A novel aerated surface flow constructed wetland using exhaust gas from biological wastewater treatment: performance and mechanisms

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Abstract

In this study, a novel aerated surface flow constructed wetland (SFCW) using exhaust gas from biological wastewater treatment was investigated. Compared with un-aerated SFCW, the introduction of exhaust gas into SFCW significantly improved NH4+-N, TN and COD removal efficiencies by 68.30 ± 2.06%, 24.92 ± 1.13% and 73.92 ± 2.36%, respectively. The pollutants removal mechanism was related to the microbial abundance and the highest microbial abundance was observed in the SFCW with exhaust gas because of the introduction of exhaust gas from sequencing batch reactor (SBR), and thereby optimizing nitrogen transformation processes. Moreover, SFCW would significantly mitigate the risk of exhaust gas pollution. SFCW removed 20.00 ± 1.23%, 34.78 ± 1.39%, and 59.50 ± 2.33% of H2S, NH3 and N2O in the exhaust gas, respectively. And 31.32 ± 2.23% and 32.02 ± 2.86% of bacterial and fungal aerosols in exhaust gas were also removed through passing SFCW,
respectively.

Keywords: Surface flow constructed wetlands; Intermittent aeration; Exhaust gases; Nitrogen transformation; Odor.

1. Introduction

Over the last few decades, rapid urbanization and economic growth has caused a series of severe environmental issues such as river pollution and water blooms in lakes, especially in developing countries. Considering the stringent discharge guidelines and standards for conventional wastewater treatment plants (WWTPs), WWTPs still face challenges in removing excess nutrients effectively from wastewater in an economical way, resulting in negative environmental consequences (Wu et al., 2016). On the one hand, WWTPs have not been constructed or fully operated due to their large capital investments and operating costs (aeration is the highest energy consumption period, accounting for 40-60% of the total plant operating costs) (Gu et al., 2008). On the other hand, constructed wetlands (CWs), which are regarded as a tertiary treatment process, have offered the greatest potential for secondary effluent treatment owing to their good efficiency, low costs and low maintenance (Vymazal, 2010).

Pollutant removal within CWs is a complex process that primarily includes substrate adsorption, plant absorption and microbial degradation. It is widely known that microbial nitrification and denitrification represent the major nitrogen removal mechanisms in CWs (Coban et al., 2015). According to the water level, CWs can be classified as surface flow (SF) CWs and subsurface flow (SSF) CWs. Compared to
SSF wetlands, an SF wetland can better simulate natural systems, as the water flows over the bed surface and is filtered through a dense stand of aquatic plants, so SFCWs have often dominated in North America (Brix, 1994). However, the pollutant removal efficiency of CWs is not viewed optimistically, and the removal efficiencies of ammonia nitrogen (NH₄⁺-N) and total nitrogen (TN) by 268 wetlands are 30.0% and 39.6%, respectively. The removal percentage of total phosphorous (TP) is within a range of 40-60% (Zhang et al., 2011). Thus, the nitrogen (N) removal efficiency still remains low in conventional CWs. In general, the purification capacity of SSFCWs is better than that of SFCWs. Improving the removal performance of pollutants in SFCWs has become a very important topic.

Artificial aeration has been widely used as one of the most effective and economic operation strategies for the intensification of CWs (Wu et al., 2014). Artificial aeration is promising not only for improving pollutant removal but also for turning the flow pattern in SFCWs from laminar to turbulent. Turbulent flow positively affects some of the mechanisms that are responsible for pollutant removal in SFCWs (Yin et al., 2016). Additionally, plants play an important role in CWs, and they generally have positive effects on purification. Airflow is one of the important environmental factors involved in plant growth, and it can affect plant photosynthesis, transpiration and leaf temperature (Nishikawa et al., 2013). Chyan et al. (2016) reported that an effective increase in the ammonium nitrogen removal from 32.9-54.8% to 78.2-78.6% was achieved in aerated SFCWs. Even though artificial aeration is the most effective method of ensuring a sufficient oxygen supply, the corresponding
operational cost greatly limits its popularity (Zhang et al., 2010). Numerous studies have focused on improving the pollutant removal performance by using artificial aeration in SSFCWs (Hu et al., 2012a; Ong et al., 2010; Uggetti et al., 2016), but few studies have focused on using aeration to intensify the removal processes in SFCWs. Hence, it is necessary to further optimize the oxygen supply strategy in SFCWs.

