Application of phyto-Fenton process in constructed wetland for the continuous removal of antibiotics

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Abstract. Phyto-Fenton process utilizes the endogenous hydrogen peroxide in plants to degrade organic pollutants in presence of iron catalyst. In this study, we have applied the magnetite particles in continuous treatment system of constructed wetland (CW) to study the effectiveness in removing sulfamethoxazole (SMX) antibiotics. Experimental results demonstrated that SMX was removed by constructed wetlands in the presence and absence of magnetite fine particles. OH radical formation was observed in the plant+Fe system with electron spin resonance spectroscopy. The magnetite addition favoured the plant growth and endogenous H₂O₂. However, enhanced treatments by phyto-Fenton process were not enhanced in the presence of magnetite particles, but the plants+soil CWs showed better removal efficiencies compared to the soil CWs.

1. Introduction
Water consumption tends to increase with the increasing world population and economic growth. Accordingly, waste water generation and pollution problems become severe. Installation of modern treatment systems becomes very necessary worldwide to remove nutrients as wells as emerging pollutants from the polluted water to maintain hygienic life. Treatment of nutrients from waste water have been practiced on-site based on different biological processes including constructed wetlands and lagoons (Wu et al., 2011; Almuktar et al., 2018), but endocrine disrupting chemicals removal technologies such as advanced oxidation processes have been scarcely practiced in decentralized areas (Chong et al., 2012). Even though biological remediation technologies are not designed for the emerging organic contaminants (EOCs) removal, but in some cases they are able to decrease these specific contaminant compounds with varied efficiencies (Luo et al., 2014). Installation of modern sewage treatment systems in developing countries are not always viable in developing countries due to economic constraints (Quasar et al., 2013). Reis et al., (2011) and Inagaki et al., (2016) proposed a low cost/high performance water and wastewater treatment process, phyto-Fenton process, in which highly reactive OH radicals can be produced through Fenton and Fenton like reaction utilizing endogenous H₂O₂ in aquatic plants. Equations (1) and (2), shows the Fenton and Fenton-like reactions respectively. Hydrogen peroxide produced inside a plant is converted to OH radicals in presence of an iron catalyst which can decompose almost every organic pollutant.
Nowrotek et al., 2016 has reported 24-30% removal of sulfamethoxazole antibiotics in down flow constructed wetlands with no significant role for vegetation. In our research, we have applied phyto-Fenton process in constructed wetlands to remove sulfamethoxazole antibiotics. We have provided iron materials to improve the performance of vegetation. In addition to the hogland growth media contained Fe, magnetite fine particles were used as iron catalyst. In order to confirm the phyto-Fenton reaction in plants, Inagaki et al., 2018 has conducted confocal fluorescence spectroscopy with plant samples, and they could clearly demonstrate the occurrence of phyto-Fenton reaction in plants. Also, Inagaki et al., 2016 reported positive fluorescence microscopy results of plants in presence of magnetite particles. Following these researches, we have applied phyto-Fenton reaction in constructed wetlands for the first time, to treat sulfamethoxazole antibiotics continuously. The effectiveness of magnetite particles for the phyto-Fenton reaction was also studied.

Sulfamethoxazole antibiotics, SMX, is low absorptive, polar sulfonamide which is commonly prescribed and thus it is widely detected in both water and soil (Muller et al., 2013). It reaches the environment mainly through human excretion, and the maximum reported concentration in wastewater is up to microgram per liter levels (Larcher and Yargeau, 2012)

2. Materials and methods

2.1. Constructed wetland

A laboratory scale artificial wetland was constructed to which the antibiotic feed was given. Continuous treatment experiments were performed in the greenhouse, and the ratio of the commercial black soil and the straw in the wetland was maintained as 1: 1. In addition, there were nine reactors: three for soil-only systems (Control), three for plant+ soil, and three for plant+soil+magnetite. Synthetic wastewater containing SMX was introduced to the system as trace pollutants (Table 2.1 and table 2.2). Makomo reeds (phragmites australis) were used as plant, under good growth conditions, the rhizomes grow about 5 m per year and take root at appropriate intervals. In this experiment, commercially available seedlings were used. Makomo is distributed in East Asia and Southeast Asia, and can be found nationwide in Japan. It thrives on the waterfront and grows in swamps, rivers and lakes.

