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ABSTRACT

This study aims to examine the treatment of graywater (GW), especially bathroom wastewater, using subsurface-flow constructed wetlands (CWs) in an apartment complex. It analyzed the removal of organic matters and nitrogen, the status of a wetland by depth, and microbial communities under the conditions of aeration and non-aeration at the inlet section, and planting and non-planting. The results of this study show that aeration at the inlet section and planting affect ORP, SO_4^{2-}, and nitrogen by water flowing distance and water depth in a wetland. The result of COD_{cr} removal seems to satisfy the Korean standard (below 20 mg/L (COD_{mn})) of reuse water quality in all three operational conditions. The aeration at the inlet section has the advantages of effectively removing organic matters at the inlet section, thus preventing its clogging, and removing nitrogen through the nitrification. Aeration at the inlet section and planting are expected to have a positive effect on the wetland status and GW treatment. Therefore, aeration at the inlet and planting will be helpful to construct and operate an artificial wetland in residential areas such as a multi-family housing complex. Ammonia-oxidizing bacteria including *Nitrosomonas*, *Nitrosospira*, *Nitrosococcus* and denitrifying or nitrate-reducing bacteria such as *Thiobacillus*, *Achromobacter*, *Pseudomonas*, *Micrococcus* belong to the classes of β-proteobacteria, γ-proteobacteria, and Actinobacteria. These microbial communities found in this study seem to contribute to nitrogen removal. But, the roles of microbial community in CWs need to be further investigated, both qualitatively and quantitatively.

Keywords: Greywater; Constructed wetland; Planting; Aeration; Microbial community; Apartment complex

1. Introduction

The shortage of usable fresh water is a big issue, not only in Korea but also worldwide. More than ever before, effective water use is required. In terms of solutions for this issue, the treatment and reuse of greywater (GW) and wastewater, the harvesting of rainwater and the desalination of sea water are some of the main topics for discussion.

GW refers to wastewater from bathroom, kitchen, and laundry water, with the exception of toilet water. Blackwater (BW) is toilet water. GW is less polluted than BW in terms of solid matters, nitrogen, and

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organic matters, although its content such as salts and surfactants can depend much on its origin. Given the difference in the contents of GW and BW, separate pipelines for GW and BW can increase the effective collection of GW, reduce the cost required to collect, treat and reuse GW [1–3]. The characteristics of GW and its separation from BW increase the potential of adapting decentralized water management systems to save water resources by reusing treated water for purposes other than drinking in many places with water shortages [4,5]. It has been suggested that constructed wetlands (CWs) can be a competitive solution to treat GW generated in cities in terms of source control [6].

GW is increasingly being used for gardening, and studies have been conducted on the treatment of GW using horizontal and vertical flow CWs. Although there have been no reports of infectious diseases caused by the reuse of GW for non-drinking purposes, it is important to properly treat GW in order to eliminate the risks of infectious diseases caused by pathogens in the water and to sustain the disinfection effect. It is also necessary to consider the removal of fecal coliform in GW treatment using CWs, as the pathogens are sometimes found in GW [7–10].

CWs can be operated using low amounts of energy, and they have both ecological and gardening advantages. The operator of CW does not need to be an expert. For these reasons, studies have been conducted to examine the treatment of less polluted river water or lake water using CWs, in order to solve the problems of eutrophication in artificial water spaces and lakes in cities [11,12].

Research has also been conducted with various methods of aeration to enhance the effectiveness of water treatment in CWs. The methods include the aeration before inlet, continuous aeration in the first half-section, aeration in the total bottom area, the oxygen provision effect of plant roots in CWs, etc. [13–16]. The relationship between plant roots and microorganisms can either be complementary or competitive, and the relationship between the roots of bean plants and rhizobium is well known. Sometimes, various types of microorganisms compete with bacteria for nutrition.

CW is an economical way to treat small amounts of wastewater with low construction and operation costs. However, CWs require a relatively large area and a long hydraulic retention time (HRT) to secure satisfactory water quality [17].