At present, the subject of focus in WWTPs is their pollutant removal efficiency and treatment cost but not the emission of exhaust gases. The exhaust gas produced by the aeration process often directly diffuses to the atmosphere, resulting in a nuisance to adjacent populations and a risk of serious environmental pollution. The exhaust gas can include bad odours (e.g., hydrogen sulphide (H$_2$S) and ammonia (NH$_3$)) (Burgess et al., 2001). In the work of Sui et al. (2008), the emission concentrations of H$_2$S were 0.01-22 mg/m$^3$ and the emission concentrations of NH$_3$ were 0-0.67 mg/m$^3$ from the municipal wastewater treatment plant. Recently, the high emission of nitrous oxide (N$_2$O) during the wastewater treatment process has attracted a great deal of attention. Kampschreur et al. (2009) estimated that 0-95% of N was converted to N$_2$O for release at the lab scale and 0-14.6% of N was converted for N$_2$O release at the full scale. N$_2$O has been listed as an important greenhouse gas that acts as the leading ozone depletion substance. Its 100-year global warming potential is 298 times higher than that of carbon dioxide (CO$_2$) (IPCC, 2013). In addition, microbial aerosols cannot be ignored, which is causing broad concerns all over the world (Brandi et al., 2000). In particular, pollution and the control of microbial aerosols from WWTPs have gradually become the focus of people's attention. Microbial
aerosols are generated from the bursting bubbles produced by the aeration system. The possible downwind movement of a microbial aerosol can increase the dispersion of airborne bacterial, viral and fungal species, which may represent a health risk for occupationally exposed personnel (Carducci et al., 1995). Srikanth et al. (2008) describes the impact of microbial aerosols on human health, and it is believed that the threshold limit value for microbial aerosols is very important for human health risk assessments. Thus, most WWTPs have become new pollution sources while also protecting the urban environment. However, the exhaust gas from biological wastewater treatment contains oxygen (O₂), CO₂, volatile organic compounds (VOCs) and microorganisms, which are beneficial for improving plant growth and enhancing the microbial abundance in CWs. Until now, few people have realized that exhaust gas is a type of “available resource”, and there have been no reports on using sewage treatment plant exhaust gas as a gas source for aerating SFCW.

As an initial attempt, the aim of this study was to design a novel aerated SFCW using exhaust gas from a biological wastewater treatment and investigating the treatment performance of the novel SFCW. For this purpose, three lab-scale SFCWs were operated under different conditions (un-aeration, intermittent aeration with air and intermittent aeration with exhaust gas). Simultaneously, the pollutant removal mechanisms in the SFCWs were investigated by measuring the microbe abundance.

2. Material and methods

2.1 Experimental system configuration

Experimental aerated SFCWs using exhaust gas from biological wastewater
treatment which were built in Shandong University in Jinan, China (36°40'36"N, 117°03'42"E). The experimental system consisted of one sequencing batch reactor (SBR) and three parallel laboratory-scale reactors (SFCW A: un-aerated CW; SFCW B: intermittent aeration CW with air; SFCW C: intermittent aeration CW with exhaust gas). The schematic diagram of the combined experimental setup is shown in Fig. 1.

The laboratory-scale SBR had a working volume of 15 L with an internal diameter and a working height of 25 cm and 30 cm, respectively. The schematic diagram of the reactors was presented in a previous paper (Zhang et al., 2015). A constant airflow was introduced for aeration through a fine air diffuser at the bottom of the reactor and the aeration rate was maintained at 0.12 m³/h. The seeding sludge was obtained from the Second Wastewater Treatment Plant of Everbright Water (Jinan) Ltd., China. A mixed liquor suspended solid (MLSS) from the reactor was maintained at 4500-5000 mg/L.