| Table 2.1 Experimental design |
|--------------------------------|
| soil and straw(kg) | 1.5, 1.5 |
| water(mL) | 3000 |
| Average temperature(℃) | 28.0 |
| Illumination | 12dark/12light |
| Average flow rate (mL/h) | 18.3 |
| Light intensity(lux) | 1200 |
| Average water temperature (℃) | 23.0 |
| HRT(days) | 7 |
| Weight of makomo and yoshi(g) | 234±15.25.7±10.6 |

| Table 2.2 Experimental specifications |
|--------------------------------------|
| Magnetite (g/kg) | Control | plant | plant+Fe |
| Makomo, the number of reeds | 10 | 0 | 10 |
|  | 0 | 2(each) | 2(each) |
2.2 Iron particles

Fine particles of magnetite (Fe₃O₄) were used as iron catalyst in our experiment. Iron nanoparticles are used in dietary supplements as well as in soil and groundwater remediation technology, and considered to be safer than other metal nanoparticles (Koo et al., 2017). However, recent studies have suggested that, it may affect humans and other organisms via the atmosphere. Therefore, in this research, to avoid environmental damages from nanoparticles, micro-sized magnetite fine particles are used as iron material. Magnetite has high surface area ratio and volume ratio, magnetism, and is widely used in scientific and industrial fields.

2.3 Sulfamethoxazole (SMX)

In this experiment, the antibiotic SMX was dissolved in the feed water and continuously supplied to the constructed wetland. The chemical properties of SMX are shown in Table 2.3. SMX is a sulfonamide bacteriostatic antibiotic, which is white crystals and odorless. It is poorly soluble in water, ethanol and diethyl ether, but can be dissolved in aqueous sodium hydroxide. The main drug effects can be applied to urinary tract infections and sinusitis, gram positive bacteria such as staphylococci, and gram negative bacteria such as Escherichia coli and Shigella.

| Table 2.3 Sulfamethoxazole chemical characteristics |
|-----------------------------------------------|
| Sulfamethoxazole(SMX)                         |

| Molecular formula | C₁₀H₁₁N₃O₃S |
|-------------------|-------------|
| Molecular weight  | 253.2776    |
| pKa               | 5.7         |
| Log Kow           | 0.89        |
| Specification     | Antibiotic  |
| Classification    | Sulfonamide |

2.4 Analytical methods
Plant growth was quantified by measurement of chlorophyll value using chlorophyll meter (SPAD-502 Plus) and leaf lengths. SMX concentration was analyzed by HPLC (Infinity II, Agilent). The magnetite concentration in the waste water collected during the continuous decomposition experiment was quantitatively analyzed by an absolute calibration curve method using an ICP emission analyzer (Inductively Coupled Plasma, Optical Emission Spectrum (ICP-OES); SHIMADZU). The endogenous hydrogen peroxide in the plant root was quantified following the procedure by Uchida et al., 2002. The electron spin resonance (ESR; JES-TE200, JEOL) analysis was employed to confirm the signals of OH radicals. The conditions were, microwave power, 4 mW; modulation amplitude, 0.1 mT; field, 336.250 mT; sweep width, 5 or 7.5 mT; gain, 200; and time constant, 0.1 s were set. DMPO (5, 5-dimethyl-1-pyrroline N-oxide, Sigma Aldrich) was used as ESR trapping agent.

3. Results and discussion

In this research, we have studied various parameters as plant growth, endogenous hydrogen peroxide concentrations, and antibiotic removals under different conditions of constructed wetlands (CWs). Three types of constructed wetlands was used for the removal experiments of SMX- CW contained soil and magnetite particles, CW contained soil and plants, CW contained soil, plants and magnetite particles. The CW contained soil and magnetite is referred as control reactor, in which the SMX removal mechanism is mainly adsorption by soil and removal by soil microorganisms. Considering the second one, CW contained soil and plant, referred as plant only system, the SMX removal mechanism is soil absorption, uptake by soil microorganism and plant uptake. Even though magnetite was not added in this system, the provided hogland media for plant growth contained low concentrations of Fe. The plant and Fe system contained soil, magnetite and plant. In this CW, the mechanism is a combination of soil adsorption, plant uptake, uptake by soil microorganism and phyto-Fenton process. The addition of magnetite particles enhanced the plant growth and hydrogen peroxide production. Also, all the three types of constructed wetlands in our study was effective in removing the SMX, and the removal patterns in CWs using plants, with or without magnetite particles showed better efficiencies than the soil+magnetite system. Nowrotek et al., 2016 has reported that vegetation has no significant effect on improving removal efficiencies of SMX, but in our study, the added hogland growth media might have enhanced the Fenton and fenton-like reactions in plants, so the plant contained CWs showed 30-86% removal efficiency. Inagaki et al., 2018 has conducted confocal laser scanning microscope experiments to demonstrate the OH radical formation in plant using an OH radical probe, 3’-(p-aminophenyl) fluorescein (APF). They could clearly demonstrate the OH radical formation in plant, and confirmed the Fenton and Fenton-like reaction in plants using ferrous iron. Inagaki et al., (2016) has demonstrated OH radical formation in plant routes implicated by magnetite fine particles by fluorescence microscope results. These studies confirm the phyto-Fenton reaction in plants in presence of different iron materials. In our study, in plant systems, the Fe particles contained in the media, and the additional magnetite supply would have enhanced the phyto-Fenton reaction, and thus gave better removal results than the soil+magnetite CW. In the plant+ soil+magnetite reactor, no pronounce effect was found in the removal compared to the plant+soil CW, but this result may be attributable to coagulation of iron compounds which reduced the internal reaction.

