Enhanced performance of constructed wetlands by incorporating magnetic Fe₃O₄ nanoparticles and the effects on the microbial community on the surface of the substrate

Zhou Qingwei^{1,2,3†}, Wu Weihong¹

¹School Institute of Environmental Materials and Applications, College of Materials and Environmental Engineering, Hangzhou Dianzi University, Hangzhou, Zhejiang 310018, PR China

²School of Environmental Science and Spatial Informatics, China University of Mining and Technology, Xuzhou, Jiangsu 221116, PR China

³Zhejiang Huachuan Industrial Group CO. LTD, Yiwu, 322000, PR China

ABSTRACT

Constructed wetlands (CWs) have been successfully employed to treat domestic sewage, while it is reported that inconsistent and low nutrient removal efficiency has been achieved. Due to the good performance in other treatment systems, magnetic Fe₃O₄ nanoparticles (Fe₃O₄ NPs) can provide a new pathway for the nutrient removal in CWs. In this study, the influences of Fe₃O₄ NPs on the nutrient removal and variation of microbial community were investigated. The CWs with Fe₃O₄ had better NH₄⁺-N (ammonia nitrogen, 95.9%), NO₃⁻-N (nitrate nitrogen, 83.8%), and TP (total phosphorus, 90.4%) removal percentages than that without Fe₃O₄. High-throughput sequencing analysis indicated that the microbial community structure could be changed by Fe₃O₄ NPs. For example, at class level, the major bacteria such as *Betaproteobacteria*, *Gammaproteobacteria* and *Deltaproteobacteria* were promoted by Fe₃O₄ NPs. On the other hand, at genus level, some abundant genera disappeared, while other abundant genera became dominant in CWs with Fe₃O₄ NPs, including *Desulfuromonas* (3.0%), *Azospirillum* (1.9%), *Chryseolinea* (1.5%), and *Desulfomicrobium* (1.8%). The latter genera essentially improved the microbial community involved in the nitrogen removal. Therefore, the CWs with Fe₃O₄ NPs showed higher NH₄⁺-N and NO₃⁻-N removal percentages than that without Fe₃O₄ NPs.

Keywords: Constructed wetland, High-throughput sequencing, Magnetic Fe₃O₄ nanoparticles, Microbial community

1. Introduction

Over the past few decades, excessive amounts of nitrogen and phosphorus from untreated wastewater caused serious ecological problems [1]. Especially, nitrogen compounds cause eutrophication in the water environment, resulting in bad water quality [2]. Therefore, effective wastewater management and treatment are critical for maintaining public health and environmental safety [3]. The scarcity of useable water necessitates sustainable wastewater treatment techniques, such as microbial fuel cell [4-6]. However, its upscaling is challenging primarily due to its capital expense and CWs are considered as an alternative to sewage treatment. [7-10]. It is reported that Constructed wetlands (CWs) have been successfully employed to treat domestic sewage for

its easy operation and maintain [11, 12]. Consequently, the application of CWs for the purification of wastewater has been recently proposed as an alternative for the wastewater treatment and reuse [13-15].

The substrate plays a significant role in CWs, because can provide a suitable growth medium for plants and microorganism that play an important role in the removal of pollutants from wastewater. Some studies display that CWs with general media (e.g. sand and gravel) can meet the requirements for the reduction of organic matter and suspended solids [16]. However, it is also reported that inconsistent and low nutrient removal efficiency has been achieved [17]. Therefore, researchers have paid attention to other substrates that can be used to enhance the removal efficiency of nitrogen and organics [18]. Recently, magnetic Fe₃O₄ nanoparticles

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/3.0/>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

Copyright © 2022 Korean Society of Environmental Engineers

Received September 03, 2021 Accepted November 08, 2021

† Corresponding author

E-mail: zhouqw@hdu.edu.cn

Tel: +86-18758275070 Fax: +86-571-88809031

ORCID: 0000-0002-9659-7838

(Fe_3O_4 NPs) have been extensively applied to environmental remediation due to their small particle size, high surface area and good magnetism [19]. An appropriate amount of Fe_3O_4 NPs in CWs can improve biological activity. In addition, the magnetic property may provide some benefit for the microorganisms distribution and balancing the growth and death of microorganisms, which can lead to the improvement of pollutant treatment rate [20]. It has been reported that the TN removal efficiency (from 69 to 92%) can be significantly improved by the existed magnetic NPs [21–24]. Due to the good performance in other treatment systems, conventional substrates with Fe_3O_4 NPs may improve the performance of CWs, and the investigation can provide valuable information.