This study was conducted to examine the proper treatment of GW using a CW within an apartment complex. There is a need to review the feasibility of wetland construction in apartment complexes, considering the availability of green spaces based on the land use plan, in order to reduce the construction cost. This study aims to produce the water resources necessary for water-friendly facilities using CWs as a naturally purifying and ecological method for the treatment and reuse of a portion of the GW generated within an apartment complex. A study to examine the treatment and reuse of GW within the apartment complex in which it is generated can also help to enhance the ecological environment in cities. Against this backdrop, this study was conducted to examine the treatment of GW, particularly bathroom wastewater, using subsurface-flow CWs with different methods of aeration, including non-aeration at inlet, planting and non-planting. In these different conditions, the removal of organic matters and nitrogen, the internal status of wetlands and microbial communities were analyzed. Compared to a free water surface wetland, the subsurface-flow CW is effective for preventing unpleasant smells, mosquitoes, and harmful insects [18].

The study investigated the effects of the conditions of aeration and non-aeration at the inlet section, planting and non-planting on the water quality and the internal environment in the CW. It also attempted to assess the risk of the CW generating factors that could cause complaints from residents in an apartment housing complex, such as insects or unpleasant odors.

2. Materials and methods

2.1. Experiment apparatus and method of CW

The experiment apparatuses were set on the basement floor of apartment buildings, to maximize the ease of obtaining GW. An artificial wetland was constructed and operated, with a subsurface flow underneath a layer of gravel.

A 1 ton FRP container was set before water was flowed into a CW basin. This container was set up for the storage of collected bathroom wastewater before the water flowed into the wetland, and it helps to control the amount of water flowing into the wetland, and deposits and removes suspended solids, such as hair strands, etc. Bathroom wastewater flowed into each wetland from the storage using a peristaltic pump. Pea gravel (5–8 mm in size) was laid inside the wetland to a depth of 0.5 m. In the inlet and outlet sections, coarse gravel (3–4 cm in size) was laid to help smooth the water flow. The porosity of pea gravel is about 36%, and HRT is about 5–6 d.

The pipes for sample collection were installed in each wetland in order to analyze water quality. Sampling pipes were installed at the distances of 20, 60, 100, and 140 cm from the inlet, and three pipes were installed at the depths of 15, 30, and 45 cm from the water's surface. Wetlands were adjusted to maintain a water level of 45 cm from the bottom.

Seedlings of aquatic plants such as *Iris pseudacorus* and *Scirpus tabernaemontani* were planted in the layer of pea gravel. To promote the growth of the plants, five artificial lights (Metal Halide Lamp 250 W; white light) were installed on the ceiling of the basement. The artificial lights were illuminated from 8 AM to 6 PM The sampling for water quality analysis was conducted after each CW had been stabilized.

Experiments were carried out in different wetlands basins with different conditions. The CWs were operated in three different conditions. Case I was operated in a condition of aeration at the inlet and planting from February 2006 to February 2007. The aeration was made through an air pump for 8 h, with a suspension of 4 h. The aeration was made within 20 cm from the inlet, about 10% of the total length from the inlet. Case II was operated in a condition of non-aeration and non-planting from August 2005 to February 2007. Case III had non-aeration and planting condition from August 2005 to February 2007. The CWs were 1.7 m in length, 0.8 m in width, and 0.45 m in depth, with a total surface area of 1.36 m^2. The water quality analysis was conducted by collecting samples from the inlet, outlet, and sampling pipes p1, p2, and p4 at the depths of 15 and 45 cm. This study used the average of the sampling data by location and depth. The sample data (Case I) were collected from the wetlands operated for about 9–11 months. The sample data of Cases II and III were collected from the wetlands operated for about 14–17 months under each condition. And, to analyze the microbial community, samples were collected from sampling pipes p1 and p4 at the depths of 15 and 45 cm. (Figs. 1 and 2).