3.1. Plant growth in Constructed wetland

In the continuous treatment system of constructed wetland, the plant growth was mainly determined by the chlorophyll values and leaf length. In the experiment system the plants were able to grow (Figure 3.1& 3.2) however, about 51st day, the growth was decreased or almost stopped, in terms of leaf length, the chlorophyll value found decreased especially in the plant only system. But above the ground rhizome part, new sprouts were emerging.
Figure 3.1 State of plants in the constructed wetland equipment for each elapsed day (A: Day0, B: Day 7, C: Day 14, D: Day 51)

Figure 3.2 Chlorophyll value for each elapsed day

3.2 Magnetite concentration
Figure 3.3 shows the iron concentration in the effluent waste water for each treatment day. Here, control refers to the reactor contains soil and magnetite, while the other contains soil, plant and magnetite. Considering that relatively large amount of magnetite with an initial iron concentration of 10 g/kg was given to the soil, it can be seen that magnetite is not flowing out so much. It can be inferred that magnetite (measured as Fe) outflow is largely related to physical factors by feed water inflow rate and chemical factors such as pH of water quality in the wetland, but in this experiment the inflow rate was relatively slow, and the pH is near neutral region. Here, the soil+plant+magnetite system is withholding the magnetite particles than the soil+Fe reactor, it is good for the continuous phyto-Fenton process, thus reduce the iron concentration in the effluent.

3.3. Change in hydrogen peroxide concentration
In phyto-Fenton process, endogenous hydrogen peroxide is the key element in the reaction. In our study, we measured the hydrogen peroxide concentration in the root of the plant. As can be seen from the figure 3.4, the \( \text{H}_2\text{O}_2 \) production in phyto-Fenton system was more compared to the plant only system. Based on the fact that plants work with iron as a nutrient, adding magnetite fine particles into the soil makes it possible for plants to take it as a nutrient source or to recognize it as a stress source, a factor that generates a large amount of hydrogen peroxide. It inferred that the addition of iron, enhanced the production of hydrogen peroxide inside the roots. In the growth analysis, the reeds of the plants were not progressive during 51st treatment day, but the sprouts were emerging, and the root system was still active. 60 to 100 nmol / g-FW of endogenous \( \text{H}_2\text{O}_2 \) was quantified.
3.4. Comparison of signals of OH radicals in ESR
The ESR signals of plant extract (Figure 3.5-A) and plant+Fe extract (Figure 3.5-B) was compared to the ESR signals of Fenton reagent (Figure 3.5-C), with DMPO as trapping reagent. In the case of Fenton reagent, the OH radical peaks are very clear. Since the concentration of H2O2 is very low in plants, in the Fenton-plant system, we could observe the small knob peaks to confirm the OH radical production (Figure 3.5-B)
3.5. Treatment of SMX

The continuous treatment system was run for 51 days in the laboratory for the removal of SMX. The control reactor contained soil and magnetite, plant only system contained soil and plants, and the plant+Fe system contained soil, Fe and plants. To all three systems, influent wastewater containing SMX was fed. Effluents were taken on 7th, 14th, 29th and 51st days of treatment. In this, both the plant only and plant+Fe systems removed SMX than the soil+magnetite system, but we couldn’t observe more removal in the phyto-Fenton, i.e., plant+magnetite system. Even though, magnetite enhances growth of plants and it produces OH radicals, it did not enhance the removal of SMX in the continuous treatment of SMX. Also, towards 51st day, the concentration of SMX increased in both plant and plant+magnetite systems. Further studies will be needed in future research to demonstrate the proper removal mechanisms, and to understand the phyto-Fenton removal, other treatment conditions and soil properties.