In CWs, microbial processes play a crucial role in the nutrient removal. However, most current studies on nutrient removal by CWs focused on the effects of different types of wetland configurations, plants and treatment strategies on the removal efficiency [25, 26]. Molecular biology techniques, including fluorescence in situ hybridization (FISH) and denaturing gradient gel electrophoresis (DGGE), have been used to monitor microbial community changes. However, these detection methods cannot obtain comprehensive information due to the insufficient sequences [27]. High-throughput 16S rRNA amplicon sequencing can help us better understand the microbial community structure and microbial diversity in CWs for the valuable information on bacterial community structures [28]. Therefore, Fe_3O_4 NPs can improve the performance of CWs by the mechanism, which can be effectively revealed.

In this study, the simulated domestic wastewater was treated in the CWs with and without Fe_3O_4 NPs. The removal efficiency of conventional wastewater quality and microbial parameters was investigated. The objectives of the present work were to (i) investigate the existence of Fe_3O_4 NPs on the performance of CWs, (ii) evaluate the influence of Fe_3O_4 NPs on microbial community structure and enzymatic activity. The investigations presented herein bring insights into the nutrient removal and transformation mechanisms from the aspects of microbes, and provide valuable information regarding the application of Fe_3O_4 NPs on the performance of CWs.

2. Materials and Methods

2.1. Construction of the CWs

Parallel subsurface flow CWs were set up in the greenhouse at China Jiliang University, Hangzhou, China. The CWs system was constructed by polyvinyl butyral, and the length, width and height are 45, 30 and 35 cm, respectively, with a volume of 47 L. The slag (particle size: 10–15 mm, porosity 0.45 and thickness 30 cm) was chose as substrate (Fig. 1), and collected from a locally heated boiler and repeatedly cleaned with tap water. A 5-cm-deep layer of soil was covered on the top of the CWs and collected from the campus of China Jiliang University. In CWs with Fe_3O_4 NPs, 90 g Fe_3O_4 NPs (frequent strategy to lower the cost is the combination of a small number of Fe_3O_4 NPs and an excess of Fe_3O_4 NPs is a threat to microorganisms and plants in wetlands) was evenly distributed horizontally along the CW (at a distance of 20 cm from the bottom, because more plant roots and microbes concentrated at this distance) [22, 24]. One common type of plants *Pontederia* was planted in CWs (height 10 cm, depth 5 cm, and density 29.6 seedlings m^{-2}), which could treat polluted urban river water with high removal efficiency of COD, TN and TP [29]. Tap water was used to enable the vegetation and microbial communities to adapt to their environment for the first two months. Three replicated mesocosms were established for each CW with and without Fe_3O_4 NPs.

2.2. Experimental Operation and Sample Collection

The synthetic domestic sewage was prepared by composing NH_4Cl (18 g/m^3), KNO_3 (35 g/m^3), KH_2PO_4 (6.5 g/m^3), CaCl_2 (1.2 g/m^3), CH_3COONa (65 g/m^3) and some trace element ingredients (MgSO_4 0.2 mg/L and FeCl_3 0.2 mg/L etc.). The influent concentrations of NH_4^+-N , NO_3^--N , TN, TP and COD were 4.11, 4.53, 9.05, 1.21, and 50.54 mg L^{-1} , respectively. The sequencing batch feeding influent mode (operation time 6 days) was adopted. The inflow rate of domestic sewage was 2.2 L for each CW. The treated water samples from outlet were collected every day and stored in brown reagent bottle in the refrigerator before detection. The amount of water extracted from each CW was 100 mL per day. The removal performance of CWs was evaluated in terms of NH_4^+-N , NO_3^--N , TN, TP and COD in the influent and effluent concentration. The

Fig. 1. Schematic of CWs. (Right represents CW with Fe_3O_4 NPs; Left represents CW without Fe_3O_4 NPs)

