA gene (targeted by 926F: 5′-AAACTYAAAKGAATTGRCGG-3′ and 1392R: 5′-ACGGGCGGTGTGTRC-3′ primers) was performed by Macrogen (Seoul, Korea) using Miseq sequencing technology (Illumina, Inc., San Diego, CA).[34]

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Environ. Sci. Technol. XXXX, XXX, XXX−XXX

Table 1. Description of Operating Conditions of the Biofilter Configured with Continuous Flow-through of Synthetic Wastewater

phase[a]	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
N_2O (ppmv)	100	100	200	200	200	200	200	200	100	100	100	100	100	100	100	100	100	100
O_2 (%)	0	0	0	0	0	0	0	0	0	5	10	15	21	21	21	21	21	21
gas flow rate (mL·min^{-1})	200	200	200	400	600	1000	2000	200	200	200	200	200	200	400	600	1000	2000	200
liquid	DW[b]	RWW[c]	SWW[d]	SWW	SWW	SWW	SWW	SWW	SWW	SWW	SWW	SWW	SWW	SWW	SWW	SWW	SWW	SWW
liquid flow rate (mL·min^{-1})	89	89	89	89	89	89	89	89	89	89	89	89	89	89	89	89	89	89
EBRT (min)	80.5	80.5	80.5	40.3	26.8	16.1	8.1	80.5	80.5	80.5	80.5	80.5	80.5	40.3	26.8	16.1	8.1	80.5
duration (h)	22.5	97	49	26	9.5	8.5	4	62	20	24	24	26	77	33	16	16	5	12

[a]These phase numbers correspond to the numbers in the header of Figure 4. [b]DW, double-distilled water. [c]RWW, raw wastewater. [d]SWW, synthetic wastewater.

RESULTS

Performance of the Lab-Scale N_2O Biofilter with Raw Wastewater As the Nutrient Medium. The performance of the biofilter constructed in a cocurrent configuration with recycling of actual raw wastewater was monitored under varying operation conditions (Figure 3). In the abiotic operation with double-distilled water, N_2O removal was kept to minimal level. Even after the circulation of wastewater was switched on, a minimal removal efficiency, 4.26 ± 0.86%, was observed when air containing 100 ppmv (nominal) N_2O was passed through the biofilter at a flow rate of 500 mL min^{-1}. As soon as the influent gas was switched to 100 ppmv N_2O in N_2, the removal efficiency increased to >80% within 25 h. The elevated removal efficiency was sustained until the gas was switched back to air containing 100 ppmv N_2O; however, immediately upon switching, the removal efficiency dropped to around 11.0 ± 0.01%. As the gas flow rate was gradually decreased to 37.5 mL·min^{-1} over the next 52 h, a gradual increase in the N_2O removal efficiencies were observed; the removal efficiencies of 16.2 ± 1.0%, 29.8 ± 2.7%, 35.2 ± 1.9%, and 49.3 ± 2.1% were recorded at the gas flow rates of 300, 150, 75, and 37.5 mL·min^{-1}, respectively. Despite the lower removal efficiencies, elimination capacities did not decrease at the higher flow rates, suggesting that N_2O reduction was not completely inhibited when the reactor was operated with higher gas flow rates (SI Figure S2b). In fact, the maximum elimination capacity, 0.476 ± 0.003 $gN_2O·(m^3)^{-1}·h^{-1}$, was recorded when the reactor was operated at 300 mL·min^{-1}. The performance of the biofilter operated in a counter-current configuration was virtually indistinct from the performance of the cocurrent biofilter, confirming that the main mode of N_2O removal in the biofilter was biological (SI Figure S3). The results from the counter-current operation of the biofilter are summarized in the SI.