Three polyethylene tanks (22 cm in length, 45 cm in width and 40 cm in depth) were used to build the SFCW systems. Each microcosm was filled with two layers of substrate, with a 10 cm bottom layer of gravel (3-4 cm in diameter) for the supporting layer and a 25 cm top layer of washed river sand (1-2 mm in diameter) to facilitate the dispersion of wastewater and the growth of plants. In SFCWs B and C, a perforated pipe sparger was installed at the surface of the substrate for use as an oxygen supply. Sweet flag (Acorus calamus L.), a typical wetland plant, was selected as the experimental plant. Healthy plants with heights of approximately 30 cm were weighed and then transplanted into the SFCWs in March of 2016. The plant density was 30-35
rhizomes per unit. After the transplantation, the CW systems were flooded using tap water and watered for two months until the sweet flag was well established.

2.2 Experimental system operation

During the experiment, the SBR was fed with synthetic wastewater (at approximately 300 mg/L of chemical oxygen demand (COD), 60 mg/L of NH$_4^+$-N). Details about the synthetic wastewater and the characteristics of the influents are shown in Supplementary Materials. The influent pH values were adjusted to 7.5-8.0 by adding NaHCO$_3$. The influent was prepared in a storage tank (100 L) and introduced into the reactor by using a peristaltic pump. The volumetric exchange ratio of the SBR was 50% with a cycle of 4 h, resulting in a hydraulic retention time (HRT) of 8 h. The reactor was operated sequentially under alternating anoxic and aerobic conditions, and each cycle consisted of feeding (10 min), an anoxic process (60 min), an aerobic reaction (120 min), settling (30 min) and decanting (20 min).

The effluent of SBR flow into a setting tank was then conveyed to the three SFCWs at a flow rate of 3 mL/min using a peristaltic pump. All the wetland systems were fed continuously with an HRT of 3 days. The pre-punched inflow and outflow tubes were positioned at the top of each tank at the same height. SFCWs B and C were intermittently aerated at an airflow rate of 0.012 m$^3$/h for 2 h each cycle, and this rate was consistent with the SBR aeration time. SFCW A was operated without aeration.

2.2.1 Water sampling and analysis
Water samples were collected from the reactor influent tank and the effluent of each system (SBR and three SFCWs) every 3 days to analyse the transformation of NH$_4^+\text{-N}$, NO$_3^-$-N, NO$_2^-$-N, TN and COD according to standard methods (APHA, 2005). The DO and pH values were measured using a DO meter (HQ40d, Hach, USA) and a pH meter (SG2, METTLER TOLEDO, Switzerland), respectively.

2.2.2. Plant sampling and analysis

At the end of June, July, August, September and October, plant leaves were harvested randomly from the different SFCWs and rinsed with distilled water to measure their chlorophyll contents. The leaf samples were cut into 1-2 cm square pieces and extracted for 24 h in the dark with 25 mL of 80% acetone. The total chlorophyll contents of the leaves were then determined using an ultraviolet spectrophotometer at 652 nm, as described by Bruinsma (1963). All the analyses were conducted in triplicate. The chlorophyll content was expressed based on the fresh weight (FW) (mg g$^{-1}$).

2.2.3. Gas sampling and analysis

2.2.3.1 The emission fluxes of N$_2$O and CO$_2$

The N$_2$O and carbon dioxide (CO$_2$) emission fluxes from the SBR and three SFCWs have been investigated in this study. When the experimental system tended to be stable, gas samples were taken in gas sampling bags (PV-500 ml; Delin, China) using an aspirator pump at intervals of 10 min according to the closed static-chamber method during the un-aeration phase and at 15 min intervals according to the open chamber during the aeration phase (Wu et al., 2009; Zou et al., 2016). The N$_2$O
concentration was measured using gas chromatography (7890B; Agilent, USA), and a
detailed method can be found in our previous study (Zhang et al., 2016). The CO₂
concentration was measured simultaneously using a thermal conductivity detector
(TCD) within the vials, and the operating condition details about the gas
chromatography were performed according to the method described in the previous
study (Wu et al., 2016). The N₂O and CO₂ emission fluxes of SFCWs B and C
(aeration phase) were obtained based on the average N₂O and CO₂ concentrations
during the sampling period, and the equation was modified according to methods
described by Hu et al. (2010), but with the sludge volume being replaced by the
chamber recovery area. The N₂O emission flux of three SFCWs (un-aeration phase)
was calculated by means of linear increases in the sampling period according to the
equation given by Jones et al. (2011). The emission rate and quantity of N₂O from
SBR was obtained from the method described by Kong et al. (2013).