removal percentages were calculated by the influent and effluent concentration of $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, TN, TP and COD. $\text{NH}_4^+\text{-N}$ (HJ 536-2009), $\text{NO}_3^-\text{-N}$ (HJ/T 346-2007), TN (HJ 636-2012), TP (HJ 671-2013) and COD (GB 11914-89) were determined as previous study [30, 31]. During the operation, soil samples were also collected every day to investigate the microbial activity that was consist of five soil samples from four corner directions and the center of each CW. The soil samples were immediately collected in separated plastic Ziploc bags and stored in a freezer at 4°C. Urease activity was measured by incubating 5 g dry soil with 10 mL 10% urea solution and 20 mL citrate buffer (pH 6.7) for 24 h at 37°C. In addition, sucrase activity was measured by incubating 5 g dry samples with 15 mL of 8% sucrose solution, 5 mL phosphate buffer (pH 5.5) and 5 drops of toluene for 24 h at 37°C. Then, 1 mL of the supernatant was treated with 3 mL of 3,5-dinitrosalicylic acid for 5 min at 100°C. The urease activity and sucrase activity were spectrophotometrically determined at 578 nm and 508 nm, respectively. At the end of the experiment, substrate samples were also collected. A total of 5g substrate samples were captured from each CW and collected from four corner directions and the center of each CW. The substrate samples from CWs with and without Fe_3O_4 NPs were respectively combined into one sample for the analysis of microbial structure and sent to Lianchuan Biology Technology Co. Ltd., China.

2.3. High-throughput Sequencing Analysis

In this study, the high-throughput sequencing was used to analyze the microbial structure of CWs. The extraction steps for DNA were based on the methods described in previous studies [26, 32]. According to the manufacturer's instructions, DNA was extracted from substrate with PowerSoil® DNA Isolation kit (MoBio Laboratories, Catalog No. 12888-50, USA). During the PCR amplification process, the V3 and V4 hypervariable regions of bacterial 16S rRNA were amplified. The first amplification PCR conditions were 95°C for 5 min and 27 cycles of three steps, 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s, followed by a final step at 72°C for 10 min. The universal bacterial primers were 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'). The amplification process and the analysis of the pyrosequencing data were similar to previous studies [33-35]. The extraction and amplification of DNA samples were performed with an Illumina MiSeq PE300 platform (Hangzhou Lianchuan Biology Technology Co. Ltd., China). A similarity of 97% was used to illustrate the same operational taxonomic units (OTUs). The biodiversity of microbial communities in CWs was analyzed by OTUs, Shannon and Simpson estimators [36].

2.4. Statistical Analysis

According to the influent and effluent concentration of $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, TN and COD, the removal efficiency of pollutants was calculated. The statistical difference of the mean removal efficiency and enzymatic activity values were investigated (The urease and sucrase activity were expressed as $\text{NH}_4^+\text{-N}$ mg g^{-1} soil and mg glucose g^{-1} soil, respectively.) in CWs with and without Fe_3O_4 NPs. Meanwhile, the significance difference was calculated using ANOVA. Statistical analyses were performed using Origin 8.0 for Windows.

3. Results and Discussion

3.1. The Removal Performance of CWs

Fig. 2 shows the removal efficiency of nutrients and COD in CWs with and without Fe_3O_4 NPs. Overall, the nutrient and COD removal efficiency gradually increased with the operation time. CWs with Fe_3O_4 NPs achieved higher removal efficiency of $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, TN, TP and COD. When the operation time was at 6th day, the removal efficiency of $\text{NO}_3^-\text{-N}$, TN and COD in CWs with Fe_3O_4 were 83.8, 91.2 and 90.1%, respectively, that were 14.6, 15.4 and 8.1% higher than those of CWs without Fe_3O_4 NPs. Previous study also proved that the presence of magnetic NPs increased TN removal efficiency (94.4%) compared with the control (80.3%) [21]. Fe_3O_4 , known as a magnetic material, can produce magnetic field and be ionized into Fe (II) and Fe (III) and a certain concentration of Fe^{3+} can promote the growth of anammox bacteria [37-40]. In this study, Fe-anammox bacteria (such as, *Desulfosporosinus*) was increased by the existence of Fe_3O_4 NPs, which could explain why higher TN removal efficiency was achieved. In the reduction of TP, mesocosms with Fe_3O_4 NPs also exhibited significantly higher ($p < 0.05$) TP (90.4%) removal percentages than mesocosms without Fe_3O_4 NPs. Ma et al. [41] also proposed that Fe_3O_4 NPs could promote the phosphorus removal due to the dissolution of Fe ion from Fe_3O_4 , which could be effective precipitants for phosphorus removal. Overall, the results demonstrated that Fe_3O_4 NPs could significantly improve the removal efficiency of $\text{NH}_4^+\text{-N}$ and TP. The microbial growth and activity were accelerated, which could affect the release and uptake rate of PO_4^{3-} in CWs.