N_2O Biofilter Operation with Continuous Flow-through of Synthetic Wastewater. In order to examine the performance of the biofiltration system in an experimental setup that better emulates the process design for the full-scale biofiltration system, the biofiltration experiment was performed with a continuous flow-through of synthetic wastewater introduced in a cocurrent manner with the influent N_2O-containing gases (Figure 4, SI Table S2). This configuration precluded the possibility of N_2O removal taking place outside the biofilter, as no liquid or gas was recirculated after passing through the biofilter. Abiotic removal of N_2O due to dissolution into the liquid phase was higher with this continuous flow-through configuration, as 29.8 ± 3.0% of N_2O influx was removed from the gas stream with a flow rate of 200 mL min^{-1} (equivalent to an elimination capacity of 45.2 ± 4.3 $mgN_2O·(m^3)^{-1}·h^{-1}$). The relatively large abiotic removal was presumably due to the high solubility (a dimensionless Henry's constant of 1.89 at 25 °C) and rapid dissolution (k_La = 450 d^{-1}) of N_2O into water. After inoculation with the wastewater, complete removal of N_2O from the anoxic gas stream (at nominal N_2O concentrations of 100 ppmv and 200 ppmv) was observed with flow rates up to 2000 mL·min^{-1}. As air:N_2 mixing ratio was gradually increased with the flow rate fixed to 200 mL·min^{-1}, a decrease in N_2O removal efficiency was observed; the removal efficiencies of 76.9 ± 0.9%, 71.0 ± 1.0%, 61.6 ± 2.0%, 54.5 ± 1.3% were recorded at the O_2 mixing ratios of 5, 10, 15, and 21%, respectively (Figure 4, SI Figure S5a). The gradual increase in the gas flow rate with air containing 100 ppmv N_2O resulted in reduced removal efficiencies; 39.4 ±

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Environ. Sci. Technol. XXXX, XXX, XXX−XXX

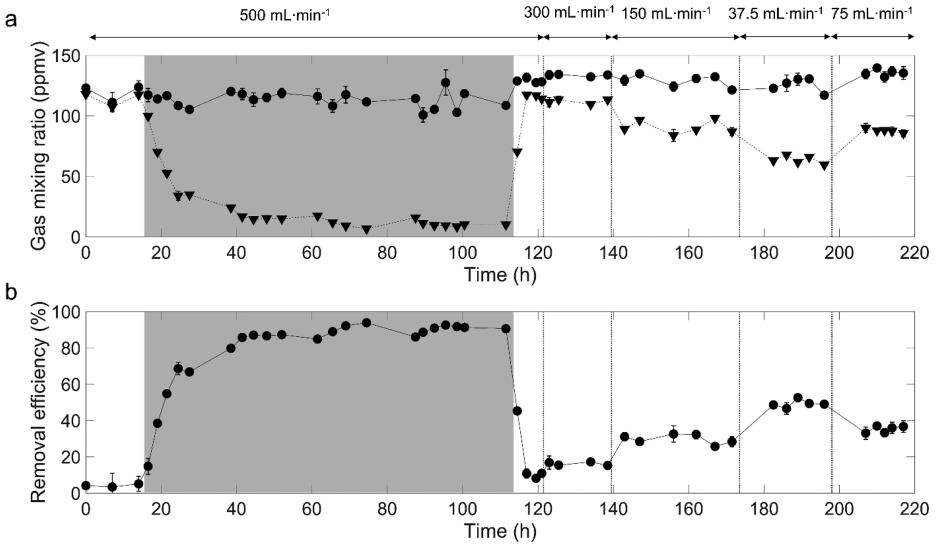

Figure 3. (a) N_2O mixing ratios at the inlet (●) and outlet (▼) of the biofilter and (b) the calculated removal efficiency when the biofilter was operated in a cocurrent manner with untreated wastewater as the nutrient medium. Shaded areas are the data acquired from initial anoxic operation with N_2O-containing N_2 gas and the white areas represent the data from oxic operation with N_2O-containing air. The averages of three consecutive measurements with 5 min intervals are presented with the error bars representing the standard deviations.