2.2.3.2 The concentrations of odors gas and O₂

The odors gas (i.e., H₂S and NH₃) samples from the SBR and SFCW C were
taken at the beginning of the aeration phase. The sampling time was 40 min at the
same time of day, with a constant sampling flow rate of 1.2 L/min. There were three
replicates for each system. The H₂S and NH₃ concentrations were determined
according to the methylene blue spectrophotometric method (Fogo et al., 1948) and
the indophenol blue spectrophotometric method (Ivančič and Degobbis, 1984),
respectively. The oxygen concentrations in the air and exhaust gas were determined
by using an O₂ meter (CY12C, KREVOR, China).
2.2.3.3 Microbial aerosol

The bacterial and fungal aerosols from the SBR and SFCW C were collected using a six-stage Andersen sampler (Thermo-Andersen, Smyrna, GA, USA) which contained six Petri dishes filled with an appropriate agar medium. The Andersen sampler has six stages with different cutoff ($D_{50}$) sizes from higher to lower as follows: 7.0, 4.7, 3.3, 2.1, 1.1, and 0.65μm, which are representative of the human respiratory system. At the beginning of the aeration phase (8:00-8:15 AM), the Andersen sampler was operated at 28.3 L/min for 15 min for each sampling at the same time of the day. Bacteria from the collected air samples were cultivated in nutrient agar (Hope Biotech Co., China) at 37°C for 24 h. Fungi were incubated in Rose Bengal Medium at (Hope Biotech Co., China) 28°C for 72 h. After the incubation, the numbers of colonies on the plates were determined by positive-hole correction method (Macher, 1989). The results were calculated as the geometric mean of the replicates and expressed in colony-forming units per cubic meter of air (CFU/m$^3$). All the analyses were conducted in triplicate.

2.2.4 Microbe sampling and analysis

At the end of the experiment, the sediment samples were collected in the top layer (10-15 cm) of every SFCW from five spots and then mixed as one composite sample for microbial analysis. After their collection, the sediment samples were dried at -60°C using a freeze-dryer (Unicryo MC 2 L freeze dryer, Germany) for 36 h and then stored at -20°C for other analyses. Simultaneously, sludge-mixed liquor (30.0 mL) was collected at the end of the oxic phase of the SBR. According to the
manufacturer’s instructions, the collected sediment and the sludge-mixed liquor were treated with a MOBIO PowerSand™ DNA Isolation Kit to extract the total genomic DNA and then stored at -20 °C for further analyses. The relative abundances of the total bacteria (16S rRNA gene), nitrifying bacteria (amoA and nxrA genes) and denitrifying bacteria (nirk, nirS and nosZ genes) were quantified by quantitative polymerase chain reaction (q-PCR).

2.4 Statistic analysis

All the statistical analyses were performed using SPSS statistical software package 11.0 (SPSS Inc., Chicago, USA). Two-sample t-tests were used to evaluate the significance of the differences among the means. The tables and figures show the results of the averaged data. In all the tests, the differences and correlations were considered statistically significant when P<0.05.