2.2. Method of water quality analysis

Total nitrogen (TN), $NH_4^+ - N$, $NO_3^- - N$, SO_4^{2-}, dissolved oxygen (DO), and CODcr were analyzed. DO was analyzed using KRK DO-2F, pH and oxidation and reduction potential (ORP) were analyzed using HANNA HI 8424 (pH sensor HI 1230, ORP sensor HI 3230). CODcr, TN, $NH_4^+ - N$, $NO_3^- - N$, and SO_4^{2-} were analyzed using an DR 4000 Spectrophotometer, an analyzer of HACH company, and all other tests were conducted pursuant to the standard method.

2.3. Analysis method of microbial community

2.3.1. Extraction of DNA

100 mL water samples of each operation type were filtered using a Millipore filter membrane with a membrane pore size of 0.2 μM under sterile conditions. Individual filter membranes were cut into pieces and transferred to their corresponding sterile eppendorff tubes, and DNA extraction was preceded using the UltraClean™ Soil Isolation Kit from MO-BIO (MO-BIO Laboratories Inc., CA, USA) according to the recommendations of the manufacturer. Approximately 5 g of the filter containing the filtered sediment was extracted, and the DNA was precipitated and washed with 70% ethanol. The extracted DNA concentration was measured using a Nanodrop ND-100 spectrophotometer, and the size of the DNA was checked on 1% agarose gels. DNA preparations were stored at −20℃ for further analysis.

2.3.2. 16S rDNA amplification for terminal-restriction fragment length polymorphism (T-RFLP)

The SSU rRNA gene was polymerase chain reaction (PCR) amplified from the genomic DNA extracted from the isolates using universal primers labeled forward FAM-27F (AGAGTTTGATCATGGCTCAG) and unlabeled reverse 1492R (TACGGTTACCTTGTTACG-ACTT) [19]. PCR reactions (25 μL) contained 0.1 ng of template DNA, 1X PCR buffer, 2 mM $MgCl_2$, 200 μM concentration of deoxy nucleoside triphosphates (dNTPs), 100 pM concentration of primer, and 0.025 U of Taq enzyme. An MJ Mini Thermal Cycler (BIO-RAD, USA) was used to incubate reactions through an initial denaturation step consisting of 94℃ for 3 min, and this was followed by 25 cycles of 94℃ for 1 min, 50℃ for 1 min, and an extension at 72℃ for 2 min. The annealing temperature was selected as the temperature that consistently yielded a single PCR product of the expected (~1,400 bp) size. The PCR products were purified using QIAGEN DNA purification spin columns (QIAGEN Inc., Valencia, CA) in a dark room.

2.3.3. Analysis of the PCR products

PCR products amplified with FAM-27F from each sample were purified and combined. Approximately 40 ng of purified PCR products was digested for 12 h at 37℃ with *Hha*I restriction enzyme in a 20 μL reaction mixture. The reaction mixture contained 2 μL of 10X Tango enzyme buffer (Fermentas), 1 μL of restriction enzyme (Fermentas), 5 μL of amplified template DNA, and sufficient ultrapure water to reach a final volume of 20 μL. Reactions were inactivated at 65℃ for 5 min and stored at −20℃ until electrophoresis. Digestions were tested for completeness with a pure culture control (*Pseudomonas* - LB400). The digested

Fig. 1. Planted, aerated at inlet(Case I), non-planted, non-aerated (Case II) and planted, non-aerated (Case III) CW.

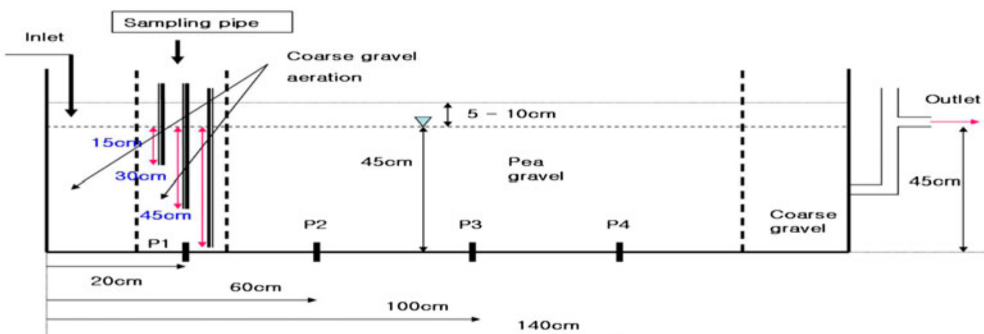

Fig. 2. Cross section of CW.