Figure 3.5 ESR signals of (A) plant extract, (B) Plant+Fe extract and (C) Fenton reagent.
4. Conclusion
In our study, we have used constructed wetlands systems for the treatment of SMX. Phytoremediation was applied in CW system and it was further enhanced by the application of phyto-Fenton process. In presence of magnetite fine particles, plants growth was enhanced and the endogenous H$_2$O$_2$ concentration was slightly better compared to the plant only system. The SMX was treated with better efficiencies in both plant and plant+Fe CWs, compared to the soil+magnetite CW. Addition of iron particles to the CW plant systems can enhance the production of hydrogen peroxides and thus OH radical formation, which can contribute to the degradation of antibiotics. Magnetite induced phyto-Fenton reaction did not enhance the removal of SMX in the constructed wetland system. So further studies will be conducted about more parameters to improve the endogenous OH radical production in plants with magnetite particles.

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References
[1] Almuktar S.A.A.A.N, Abed S.N and Scholz M, 2018. Wetlands for wastewater treatment and subsequent recycling of treated effluent: A review. Environ Sci Pollut Res 25:23595.
[2] Chong M.N, Sharma A.K, Burn S and Saint C.P, 2012. Feasibility study on the application of advanced oxidation technologies for decentralised wastewater treatment. J. Cleaner Prod. 35, 230-238.
[3] Inagaki Y, Cong V. H, and Sakakibara Y, 2016. Identification and Application of Phyto-Fenton reactions, Chemosphere 144 1443-1450.
[4] Inagaki Y, Nara S and Sakakibara,Y, 2018. OH radical generation by a novel phyto-remediation process and its application to remove POPs. Journal of water and waste, The Industrial Water Institute, Vol.60, No.8,39-45. (In Japanese)
[5] Koo H, B. K. Salunke, B. Iskandarani, W. G. Oh, and B. S. Kim 2017. Improved degradation of lignocellulosic biomass pretreated by Fenton-like reaction using Fe3O4 magnetic
nanoparticles. Biotechnol. Bioprocess Eng. 22: 597–603.

[6] Larcher S and Yargeau V, 2012. Biodegradation of sulfamethoxazole: current knowledge and perspectives. Appl. Microbiol. Biotechnol. 96 (2), 309–318.

[7] Luo Y, Guo W, Ngo H.H, Nghiem L.D, Hai, F.I, Zhang J, Liang S, and Wang X.C, 2014. A review on the occurrence of micropollutants in the aquatic environment and their fate and removal during wastewater treatment. Sci. Total Environ. 473, 619–641

[8] Müller E, Schüssler W, Horn H and Lemmer H., 2013. Aerobic biodegradation of the sulfonamide antibiotic sulfamethoxazole by activated sludge applied as co-substrate and sole carbon and nitrogen source. Chemosphere 92 (8), 969–978.

[9] Nowrotek M, Sochaki A, Felis A and Miksch K. 2016. Removal of diclofenac and sulfamethoxazole from synthetic municipal waste water in microcosm downflow constructed wetlands: Start up results. International Journal of Phytoremediation, Volume 18, No.2, 157-163.

[10] Qaisar M, Arshid P, Bibi S Z, Habiba Z, Hajra Y, Muhammad W, Zahidullah, and Sumera A. 2013. Natural Treatment Systems as Sustainable Ecotechnologies for the Developing Countries. Biomed Research International.

[11] Reis A.R and Sakakibara Y. 2011. Continuous treatment of endocrine disrupting chemicals by aquatic plants and biological Fenton Reaction, JSCE, Ser. G (Environmental Research), 67(7), 725-734.

[12] Uchida A, A.T. Jagendorf, T. Hibino, T. Takabe. 2002. Effects of Hydrogen Peroxide and Nitric Oxide on both Salt and Heat Stress Tolerance in Rice. Plant Science. 163(3): 515-523.

[13] Wu H, Fan J, Zhang J, Ngo H.H, Guo W, Hu Z and Liang S., 2015. Decentralized domestic wastewater treatment using intermittently aerated vertical flow constructed wetlands: impact of influent strengths. Bioresour. Technol. 176, 163–168.