3. Results and discussion

3.1 Nitrogen and organic removal from different SFCW systems

3.1.1 Nitrogen removal

After one month of operation, the effluent contaminants concentrations tended to be stable and the combined system was in steady state. The influent and effluent concentrations of NH₄⁺-N, NO₂⁻-N, NO₃⁻-N and TN in the wastewater for each SFCW are shown in Fig. 2a. The average NH₄⁺-N influent concentration was 28.55 ± 2.51 mg/L. The NH₄⁺-N removal was clearly observed in the three SFCWs in which the average discharge concentrations of SFCWs A, B and C were 13.66 ± 2.08, 4.89 ± 1.64 and 3.49 ± 2.04 mg/L, resulting in NH₄⁺-N removal efficiencies of 52.15 ±
1.13%, 82.87 ± 2.23% and 87.77 ± 1.92%, respectively. The NH$_4^+$-N removal efficiencies in SFCWs B and C were clearly improved due to the oxygen supply via intermittent aeration and the temporal profile of the DO distribution in the three SFCWs for the aeration phase (2 h) is illustrated in Fig. 2b. The DO concentrations of SFCWs B and C increased from 1.51 to 2.38 mg/L and 1.35 to 2.25 mg/L over the first 0.5 h aeration time (0-30min), respectively, which was primarily attributed to the excess power of the compressor used for air and exhaust gas injection. During the aeration phase (0.5-2 h) the DO concentration decreased rapidly due to the NH$_4^+$-N oxidation process. More precisely, the DO concentration decrease in SFCW C was faster than that in SFCW B. This finding implied that SFCW C has a better nitrification performance. The DO was 1.12 ± 0.05 mg/L in SFCW A due to atmospheric reaeration (AR), which caused an anaerobic environment in SFCW and thus may result in poor nitrification. Thus, NH$_4^+$-N was the primary form of nitrogen in SFCW A. The major form of nitrogen in SFCWs B and C was NO$_3^-$-N, implying that nitrification (NH$_4^+$-N → NO$_3^-$-N) was well achieved in wetland systems B and C. This result could indicate a greater oxidizing condition for boosting the growth of nitrifying bacteria. It is generally accepted that DO concentration above 1.5 mg/L are essential for nitrification to occur (Saeed and Sun, 2012).

Regarding TN removal, it is first dependent on complete nitrification, and the nitrified N is permanently removed via denitrification. TN removal could be limited by various factors such as excess inorganic N (including nitrate and nitrite) in effluent, excess oxygen and insufficient organic carbon sources. A TN removal trend similar to
that of NH$_4^+$-N removal was observed in different SFCW systems. In more detail, SFCW C (48.97 ± 3.42%) reached the highest TN removal efficiency, followed by SFCW B (41.16 ± 2.12%) and SFCW A (39.20 ± 2.35%). This result indicates that intermittent aeration with exhaust gas significantly intensifies the TN removal, which is consistent with other research (Maltais-Landry et al., 2009c). This finding could be attributed to three possible reasons. First, intermittent aeration developed alternating aerobic and anaerobic conditions for nitrification and denitrification. In particular, the DO concentration in SFCW B was higher than that of SFCW C under the same aeration flow (Fig. 2b), which was ascribed to the oxygen content differences between the air (21.00 ± 0.13%) and exhaust gas (18.96 ± 0.16%). This result indicated that SFCW C could be beneficial in reaching full denitrification. Second, the CO$_2$ and volatile organic compounds (VOCs) in the exhaust gas could help contribute to the available carbon supply to promote the growth of nitrifying and denitrifying bacteria. Third, in aerated SFCWs, aeration could cause a flow pattern change from laminar to turbulent so the mixing of pollutants in wastewater and microorganisms in the substrate would be extensive (Hu et al., 2012b). Moreover, the low TN removal efficiency in un-aerated CWs is primarily due to the poor nitrification caused by the DO deficit (Fig. 2b).

3.1.2 Organics removal

Fig. 3 shows characteristics of COD removal from the different SFCW systems throughout the experiment. In intermittently aerated SFCW B (39.93 ± 2.31%) and SFCW C (42.21 ± 1.89%), the removal efficiency of the organic matters was much
higher than that of un-aerated SFCW A (24.27 ± 1.62%), suggesting that intermittent aeration had obvious impacts on enhancing the removal of COD in SFCW. Organic matter is decomposed in constructed wetlands by both aerobic and anaerobic microbial processes as well as by the sedimentation and filtration of particulate organic matter. In particular, aerobic heterotrophic bacteria played an important role in the aerobic degradation of organic matters. Saeed and Sun (2012) reported that intermittent aeration could not only create the substantial oxygenated conditions inside the CWs to foster aerobic bio-degradation pathways of organics but could also stimulate anaerobic organics degradation. In addition, by comparison with SFCW B, a high COD removal rate was observed in SFCW C, which could be ascribed to the aeration with exhaust gas in the wetlands, promoting the growth and reproduction of heterotrophic bacteria.