samples were sent to the National Instrumentation Center for Environmental Management (NICEM, Korea) for sequence analysis. Terminal fragments smaller than 50 bases or larger than 600 bases were deleted from the analysis, the former because of interference from unincorporated labeled primer and the latter because of sizing inaccuracies for such large fragments. Finally, a level of 50 fluorescence units was imposed as a minimum threshold value for all peaks in the selected size range. Profiles were visually inspected and aligned based on relative peak distribution. For each enzyme digestion, duplicates were run as a means of confirming the reproducibility of the method. The peak height and area of fragments were analyzed using DNA sequencer 377, and phylogenetic assignment tool, a web-based program, was used for T-RFLP analysis [20].

3. Results and discussions

3.1. *Effect of aeration at the inlet and planting on treatment of bathroom wastewater in CWs*

The internal status, water quality, and microbial community (class level) in the CWs were analyzed. Water quality was measured in three wetlands, which will be called Case I (aeration at the inlet and plating), Case II (non-aeration and non-planting), and Case III (non-aeration and planting) in this paper. Bathroom wastewater from showers, hand basins, and bathtubs includes soaps, body-fats, shampoos, soils, urine, feces, etc. [21]. These constituents may be the major sources of nitrogen and carbons as Fig. 3 shows.

In Case I, the DO is at a maximum at the inlet section and goes down in the middle section. DO shows the same level as Cases II and III which had no inlet aeration in the end section. These indicate that the influence of the aeration at the inlet does not reach the end section. In Case I, the wetland has the effect of maintaining a high level of DO to the middle section, 0.6 m from the inlet. The aeration at the inlet influences the levels of ORP, SO_4^{2-}, and NO_3^- by water flowing distance and water depth in the wetland. The DO level is higher at a depth of 15 cm than of 45 cm in Cases I, II, and III because of contact with atmosphere. In a wetland with planting, the roots of plants are the source that provides oxygen in a limited amount [9,22]. The reason that DO levels at a depth of 15 cm in Case III are slightly lower than DO levels at the same depth in Case II could be due to the blocking