3.2. Enzymatic Activity in CWs

In this study, the enzymatic activity was quantified to compare the effects of Fe_3O_4 NPs on microbial activity in CWs (Fig. 3). The urease activities in CWs with Fe_3O_4 NPs were higher than those of CWs without Fe_3O_4 , ranging from 0.37 to 0.50 and 0.29 to 0.35 mg $\text{NH}_4^+\text{-N}$ g^{-1} soil, respectively. This observation might illuminate why CWs with Fe_3O_4 NPs had higher removal percentage of $\text{NH}_4^+\text{-N}$. He et al. [42]. also proposed that urease activities in Fe_3O_4 NPs treatments were significantly higher than those without Fe_3O_4 NPs. The sucrase activities in CWs with and without Fe_3O_4 NPs were ranged from 0.18 to 0.11 and 0.291 to 0.15 mg glucose g^{-1} soil, respectively. The results suggested that the Fe_3O_4 NPs also had effects on sucrase activities in CWs. Li et al. [43] also held the view that sucrase activity was positively correlated with the rate of C mineralization and accelerate COD removal, which might imply that the removal of organic matter was positively consistent with sucrase activity. Therefore, the results confirmed that Fe_3O_4 NPs played a certain role in $\text{NH}_4^+\text{-N}$ and COD removal efficiency due to the changes in enzymatic activity in CWs.

3.3. Biodiversity and Distribution of Microorganisms in CWs

Table 1 presents the richness and evenness of microorganisms in the CWs. The total number of usable sequences for all the samples was 112310, and the number of operational taxonomic units (OTUs) was 8741 with a similarity level of 97%. Compared with CWs with Fe_3O_4 NPs, the samples collected from CWs without Fe_3O_4

Fig. 2. Removal efficiencies of CWs. (Fe₃O₄ NP represents CW with Fe₃O₄ NPs; KB represents CW without Fe₃O₄ NPs)

Fig. 3. The variation of urease and surcuse activities in the CWs. (Fe₃O₄ NP represents CW with Fe₃O₄ NPs; KB represents CW without Fe₃O₄ NPs)

Table. 1. The Richness and Evenness of Bacterial Samples Collected from the CWs Incorporated with and without Fe₃O₄ NPs

Sample	Sequence	OTU	Chao	Shannon	Simpson	Coverage
Fe ₃ O ₄ NPs	46,633	3,980	4,682.7	9.97	1.0	0.97
KB	65,677	4,761	5,107.6	10.13	1.0	0.98

Fe₃O₄ NPs represents CWs incorporated with Fe₃O₄ NPs; KB represents CWs incorporated without Fe₃O₄ NPs.

Fig. 4. Bacterial communities at the phylum (a) and (b) and class level (c) and (d) from the microbial DNA sequences in the CWs. (Fe_3O_4 NPs represents CW with Fe_3O_4 NPs; KB represents CW without Fe_3O_4 NPs)

NPs yielded a higher OTU number. The richness of the microbial community was evaluated by Ebrahimi et al. [44]. In this study, Chao estimator indicated that the richness of microorganisms in CWs without Fe_3O_4 NPs was higher than that in CWs with Fe_3O_4 NPs, which was similar to OTUs. The Shannon indexes contain information about the diversity of the microbial structure [45]. The Shannon value of CWs without Fe_3O_4 NPs (10.13) was also higher than that of CWs with Fe_3O_4 NPs (9.97), which indicated that Fe_3O_4 NPs could reduce the bacterial diversity. A previous study also indicated that short-term exposure to magnetic NPs could result in acute toxicity to microorganisms [46]. Therefore, the richness of bacteria in the CWs with Fe_3O_4 NPs was much lower. On the contrary, some bacteria, such as denitrification, nitrification, or COD-reducing microorganisms, could thrive after long exposure to magnetic NPs [47]. Hence, the CWs with Fe_3O_4 NPs could still show better performance.

The relative abundance of bacteria at the phylum level is summarized in Fig. 4 and Fig. S1. The most abundant groups (relative abundance of > 5%) in CWs with Fe_3O_4 NPs were *Proteobacteria* (55.4%), *Acidobacteria* (8.9%), *Cyanobacteria* (5.9%) and *Bacteroidetes* (7.9%), accounting for 78.1% of the total effective reads. For CWs without Fe_3O_4 NPs, the major dominant group (relative abundance of > 5%) was different and *Actinobacteria* became a dominant bacterium. The results suggested that the relative abundance of bacteria was changed in CWs for the existence of Fe_3O_4 NPs. Notably, the content of *Proteobacteria* and *Bacteroidetes* in the CWs with Fe_3O_4 NPs increased compared with