3.2 Emergent plant monitoring

The chlorophyll content and plant height of the sweet flag in the three SFCWs were monitored every month during the experimental period, as shown in Table 1. Plants play an important role in constructed wetlands. Wang et al. (2016) determined that the presence of plants positively affected both the microbial abundance and community. In this study, all the plants in SFCWs A, B, and C grown well without obvious symptoms of toxicity or nutrient deficiency with the laboratory environment and synthetic wastewater, enrich with nutrients. Although no significant differences in plant growth were observed between SFCWs B and C (p > 0.05), the growth of sweet flag in the aerated SFCWs and un-aerated SFCW was significantly different as shown
in the increased heights of the emergent plants, suggesting that aeration could markedly improve plant growth. Moreover, chlorophyll is the core of photosynthetic activity, affecting plant photosynthesis and ultimately plant growth. The variations in chlorophyll contents during the five-month experiments were in accordance with the plant height. The chlorophyll content was accumulated in the aerated SFCWs, indicating that the plant growth was improved by artificial aeration because of the enhanced chlorophyll synthesis. Over time, a decrease in the chlorophyll content and plant height occurred in October, and more obvious downward trends were observed in un-aerated SFCW compared with aerated SFCWs, suggesting that aeration could markedly delay plant decline. The following three reasons contributed to the enhanced plant growth by aeration. First, the change in the plant root zone oxygen content causing by aeration is beneficial for the growth of plants (Chen et al., 2015), and long-term anoxic conditions cause plant death due to alcohol poisoning (Rzewuski and Sauter, 2008). Second, as a critical factor for plants growth, more available nutrients are provided in aerated SFCWs due to the high nitrification rate, and a turbulent flow distributes nutrients evenly throughout the SFCW, thereby promoting plant absorption and increasing nutrient removal (Adler, 2010). Third, plants also affect CO₂ emissions through the photosynthesis and respiration of their aerial parts. The CO₂ fluxes in aerated SFCWs were lower than they were in un-aerated SFCWs as a result of the higher plant biomass in aerated SFCWs. These results are consistent with the findings of Maltais-Landry et al. (2009a).

3.3 Purification of exhaust gas
Nitrogen removal has been improved, and simultaneously the exhaust gas has been purified. The change of microbial aerosol in exhaust gas after passing through SFCW was investigated, as shown in Fig. 4. A significant decrease in the particle concentrations of bacterial and fungal aerosols was observed. Results showed that bacterial and fungal aerosols in exhaust gas could be markedly reduced by $31.32 \pm 2.23\%$ and $32.02 \pm 2.86\%$ after passing through the SFCW, respectively. No significant difference in the capture efficiency was observed between the bacterial aerosol and fungal aerosol, because fungi and bacteria had the same particle size distribution between stages 4 and 5 (Sanchez-Monedero et al., 2003). The microbial aerosol removal mechanism in SFCWs primarily depends on the interception and absorption of the liquid layer, which is similar to a bioscrubber (Scharf et al., 2004). Additionally, wetland plants also contribute to the removal of aerosols, which can prevent microbial aerosol diffusion.