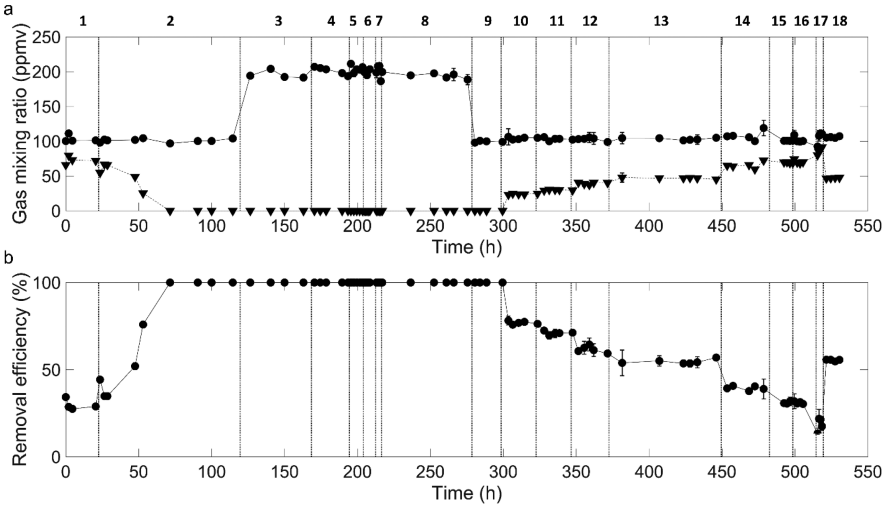

Figure 4. (a) N_2O mixing ratio at the inlet (●) and outlet (▼) of the biofilter and (b) the removal efficiency when the biofilter was operated with continuous flow-through of synthetic wastewater. Refer to Table 1 for descriptions of the operation conditions and SI Table S2 for additional results. The averages of three consecutive measurements with 5 min intervals are presented with the error bars representing the standard deviations. The snapshots of N_2O concentration profiles along the height of the biofilter at several different time points are presented in SI Figure S4.

1.2%, 31.2 ± 0.7%, 31.0 ± 0.7%, 18.5 ± 3.9% were measured at the gas flow rates of 400, 600, 1000, and 2000 $mL \cdot min^{-1}$, respectively. The elimination capacity increased with the gas flow rates despite the decrease in the removal efficiency; the elimination capacities of 0.125 ± 0.008 $gN_2O \cdot (m^3)^{-1} \cdot h^{-1}$, 0.138 ± 0.003 $gN_2O \cdot (m^3)^{-1} \cdot h^{-1}$, 0.233 ± 0.016 $gN_2O \cdot (m^3)^{-1} \cdot h^{-1}$, 0.291 ± 0.079 $gN_2O \cdot (m^3)^{-1} \cdot h^{-1}$ were observed at the flow rates of 400 $mL \cdot min^{-1}$, 600 $mL \cdot min^{-1}$, 1000 $mL \cdot min^{-1}$ and 2000 $mL \cdot min^{-1}$, respectively (SI Figure S5b). Assuming that the abiotic dissolution into the trickling medium occurred at the rate of 0.045 ± 4.3 $gN_2O \cdot (m^3)^{-1} \cdot h^{-1}$ and that biotic N_2O reduction occurred uniformly on the surfaces of the packing materials, the biological N_2O reduction rates were calculated per m^2 of reactive surface area (SI Table S2). The increase in biological N_2O removal rate with increasing flow rate indicated that kinetics was the limiting factor at low gas flow rates. These results suggested that the self-sustained N_2O biofiltration is a feasible option for removing low-concentration N_2O from the WWTP off-gas streams without additional energy or chemical inputs or CO_2 emissions.

Microbial Composition of the Biofilms Developed in the Biofilter. The only identified biological sinks of N_2O are the *nosZ*-possessing organisms. To investigate the organisms carrying out reduction of N_2O in the biofilter upon oxic operation, the microbial community of the biofilm operated with continuous configuration was analyzed (Table 2). After quality-trimming and assembly, 45 838 reads were obtained and subjected to the analysis. The saturated rarefaction curves indicated that almost entire diversity was captured with the amplicon sequencing (SI Figure S6). The low Shannon-Weiner index (5.13), the high Simpson index (0.92), and the low OTU richness indicated a selective pressure on the microbial