that of CWs with Fe_3O_4 NPs, and the content of *Acidobacteria* and *Cyanobacteria* decreased. Li et al. [46] also proposed that the abundance of *Proteobacteria* and *Bacteroidetes* can be significantly increased from 37.15% (control, R1) to 60.72% (1 mg/L Fe_3O_4 NPs, R2), which suggested that some bacteria (called Fe-anammox attach to *Proteobacteria* and *Bacteroidetes*) that oxidized NH_4^+ -N coupled to the reduction of ferric iron (Fe^{3+}) could be thrived by the presence of low dose Fe_3O_4 NPs [38, 46]. In a previous study, it was also reported that *Proteobacteria* and *Bacteroidetes* were denitrobacteria and ubiquitous organic nutrient bacteria, respectively, which explains why CWs with Fe_3O_4 NPs displayed higher removal percentages of NO_3^- -N and COD [48].

The relative abundance of bacteria at the class level is shown in Fig. 4 and Fig. S2. The results indicated that the presence of Fe_3O_4 NPs could change the relative abundance of bacteria at the class level. For CWs with and without Fe_3O_4 NPs, five major dominant bacteria, namely *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, *Deltaproteobacteria* and *Cyanobacteria*, were found. Overall, *Betaproteobacteria* (15.3%), *Gammaproteobacteria* (10.5%) and *Deltaproteobacteria* (11.8%) in CWs with Fe_3O_4 NPs were higher than that of CWs without Fe_3O_4 NPs. In contrast, the relative abundance of *Alphaproteobacteria* (16.5%) and *Cyanobacteria* (5.3%) in CWs with Fe_3O_4 NPs were 6.0% and 2.0% lower, respectively, than that of CWs without Fe_3O_4 NPs. Additionally, it was also found that the number of *Proteobacteria* was increased the from 50.1% to 55.3%. Microorganisms from *Proteobacteria* might be involved in the biodegradation or bio-

Fig. 5. Bacterial communities at the genus level from the microbial DNA sequences in the CWs. (Fe_3O_4 NPs represents CW with Fe_3O_4 NPs; KB represents CW without Fe_3O_4 NPs)

Fig. 6. Heat-map graph of hierarchy cluster for the top 20 genera in the CWs. (Fe_3O_4 NPs represents CW with Fe_3O_4 NPs; KB represents CW without Fe_3O_4 NPs)

transformation of numerous organic compounds or nitrate in natural or manmade ecosystems [49]. For example, *Gammaproteobacteria* was recognized as a major colony responsible for nitrate removal and *Betaproteobacteria* played an essential ecological function in reducing phosphorous [33, 50]. Our results illustrated that the major bacteria (e.g. *Betaproteobacteria*, *Gammaproteobacteria*, and *Deltaproteobacteria*) in CWs could be promoted by Fe_3O_4 NPs, thus

improving the removal efficiency of nitrate and phosphorous.

The distribution of bacteria at the genus level is presented in Figs. 5 and 6. Overall, more abundant genera were achieved in the CWs with Fe₃O₄ NPs. The genera (relative abundance of > 1%) in CWs without Fe₃O₄ NPs included *Genomatimonas* (3.6%), *Gp6* (4.5%), *Gp16* (2.2%), *Hydrogenophaga* (3.0%), *Sphingomonas* (1.0%), *Rhodobacter* (2.2%), *Pseudomonas* (1.1%), *Aridibacter*

(1.7%), *Dexia* (1.9%), *Ilumatobacter* (1.7%) and *Desulfomicrobium* (1.0%). However, the relative abundance of *Gemmatimonas*, *Gp6*, *Gp16*, *Hydrogenophaga*, *Sphingomonas*, *Rhodobacter*, *Pseudomonas*, *Aridibacter*, *Dexia*, *Ilumatobacter* and *Desulfomicrobium* in CWs incorporated with Fe_3O_4 NPs was changed, with the values of 4.4, 2.2, 1.6, 2.3, 0, 1.1, 2.0, 0, 1.6, 0 and 1.8%, respectively. Some abundant genera disappeared, and only *Gemmatimonas* and *Desulfomicrobium* increased. *Gemmatimonas* had been identified in enhanced biological phosphorus removal, and *Desulfomicrobium* was a kind of microorganism related to the process of nitrification and denitrification [26, 45]. The nitrification, denitrification and phosphorus-accumulating bacteria thrived, which was closely correlated with the phylum-level results. Most importantly, some new genera became the most abundant genera, including *Desulfuromonas* (3.0%), *Azospirillum* (1.9%), *Chryseolinea* (1.5%), *Geobacter* (1.5%), *GpXIII* (1.9%), *Desulfomicrobium* (1.8%), *Schlegelella* (1.0%) and *Fibrobacter* (1.0%). Some studies had revealed that *Desulfuromonas*, *Azospirillum* and *Desulfomicrobium* were involved in nitrogen removal and *Chryseolinea* was involved in the process of reducing COD [33, 51]. Therefore, our results proved that the bacteria at the genus level were also changed by the existence of Fe_3O_4 NPs.