Moreover, CWs also have good removal performance against H$_2$S, NH$_3$ and N$_2$O. As shown in Fig. 5a, the removal efficiencies of H$_2$S, NH$_3$ and N$_2$O were $20.00 \pm 1.23\%$, $34.78 \pm 1.39\%$ and $59.50 \pm 2.33\%$, respectively. In this study, SFCW can be seen as a bioscrubber for purifying exhaust gas by water dissolution (leading role), substrate adsorption or microbial utilization. The primary process involved in this purification is the absorption of the pollutants by the liquid layer of the SFCW, making the pollutants available to the microorganisms for aerobic biodegradation. Moreover, the turbulence flow that is induced by aeration in the liquid layer of SFCW accelerated the exchange of contaminants and washings.
Intermittent aeration had obvious impacts on N₂O emissions. More details are shown in Fig. 5b, SFCW C had the highest N₂O emission flux, followed by SFCW B and SFCW A, suggesting that aeration could markedly enhance N₂O emissions. This finding can be explained by the following two reasons: 1) N₂O can be produced through both the nitrification and denitrification processes. SFCWs B and C had higher nitrification (Fig. 2a) which accelerated the nitrogen transformations and led to high N₂O production and was consistent with other research (Itokawa et al., 2001); and 2) aerated SFCWs had a higher NO₃⁻-N concentration than un-aerated SFCW (Fig. 2a) in treated effluents, indicating that the denitrification process was inhibited and resulted in more N₂O emission. Maltais-Landry et al. (2009b) also reported that artificial aeration apparently stimulated N₂O production, potentially via incomplete denitrification. Interestingly, although SFCW C had a lower NO₃⁻-N concentration than SFCW B, the highest emission flux detected in SFCW C is approximately 1.47 times higher than that in SFCW B, because N₂O in exhaust gas is only filtered through the liquid layer and then discharged directly into the atmosphere.

3.4 Microbial analysis

Microbial processes have been determined to be important pathways that contribute to nitrogen removal. The quantities of functional genes involved in biological nitrogen transformations, i.e., all bacteria (16S rRNA), AOB (amoA), NOB (nxrA), denitrifying bacteria (nirK+nirS) and nosZ genes are shown in Table 2.

There were remarkable differences in microbial abundance of the three SFCWs. First, a large number of nitrifying bacteria (AOB, NOB) were detected in aerated
SFCWs, while fewer AOB and NOB were detected in un-aerated SFCW, indicating that intermittent aeration was beneficial to promote the growth and reproduction of AOB and NOB, and the low DO in SFCW A seriously limited the growth of nitrifying bacteria. The results could explain the high removal of NH$_4^+$-N from aerated SFCWs (Fig. 2a). Second, the gene numbers of nirK and nirS exhibited an increase associated with the enhanced denitrifying activity that was responsible for eliminating the NO$_3^-$-N concentration. The highest abundance of denitrifying bacteria was detected in SFCW C, followed by SFCW B and SFCW A. This result indicated that the aerated SFCWs only inhibited the activity of denitrifying bacteria rather than eliminating it. Third, compared with SFCWs A and B, the abundance of nitrifying bacteria, denitrifying bacteria and total bacteria in SFCW C was greatly enhanced. The reason for this enhancement is probably that the microbe from the SBR was introduced to the CWs by aerating with exhaust gas.

In addition, to explain the microbial mechanism of N$_2$O reduction, nosZ gene was conducted and the results are shown in Table 2. Reportedly, the nosZ gene that encodes the catalytic subunit of the N$_2$OR has a plausible link to the N$_2$O reduction activity, which was correlated with more bacteria being capable of reducing N$_2$O to N$_2$ (Chourey et al., 2013). The highest nosZ copy density was observed in SFCW C, primarily because it had a higher quantity of denitrifying bacteria (Table 2) and better anaerobic conditions (Fig. 2b), thus accelerating the expression of the nosZ gene.

**3.5 Feasibility analysis in a real system**

As an initial attempt, our present study designed a novel aerated surface flow
constructed wetland using exhaust gas from biological wastewater treatment for mitigating WWTP exhaust gas emission and at the same time improving the performance of wetland. The collection and introduction of exhaust gas were crucial point to apply this system in a real system. On one hand, considering the rapid development of odour management and treatment for WWTPs, and various investigations on collection system of exhaust gases in WWTPs have been conducted (Chang, 2009; Xu et al., 2012). Device of collected waste gas streams is ripe. On the other hand, adding aeration by air diffusers such as a perforated pipe sparger is a normal operation in aerated wetlands (Maltais-Landry et al., 2009c; Wu et al., 2014). Aerated constructed wetlands have been widely used in practical engineering applications. Thus, the results of present study might be easily applied in a real system without adjusting the existing technology.