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Environ. Sci. Technol. XXXX, XXX, XXX−XXX

Table 2. Taxonomic Affiliations of OTUs (Clustered with 97% Identity Threshold) Recovered From 16S rRNA Amplicon Sequencing of the DNA Samples Isolated from the Biofilms Attached to Polyurethane Cubes (Only the OTUs with Relative Abundance Higher than 0.5% Were Represented.)

phylum	order	family	genus	RA[a](%)	*nosZ*[b]
Actinobacteria		*Microbacteriaceae*	*Leucobacter*	23.45	
Firmicutes	*Clostridiales*	*Clostridiaceae*	*Clostridium*	14.03	
Bacteroidetes	*Cytophagales*	*Cytophagaceae*	*Flectobacillus*	6.40	
Bacteroidetes	*Flavobacteriales*	*Flavobacteriaceae*	*Flavobacterium*	5.92	O
Bacteroidetes	*Bacteroidales*	*Rikenellaceae*		4.93	
Proteobacteria	*Pseudomonadales*	*Pseudomonadaceae*	*Pseudomonas*	4.26	O
Firmicutes	*Clostridiales*	*Clostridiales Family XIII. Incertae Sedis*		3.12	
Bacteroidetes	*Cytophagales*	*Cytophagaceae*	*Emticicia*	2.56	
Proteobacteria	*Rhizobiales*	*Bradyrhizobiaceae*	*Bosea*	2.39	O
Bacteroidetes	*Sphingobacteriales*	*Sphingobacteriaceae*	*Pedobacter*	1.82	
Proteobacteria	*Rhodospirillales*	*Rhodospirillaceae*	*Novispirillum*	1.75	
Proteobacteria	*Campylobacterales*	*Campylobacteraceae*	*Sulfurospirillum*	1.34	O
Proteobacteria	*Burkholderiales*	*Comamonadaceae*	*Acidovorax*	1.33	O
Proteobacteria	*Rhodobacterales*	*Rhodobacteraceae*		1.18	O
Bacteroidetes	*Sphingobacteriales*	*Sphingobacteriaceae*	*Sphingobacterium*	1.07	
Bacteroidetes	*Cytophagales*	*Cytophagaceae*	*Runella*	0.98	O
Proteobacteria	*Rhodospirillales*	*Rhodospirillaceae*		0.93	O
Proteobacteria	*Legionellales*	*Legionellaceae*	*Legionella*	0.77	
Proteobacteria	*Chromatiales*	*Chromatiaceae*	*Rheinheimera*	0.73	
Proteobacteria	*Burkholderiales*	*Oxalobacteraceae*	*Undibacterium*	0.71	
Proteobacteria	*Caulobacterales*	*Caulobacteraceae*	*Brevundimonas*	0.70	
Proteobacteria	*Rhodocyclales*	*Rhodocyclaceae*	*Dechloromonas*	0.61	O
Bacteroidetes	*Flavobacteriales*	*Flavobacteriaceae*	*Chryseobacterium*	0.56	O

[a]RA: relative abundance. [b]The genus or families (for OTUs without genus-level assignments) were searched against the NCBI database for presence of species or strains with *nosZ* genes.

community developed in the biofilms. Six bacterial phyla were represented by the OTUs recovered from the biofilms; *Bacteroidetes* (25.97%), *Actinobacteria* (24.05%), *Proteobacteria* (20.24%), *Firmicutes* (17.85%), *Fusobacteria* (0.07%), and *Lentisphaerae* (0.02%). The OTUs with the largest abundance were affiliated to *Leucobacter* (23.45%), *Clostridium* (14.03%), *Flectobacillus* (6.40%), *Flavobacterium* (5.92%), and *Pseudomonas* (4.26%) at genus level. The list of the abundant genera included both obligately aerobes (*Leucobacter*), as well as obligate anaerobes (*Clostridium*), suggesting the presence of both oxic and anoxic niches within the biofilm. The genera with species or strains previously identified with a *nosZ* gene included *Flavobacterium*, *Pseudomonas*, *Bosea*, *Sulforospirillum*, *Acidovorax*, *Runella*, *Dechloromonas*, and *Chryseobacterium* (0.40%), suggesting that these organisms were responsible for biological removal of N_2O in the biofilter (Table 2). The genera under the families *Flavobacteriaceae*, *Comamonadaceae*, *Pseudomonaceae*, and *Rodocyclaceae* were also the most abundant *nosZ*-possessing organisms in the biofilm collected from the biofilter operated with circulation of raw wastewater (SI Table S3). These organisms were likely the main players for biological removal of N_2O in the biofilter. The negligible abundance (<0.1%) of the OTUs affiliated to ammonia oxidizing bacteria or archaea in either biofilm sample precluded the possibility of ammonia oxidation taking place in the biofilm.