4. Conclusions

In this study, Fe_3O_4 NPs were employed to treat the simulated wastewater in CWs. The results indicated that the simulated wastewater can be better treated by the CWs with Fe_3O_4 NPs. At the same time, the results also showed that the magnetic Fe_3O_4 NPs could improve the enzymatic activity and stimulate the development and growth of nitrification, denitrification and polyphosphate-accumulating microorganisms, thereby improving the performance of CWs, which could contribute to the large-scale application of CWs in rural China.

Acknowledgment

The authors would like to acknowledge the financial support from the National Nature Science Foundation of China (NO.42173073) and Zhejiang Huachuan Industrial Group Co. LTD.

Author Contributions

Z.Q. (Ph.D. lecturer) conducted all the experiments and wrote the manuscript. W.W. (Ph.D. Professor) revised the manuscript.

Reference

- Li D, Zheng B, Liu Y, et al. Use of multiple water surface flow constructed wetlands for non-point source water pollution control. *Appl. Microbiol. Biot.* 2018;21(3):329-341.
- Chai H, Choi Y, Kim M, et al. Trends of microbial electrochemical technologies for nitrogen removal in wastewater treatment. *J. Kor. Soc. Wat. Waste.* 2020;34(5):345-356.
- De S, Elmetwally A, Ernst E, et al. Evidence for the efficacy of complementary and alternative medicines in the management of fibromyalgia: a systematic review. *Rheumatology* 2011;50(5): 911-920.
- Nam T, Kang H, Pandit S, et al. Effects of vertical and horizontal configurations of different numbers of brush anodes on performance and electrochemistry of microbial fuel cells. *J. Clean. Prod.* 2020;124125.
- Koo B, Jung S. Improvement of air cathode performance in microbial fuel cells through the binding of metal organic framework and activated carbon using ultrasonication and solution precipitation. *Chem. Eng. J.* 2021;424:12308.
- Son S, Koo B, Chai H, et al. Comparison of hydrogen production and system performance in a microbial electrolysis cell containing cathodes made of non-platinum catalysts and binders. *J. Water. Process. Eng.* 2021;40:101844.
- Koo B, Lee S, Oh S, et al. Addition of Reduced Graphene Oxide to an Activated-Carbon Cathode Increases Electrical Power Generation of a Microbial Fuel Cell by Enhancing Cathodic Performance. *Electrochim. Acta* 2019;297:613-622.
- Jung SP, Eojin K, Bonyoung K. Effects of Wire-type and Mesh-type Anode Current Collectors on Performance and Electrochemistry of Microbial Fuel Cells. *Chemosphere* 2018; 209:542.
- Koo B, Jung SP. Characterization of Impedance and Polarization of Carbon-Felt Bioanodes and Activated-Carbon Cathodes in a Continuous-Flow Microbial Fuel Cell. *J. Kor. Soc. Urb. Environ.* 2018;18(2):177-191.
- Gupta S, Srivastava P, Patil SA, et al. A comprehensive review on emerging constructed wetland coupled microbial fuel cell technology: Potential applications and challenges. *Bioresour. Technol.* 2021;320:124376.
- Mesquita MC, Albuquerque A, Amaral A, Nogueira R. Effect of vegetation on the performance of horizontal subsurface flow constructed wetlands with lightweight expanded clay aggregates. *Int. J. Environ. Sci. Technol.* 2013;10:433-442.
- Niu S, Guerra HB, Chen Y, et al. Performance of a vertical subsurface flow (VSF) wetland treatment system using wood-chips to treat livestock stormwater. *Environ. Sci. Proc. Imp.* 2013;15(8):1553-1561.
- Zhou Q, Zhu H, Bañuelos G, et al. Impacts of vegetation and temperature on the treatment of domestic sewage in constructed wetlands incorporated with Ferric-Carbon micro-electrolysis material. *Int. J. Phytoremediation.* 2017;19(10):915-924.
- Donde O, Tian C, Tian Y, et al. Efficacy of macrophyte dominated wastewater inclosure as post-treatment alternative in domestic wastewater quality polishing for eradication of faecal pathogenic bacteria pollution. *Process. Saf. Environ.* 2018;114(1):192-205.
- Eom H, Flimban S, Gurung A, et al. Impact of carbon and nitrogen on bioclogging in a sand grain managed aquifer recharge (MAR). *Environ. Eng. Res.* 2019;25(6):841-846.
- Ahmad T, Ahmad K, Alam M. Sustainable management of water treatment sludge through 3'R' concept. *J. Clean. Prod.* 2016; 124(15):1-13.
- Park W. Integrated constructed wetland systems employing