4. Conclusions
A novel aerated SFCW using exhaust gas from biological wastewater treatment was designed and used to intensifying pollutants removal and increasing resource utilization. The introduction of exhaust gas significantly intensified the removals of organic and nitrogen. Simultaneously, the risk of exhaust gas pollution was effectively eliminated since SFCW (as a bioscrubber) could purify the exhaust gas ingredients, including H₂S, NH₃, N₂O and microbial aerosols. Microbial analysis showed that the novel aerated SFCW could improve microbial abundance. The strategy of integrating intermittently aerated SFCW with exhaust gas has a promising future in purifying WWTP effluent and exhaust gas simultaneously.
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Supporting Information

The detailed information of q-PCR, Compositions of the SBR influent (Table S1), CO₂ total emission flux in three systems (Fig. S1), Oxygen content of exhaust gas profile during aeration phase (Fig. S2) and Engineering drawings of aerated constructed wetlands (Fig. S3) can be found in the Supporting Information.
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Figure Captions:

**Fig. 1.** The schematic diagram of the combined experimental setup (SFCW A: un-aerated CW; SFCW B: intermittent aeration CW with air; SFCW C: intermittent aeration CW with exhaust gas).

**Fig. 2.** NH$_4^+$-N, NO$_2^-$-N and NO$_3^-$-N concentration variations in the influent and effluent from different SFCW systems (a) and the DO profile during the aeration phase in the three systems (b) throughout the experiment.

**Fig. 3.** Characteristics of COD removal from the different SFCW systems throughout the experiment.

**Fig. 4.** Culturable microbial aerosol concentrations for influent and effluent from SFCW C: (a) bacterial aerosol and (b) fungal aerosol.

**Fig. 5.** The concentration of odors gas (H$_2$S and NH$_3$) in SBR and SFCW C (a) and N$_2$O emission flux from the three SFCWs (b).
Table 1 The chlorophyll content and plant height in three systems during the experimental period

| Time   | Total chlorophyll concet (mg g⁻¹FW) | Plant height (cm) |
|--------|------------------------------------|------------------|
|        | A        | B        | C        | A        | B        | C        |
| June   | 1.29 ± 0.05 | 1.27 ± 0.06 | 1.30 ± 0.08 | 31.23 ± 2.36 | 30.83 ± 2.23 | 30.50 ± 1.98 |
| July   | 1.39 ± 0.06 | 1.53 ± 0.08 | 1.58 ± 0.09 | 35.09 ± 1.89 | 38.21 ± 2.01 | 39.56 ± 2.08 |
| August | 1.28 ± 0.05 | 1.59 ± 0.09 | 1.67 ± 0.08 | 37.26 ± 2.13 | 45.23 ± 2.35 | 46.13 ± 2.36 |
| September | 1.49 ± 0.07 | 1.79 ± 0.10 | 1.81 ± 0.11 | 38.98 ± 2.56 | 46.86 ± 2.11 | 47.06 ± 2.09 |
| October | 0.78 ± 0.04 | 1.58 ± 0.07 | 1.59 ± 0.06 | 33.27 ± 2.11 | 43.73 ± 2.33 | 44.06 ± 2.73 |
Table 2 Relative abundance of related genes for each system based on q-PCR analysis

| Experimental systems | amoA (AOB) | nrxA (NOB) | nirS and nirK (Denitrifying bacteria) | nosZ | 16S rRNA (All bacteria) |
|----------------------|------------|------------|--------------------------------------|------|-------------------------|
| A                    | 7.53E5 ± 5.26E5 | 3.06E6 ± 6.11E5 | 7.99E8 ± 2.52E8                      | 2.29E9 ± 3.12E8 | 5.24E10 ± 3.82E9         |
| B                    | 3.13E6 ± 8.62E5 | 1.32E7 ± 7.89E5 | 1.69E9 ± 2.78E8                      | 2.14E9 ± 3.75E8 | 7.48E10 ± 4.52E9         |
| C                    | 1.96E7 ± 9.12E5 | 2.27E7 ± 8.13E5 | 5.49E9 ± 2.69E8                      | 7.54E9 ± 3.65E8 | 1.04E11 ± 4.02E9         |
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