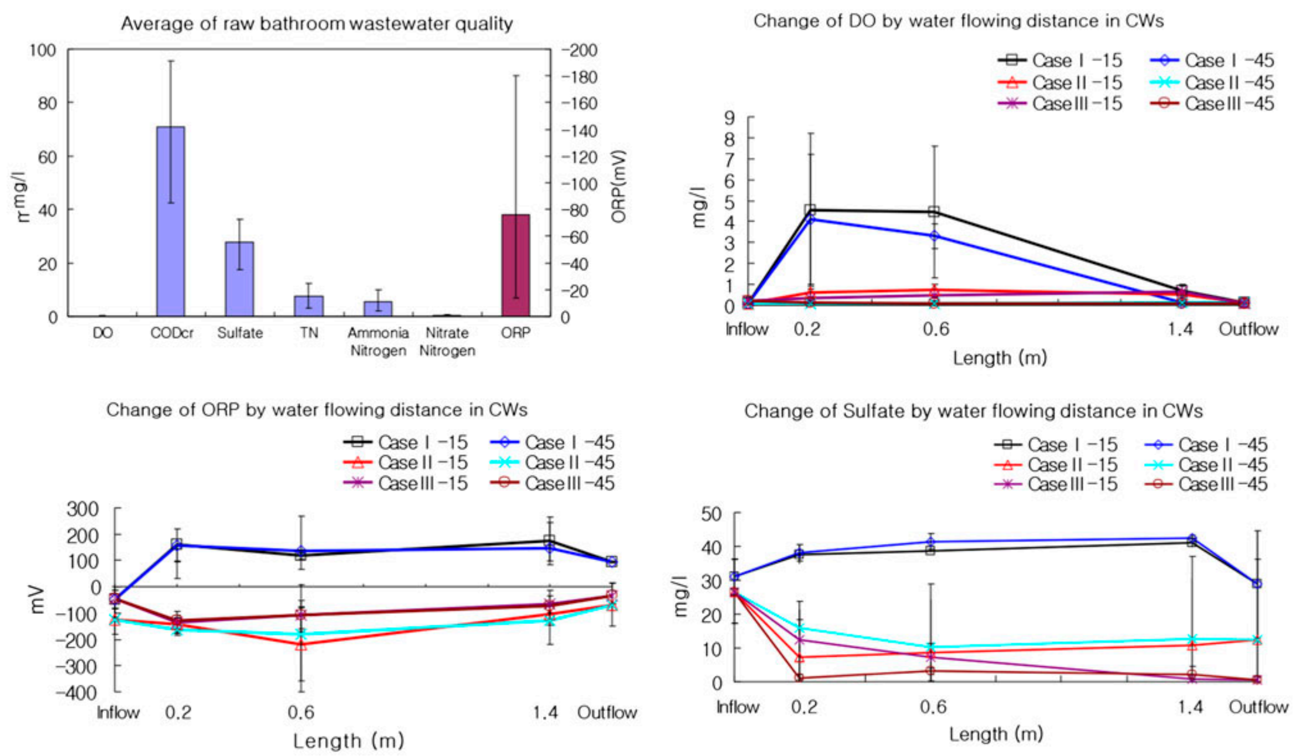

Fig. 3. Bathroom wastewater quality and change of DO, ORP, and sulfate by water flowing distance in CWs.

effect of an insulation layer with moss covering the surface of the wetland [15]. The DO levels in Cases II and III, which lacked aeration at the inlet, are consistent throughout the wetlands, although DO in the outlet area increases slightly compared to that in the inlet area.

ORP also shows a slight increase from the inlet area to the outlet area. ORP in Case I increases with aeration. The measured ORP is added by +244 mV (at 25℃) to be converted to hydrogen electrode potential (E_h). Based on the anoxic condition of $-100\text{ mV} < E_h < +300\text{ mV}$, it is an anoxic condition when ORP is in the range from −344 to 56 mV [22].

In Case I, ORP of the inflowing source water is about −50 mV, and ORP of the water flowing inside the wetland is in the range of 118–175 mV due to the aeration at the inlet. In Case II, ORP of the inflowing source water is about −125 mV and ORP of the flowing water inside the wetland is in the range of −219 to 102 mV. In Case III, ORP of the inflowing source water is −43 mV and ORP of flowing water inside the wetland is in the range of −134 to 67 mV. In Case III, better reduction conditions are not evident in deeper locations compared to the near-surface area. However, Case II shows better reduction conditions compared to Case III, which seemed to be affected by plant roots [22,23].

Sulfate, a component existing in wastewater, is generally reduced in wetlands where oxygen is lacking, but the aeration at the inlet can prevent this reduction [14]. In Case I, there is no sulfate-removal function; in fact, sulfate is increased due to the aeration at the inlet area and then is reduced at the outlet area, to reach the same level as at the inlet. In Cases II and III with the anoxic condition, SO_4^{2-} was reduced. In this connection, the measured Fe level was mostly increased according to water flowing distance (data not shown). Case III shows lower sulfate than Case II although Case II showed lower ORP than Case III. Sulfate is reduced and forms a great deal of sulfide ion under pH 7, which can cause unpleasant odors due to hydrogen sulfide [24]. However, residents did not complain about unpleasant odors because the sulfate and pH in the bathroom wastewater flowing into the wetlands were in the ranges of 20–40 mg/L and 7–8 (data not shown), respectively.