- alum sludge and oyster shells as filter media for P removal. *Ecol. Eng.* 2009;35(8):1275-1282.
18. Wu H, Fan J, Zhang J, et al. Intensified organics and nitrogen removal in the intermittent-aerated constructed wetland using a novel sludge-ceramsite as substrate. *Bioresour. Technol.* 2016;210:101-107.
19. Weng X, Li M, Guo M, et al. Removal of Doxorubicin hydrochloride using Fe₃O₄ nanoparticles synthesized by euphorbia cochinchinensis extract. *Chem. Eng. J.* 2018;353:482-489.
20. Xin X, Yang H, Guan L, et al. Responses of Nitrogen and Phosphorus Removal Performance and Microbial Community to Fe₃O₄@SiO₂ Nanoparticles in a Sequencing Batch Reactor. *Appl. Biochem. Biotechnol.* 2021;193:544-559.
21. Ni S, Ni J, Yang N, et al. Effect of magnetic nanoparticles on the performance of activated sludge treatment system. *Bioresour. Technol.* 2013;143:555-561.
22. Tao M, Zhang L, Xi B, et al. Treatment of farmer household tourism wastewater using iron-carbon micro-electrolysis and horizontal subsurface flow constructed wetlands: A full-scale study. *Ecol. Eng.* 2018;110:192-203.
23. Jung S, Kim Y, Kang H. Denitrification Rates and Their Controlling Factors in Streams of the Han River Basin with Different Land-Use Patterns. *Pedosphere* 2014;24(4):516-528.
24. Jung S, Yoon M, Lee S, et al. Power Generation and Anode Bacterial Community Compositions of Sediment Fuel Cells Differing in Anode Materials and Carbon Sources. *Int. J. Electrochem. Sci.* 2014;9(1):315-326.
25. Liang Y, Zhu H, Banuelos G, et al. Removal of nutrients in saline wastewater using constructed wetlands: Plant species, influent loads and salinity levels as influencing factors. *Chemosphere* 2017;187:52-61.
26. Zhang P, Peng Y, Lu J, et al. Microbial communities and functional genes of nitrogen cycling in an electrolysis augmented constructed wetland treating wastewater treatment plant effluent. *Chemosphere* 2018;211:25-33.
27. Hua G, Ying C, Kong J, et al. High-throughput sequencing analysis of bacterial community spatiotemporal distribution in response to clogging in vertical flow constructed wetlands. *Bioresour. Technol.* 2017;248:104-112.
28. Jung S, Cheong Y, Yim G, et al. Performance and bacterial communities of successive alkalinity-producing systems (SAPSs) in passive treatment processes treating mine drainages differing in acidity and metal levels. *Environ. Sci. Pollut. Res. Int.* 2014;21(5):3722.
29. Gu D, Xu H, He Y, et al. Remediation of Urban River Water by Pontederia Cordata Combined with Artificial Aeration: Organic Matter and Nutrients Removal and Root-Adhered Bacterial Communities. *Int. J. Phytoremediation.* 2015:1105-1114.
30. Yuan J, Dong W, Sun F, et al. An ecological vegetation-activated sludge process (V-ASP) for decentralized wastewater treatment: system development, treatment performance, and mathematical modeling. *Environ. Sci. Pollut. Res. Int.* 2016;23:10234-10246.
31. Zhu H, Zhou Q, Yan B, et al. Influence of vegetation type and temperature on the performance of constructed wetlands for nutrient removal. *Water Sci. Technol.* 2018;77(3-4):829-837.
32. Zhou Q, Zhang L, Chen J, et al. Enhanced stable long-term operation of biotrickling filters treating VOCs by low-dose ozonation and its affecting mechanism on biofilm. *Chemosphere* 2016;162:139-147.
33. Wang J, Song X, Wang Y, et al. Microbial community structure of different electrode materials in constructed wetland incorporating microbial fuel cell. *Bioresour. Technol.* 2016;221:697-702.
34. Zhou Q, Zhang L, Chen J, et al. Performance and microbial analysis of two different inocula for the removal of chlorobenzene in biotrickling filters. *Chem. Eng. J.* 2016;284:174-181.