DISCUSSION

Active abatement of N_2O emitted from WWTP is deemed difficult, due mainly to the low concentrations of N_2O in the off-gas streams, which rarely exceed 400 ppmv at typical activate sludge WWTPs.[35] As rates of biological reactions decrease precipitously with decreases in the substrate concentrations, and microorganisms usually have threshold concentrations for growth substrates, designing of bioreactors for removal of low-concentration contaminants is always challenging.[36] Often, the operational costs for such removal systems exceed their monetary benefits, rendering the removal systems economically infeasible.[37] In addition, challenges specific to N_2O removal also needed to be overcome, including oxygen inhibition of N_2O reduction and production of CO_2eq from oxidation of organic electron donors.

The innovative design of the biofiltration system proposed in this study resolved these dilemmas by (1) developing a high-affinity N_2O-reducing microbial community in the bioreactor, (2) utilizing untreated wastewater as the source of electron donor and nutrients, and 3) minimizing the electricity requirement by utilizing gravitational flow and gas pressure built up by aeration. As expected from the Michaelis−Menten kinetics of N_2O reducers,[26] the microbial community in the biofilter was capable of reducing N_2O applied at mixing ratios lower than 200 ppmv, and removal efficiency up to 95% was easily achieved regardless of the gas flow rate, when the bioreactor was operated anoxically. Albeit at significantly lower removal efficiency and elimination capacity, substantial removal of low-concentration N_2O was achieved with fully oxic operation as well, confirming that N_2O removal using a biofilm reactor is a technically feasible option for N_2O removal from the WWTP off-gases. The analysis of microbial community in the biofilm identified the presence of the N_2O-reducing populations, for example, *Flavobacterium* spp., *Pseudomonas* spp., and *Bosea* spp., suggesting that these microorganisms were responsible for reduction of the low-concentration N_2O in the anoxic niches of the biofilter.[27,38] The large abundance of *Flavobacterium* spp. was especially intriguing, as this phyloge-

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Environ. Sci. Technol. XXXX, XXX, XXX−XXX

netic group of bacteria possesses the recently discovered clade II *nosZ*, thought to be associated with high-affinity N_2O reduction.[26]

In the N_2O abatement system proposed in this study, the use of untreated wastewater and naturally occurring gas and liquid flow allows for a zero-carbon operation. Wastewater is a rich source of nutrients needed for microbial growth, including bioavailable organic carbon, nitrogen, and phosphorus, and trace metals.[39−41] The proof-of-concept experiments in this study have shown that the organic electron donor and nutrients in the wastewater should suffice as resource for N_2O-reducing organisms in the biofilter, without necessity for addition of synthetic chemicals. Utilization of elevation and pressure differentials for directing liquid and gas flows through the biofilter will minimize the energy demand. With the minimized requirement for synthetic chemicals and energy, a substantial removal of life-cycle CO_2eq by removing trace N_2O in the WWTP off-gases would be possible.