The effect of planting on the efficiency of water treatment in CWs has been studied in numerous papers. The studies have shown that the amount of oxygen provided by plants is very low compared to the amount required for wastewater treatment. In general, the oxygen-providing effect of plants is often ignored in wetland design [9,13–15,22,25]. The natural provision of oxygen to wetlands occurs through the photosynthetic activities of algae on the wetlands, the atmospheric diffusion effect on the wetland's surface, and plant roots. When straw or other plant stalks fall over the surface of wetland gravel, they form a blocking layer that prevents the atmospheric diffusion effect. Therefore, the blocking layer cannot interfere with oxygen transfer into the wetland. To resolve this, artificial aeration systems have been studied in the inlet and outlet sections of the CW [15,26].

Nitrogen is most effectively removed in Case I, which operates the aeration at the inlet section. Case I shows that the aeration at the inlet section causes the formation of nitrate and the rapid reduction of ammonia. The nitrate generated in the process is reduced by denitrification as the water goes to the end of the wetland. Nitrification takes place rapidly and reduces ammonia-nitrogen in the inlet section. The rapidly increased amount of nitrate-nitrogen in the inlet section gradually reduces as it goes through the middle to the end of the wetland. Although the aeration in the inlet section influences the middle section, the nitrate-nitrogen reduces. Therefore, the influence of the aeration in the inlet section does not seem to be significant on the denitrification that takes place in the middle section of the wetland [13,14].

However, nitrate-nitrogen is generally very low in Cases II and III. This seems to be because nitrate is consumed as an electron acceptor in an anoxic or anaerobic condition [27]. In Case III, the TN removal efficiency is lower than that of Case I, but it works relatively well and ammonia-nitrogen gradually decreases.

COD_{cr} of bathroom wastewater is in the range of 67.1–76.4 mg/L, and $SCOD_{cr}$ is about 30% of COD. In Case I, the COD_{cr} removal efficiency is the highest in the section from the inlet to 0.2 m due to the effect of aeration, but shows the same rate as Cases II and III in the other section after the 0.2 m spot. Case III shows a lower removal efficiency of organic matters than Case I in the section from the inlet to the 0.2 m spot, but shows a similar rate in the other section. That means for the treatment of bathroom wastewater containing low levels of organic matters, the aeration at the inlet influences the elimination of organic matters only in the section from the inlet to 0.2 m, but not in other areas.

All three cases show that the organic matters level is very low as water flows through the 0.6 m spot to the end of the wetlands, beginning to satisfy the Korean standard (below 20 mg/L (COD_{mn})) of reuse water quality from the 0.6 m spot and certainly satisfy the standard from the 1.4 m spot on.

The aeration at the inlet seems to have a great influence on the elimination of organic matters at the inlet section. The planting also seems to have some effects at the other section, but the difference between Case II and Case III is not so big. Nitrate-nitrogen increases slightly as it goes to the end section of the wetlands in Cases II and III, doing so especially in Case III, which is operated under the condition of planting. As seen in Fig. 4, though the planting has a positive effect on the wetland environment, the effect is not significant. The advantages of aeration at the inlet are the effective elimination of organic matters at the inlet, the prevention of clogging at the inlet, and the nitrification. The front aeration to prevent the clogging at the inlet seems useful when constructing an artificial wetland for an apartment complex or a green space in a city. The planting also seems essential for CW in an apartment complex to create an ecological green space as well as to treat bathroom wastewater.

Although the roles of plants are not clearly identified, the micro fauna in the root zone seems to play some role in forming a microbial community, purifying and filtering. Plants also seem to help form a habitable environment for aerobic microorganisms by providing oxygen from their roots and to influence the formation of a better environment for microorganisms by forming micro fauna in their root zone [14,15,25,28].