35. Yang B, Wang M, Wang J, et al. Mechanism of high contaminant removal performance in the expanded granular sludge blanket (EGSB) reactor involved with granular activated carbon for low-strength wastewater treatment. *Chem. Eng. J.* 2018;334:1176-1185.
36. Yang B, Xu H, Wang J, et al. Bacterial and archaeal community distribution and stabilization of anaerobic sludge in a strengthen circulation anaerobic (SCA) reactor for municipal wastewater treatment. *Bioresour. Technol.* 2017;224:750-758.
37. Wang Z, Qi Y, Wang J, et al. Characteristics of aerobic and anaerobic ammonium-oxidizing bacteria in the hyporheic zone of a contaminated river. *World J. Microbiol. Biotechnol.* 2012;28:2801-2811.
38. Lackner S, Horn H. Evaluating operation strategies and process stability of a single stage nitrification-anammox SBR by use of the oxidation-reduction potential (ORP). *Bioresour. Technol.* 2012;107:70-77.
39. Zhou Y, Jia X, Tan L, et al. Magnetically enhanced cytotoxicity of paramagnetic selenium-ferroferic oxide nanocomposites on human osteoblast-like MG-63 cells. *Biosens Bioelectron.* 2010;25:1116-1121.
40. Liu S, Yang F, Meng F, et al. Enhanced anammox consortium activity for nitrogen removal: impacts of static magnetic field. *J. Biotechnol.* 2008;138:96-102.
41. Ma B, Wang S, Li Z, et al. Magnetic Fe₃O₄ nanoparticles induced effects on performance and microbial community of activated sludge from a sequencing batch reactor under long-term exposure. *Bioresour. Technol.* 2017;225:377-385.
42. He S, Feng Y, Ren H, et al. The impact of iron oxide magnetic nanoparticles on the soil bacterial community. *J. Soil. Sediment.* 2011;11(8):1408-1417.
43. Li X, Hou L, Liu M, et al. Primary effects of extracellular enzyme activity and microbial community on carbon and nitrogen mineralization in estuarine and tidal wetlands. *Appl. Microbiol. Biotechnol.* 2015;99(6):2895-909.
44. Ebrahimi S, Gabus S, Rohrbach-Brandt E, et al. Performance and microbial community composition dynamics of aerobic granular sludge from sequencing batch bubble column reactors operated at 20°C, 30°C, and 35°C. *Appl. Microbiol. Biotechnol.* 2010;87(4):1555-1568.
45. Yang B, Wang J, Wang J, et al. Correlating microbial community structure with operational conditions in biological aerated filter reactor for efficient nitrogen removal of municipal wastewater. *Bioresour. Technol.* 2017;250:374-381.
46. Li H, Chi Z, Yan B. Insight into the impact of Fe₃O₄ nanoparticles on anammox process of subsurface-flow constructed wetlands under long-term exposure. *Environ. Sci. Pollut. Res. Int.* 2018;25:29584-29592.

47. Drzewicki A, Dębowski M, Zieliński M. Effect of a static magnetic field on activated sludge community. *Environ. Technol.* 2016;38:1-23.
48. Zhang P, Peng Y, Lu J, et al. Microbial communities and functional genes of nitrogen cycling in an electrolysis augmented constructed wetland treating wastewater treatment plant effluent. *Chemosphere* 2018;211:25-33.
49. Guan W, Yin M, He T, et al. Influence of substrate type on microbial community structure in vertical-flow constructed wetland treating polluted river water. *Environ. Sci. Pollut. Res.* 2015;22:16202-16209.
50. Tian H, Zhao J, Zhang H, Chi C, Li B, Wu X. Bacterial community shift along with the changes in operational conditions in a membrane-aerated biofilm reactor. *Appl. Microbiol. Biotechnol.* 2015;99:3279-3290.
51. Fang W, Yan D, Wang Q, et al. Changes in the abundance and community composition of different nitrogen cycling groups in response to fumigation with 1,3-dichloropropene. *Sci. Total. Environ.* 2018;650:44-55.