Nitrous oxide reduction is a reaction that was traditionally known to be very sensitive to O_2 concentration.[42,43] The bioreactor used in this study was able to remove N_2O despite of the presence of high concentration of O_2 and supposed inhibition of N_2O reduction activity by O_2. As physiology of N_2O reduction for many *nosZ*-possessing organisms remain virtually unknown despite the recent progresses, the possibility of 'aerotolerant N_2O reduction' cannot be completely overlooked, even though the analyses of microbial population did not reveal the abundance of genus identified as aerobic denitrifiers.[44] Several recent researches have suggested that the oxygen inhibition of nitrous oxide reduction activity may not be complete even at relatively high ambient O_2 concentrations (up to 15% O_2), not only in "aerobic denitrifiers" but also in typical denitrifiers, *Pseudomonas stutzeri* and *Paracoccos denitrificans*.[45,46] N_2O removal observed in the biofilter operated under oxic conditions may have been due to this partial activity of N_2O-reducing denitrifiers and/or nondenitrifiers in the presence of O_2. Another explanation for the N_2O reduction activity under oxic operation is the development of anoxic niches in inner biofilm. Previous model calculations and microelectrode measurements have estimated that O_2 in bulk air penetrates no farther than 100 μm into the biofilm, while biofilm thickness often exceeds this value by far.[47,48] A recent research that examined codiffusion of O_2 and N_2O into biofilm confirmed the formation of anoxic niches within the biofilm exposed to air, wherein N_2O was reduced.[49]

Scaling-up of the biofiltration system to suit a typical BNR WWTP with an average aeration rate of 2,000 m^3 day^{-1} (the average aeration rate of a BNR WWTP treating 210 000 population equivalents)[50] would require 111.8 m^3 of reactor volume to ensure an EBRT of 80.5 min (removal efficiency higher than 50%). This dimension may seem unrealistic; however, assuming a constant aeration requirement per population equivalent, this calculation result also suggest that biofilters with a feasible reactor volume (<28 m^3) would suffice for WWTPs serving less than 50 000 population equivalents. For downsizing of the reactor, which is necessary for broader implementation and cost reduction, performance enhancement would be necessary. The higher biological N_2O removal rate (per m^2 of surface area) at higher flow rates (SI Table S2) suggested that the biofiltration system may be kinetically limited. If this is the case, the performance of the biofilter may be enhanced simply by increasing the reactive surface area. Nonetheless, the major focus of performance enhancement should be on finding solutions to the "oxygen problem". The off-gases from activated sludge tanks in WWTPs always contain >10% O_2 and a significant drop in N_2O removal efficiencies and biological N_2O removal rates were observed when the experiments were performed with oxic gas streams. Reversible oxygen scavengers may be utilized to remove O_2 from off-gas stream before passing the gas through the biofilter. The choice of packing materials with narrow and deep pores disrupting oxygen mass transfer may increase the volume of the anaerobic niches in the biofilter and improve the biofilter performance. Enrichment and isolation of O_2-tolerant N_2O-reducing microorganisms would be an exciting scientific investigation, and inoculation of the biofilter with such organisms will help improve the performance of the biofilter operated under oxic condition. These potential improvements would allow for substantial downsizing of the biofiltration system. Implementation of these biofiltration systems for treatment of off-gases containing lower concentrations of O_2 can also be considered. For example, the biofiltration system would be an attractive option for treating N_2O-containing off-gases from partial nitritation processes used as the pretreatment for annamox processes, as O_2 concentration is often lower than 10% in these gases.[51,52]

The experimental results in this study suggested that the proposed biofiltration system will be a promising advancement in the environmental technology that will significantly reduce the carbon footprints of WWTPs with unprecedentedly low cost and environmental impacts. Future research is warranted, however, for long-term observation of the performance in less-than-ideal field conditions, for example, fluctuating N_2O concentration and temperature and the presence of potentially toxic compounds (e.g., volatile organic compounds, NH_3, and H_2S) in the raw wastewater, before scaling-up of the N_2O-removal system to a fully operational pilot-scale plant. A comprehensive life-cycle analysis for the pilot plant will also be necessary to examine the economic as well as technical feasibility at the commercial scale.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.7b02750.

Table S1−S3, Figure S1−S6, and the results from the counter-current operation of biofilter (PDF)

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Environ. Sci. Technol. XXXX, XXX, XXX−XXX

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Environ. Sci. Technol. XXXX, XXX, XXX−XXX