3.2. Microbial community shifts in response to the CWs

In response to the treatments by the CWs, the microbial community structures in each case were shifted (Table 1). In most of the inlet aeration and planting CW samples, spirochetes class populations were predominant. In the non-aeration and non-planting CW, spirochetes class populations were also predominant in the upstream samples (P1-15 and P1-45), while Bacilli and Clostridia class populations were predominant in the downstream sample (P4-45). However, the non-aeration and planting CW showed a significantly different pattern in community structure shifts in response to the CW treatment; the relative abundances were evenly distributed among the populations, and other than Spirochetes, Bacilli, and Clostridia became the predominant ones. These indicate that the non-aeration and planting CW provided the most influential effect on the microbial community structure and population dynamics among the three CW treatments.

Typical ammonia-oxidizing bacteria including *Nitrosomonas*, *Nitrosospira*, and *Nitrosococcus* belong to the classes of β-proteobacteria and γ-proteobacteria [29].

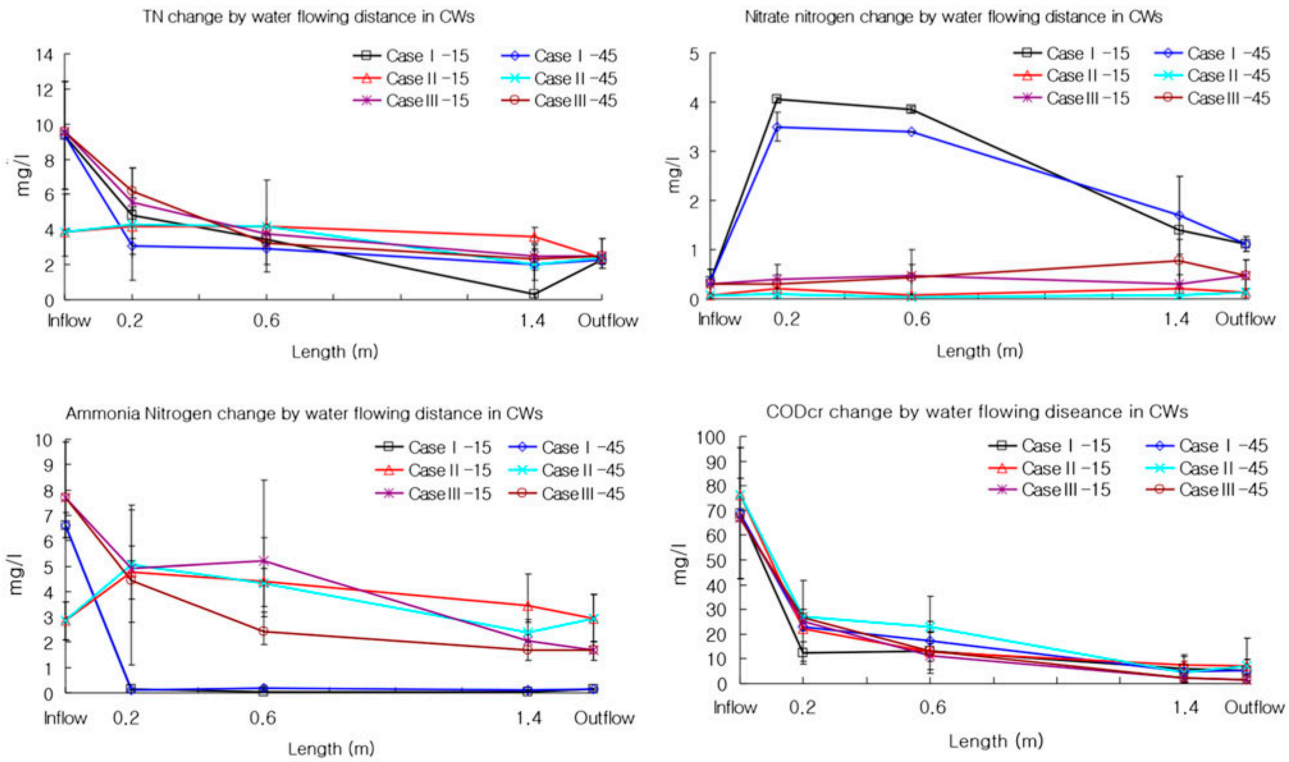

Fig. 4. Change of nitrogen and CODcr by water flowing distance in CWs.

Table 1
Microbial community structure in response to the CW. The numbers indicate the relative abundance (%) of total community

Microbial population (class level)	Case 1				Case 2				Case 3			
	P1-15	P1-45	P4-15	P4-45	P1-15	P1-45	P4-15	P4-45	P1-15	P1-45	P4-15	P4-45
Actinobacteria	ND	ND	ND	0.7	1.2	ND	NM	ND	ND	ND	ND	ND
Bacilli	ND	ND	ND	ND	ND	ND	NM	30.8	26.4	6.5	ND	ND
Bacteroidetes	ND	ND	45.2	ND	ND	0.8	NM	ND	ND	ND	ND	ND
α-Proteobacteria	2.0	ND	ND	ND	ND	ND	NM	ND	ND	ND	ND	ND
β-Proteobacteria	ND	ND	ND	0.9	ND	ND	NM	9.6	22.6	28.3	ND	ND
γ-Proteobacteria	13.6	ND	ND	14.0	ND	2.4	NM	12.6	23.5	26.6	21.7	ND
Clostridia	2.6	3.2	26.1	2.3	2.4	2.6	NM	30.4	18.6	29.1	30.5	42.8
Flavobacteria	ND	ND	ND	1.0	ND	ND	NM	ND	ND	ND	ND	ND
Spirochetes	81.7	96.7	18.5	79.3	92.4	81.5	NM	16.5	8.8	9.6	12.0	26.4
Unclassified	ND	ND	10.2	ND	3.9	2.7	NM	ND	ND	ND	35.9	30.8

Typical denitrifying or nitrate-reducing bacteria such as *Thiobacillus*, *Achromobacter*, *Pseudomonas*, and *Micrococcus* belong to the classes of β-proteobacteria, γ-proteobacteria, and actinobacteria [30–33]. β-proteobacteria, γ-proteobacteria, and actinobacteria were significantly detected in the Cases I, II, and III. This suggests that the efficient ammonia and nitrate removals (Fig. 4) might be due to microbial activities.

4. Conclusions

The aeration at the inflow portion influences ORP, SO_4^{2-}, and nitrogen removal in the wetlands according to the flowing distance and depth. ORP is more considerably reduced in Case II (non-aeration and non-planting) than in Case III (non-aeration and planting). This seems to be because Case III is affected by the plants. Sulfate in the bathroom water was in the range of 20–40 mg/L and did not cause complaints from residents about unpleasant odors.

Case I shows that the aeration at the inflow portion causes the rapid nitrification. The generated nitrate is reduced by denitrification in the middle and end section of the wetland. The aeration in the inflow portion does not seem to significantly affect the denitrification from the 0.6 m spot on. The TN removal rate of Case III is lower than that of Case I, but it goes comparatively well.

The COD_{cr} removal rate is the highest by the aeration at the inlet section in Case I, but the rate is the same as Cases II and III in the other area except for the inlet section. The aeration at the inflow portion influences the organic matters removal only in the section (from the inlet to 0.2 m), but not in the other section. All three cases satisfy the Korean standard of reuse water quality (organic concentration) through the 0.6 m spot to the end of wetlands.

The inlet aeration seems to have the merits of the removal of organic matters, prevention of clogging, and removal of nitrogen by nitrification. The planting seems to cause a positive effect on the elimination of organic matter and nitrogen compared to Case II with anoxic conditions. But, the effect is not important. Planting is required, because CWs have roles of creating a green space and ecological education in addition to GW treatment. Therefore, the aeration at the inflow portion and planting will be useful in constructing a subsurface-flow wetland in residential space such as the apartment complex.

The results of the microbial community analysis showed that the non-aeration and planting CW treatment improved the microbial diversity, and suggested that the effective ammonia and nitrate removals were attributed to microbial activities. These findings suggest that the non-aerobic and planting treatment may induce microbial nitrogen removal in CW treatments.

Acknowledgments

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