

# Sulfamethoxazole removal and fuel-feedstock biomass production from wastewater in a phyto-Fenton process using duckweed culture

Tadashi Toyama<sup>a,\*</sup>, Maki Kobayashi<sup>b</sup>, Rubiy'atno<sup>a</sup>, Masaaki Morikawa<sup>c</sup>, Kazuhiro Mori<sup>a</sup>

<sup>a</sup> Graduate Faculty of Interdisciplinary Research, University of Yamanashi, 4-3-11 Takeda, Kofu, Yamanashi, 400-8511, Japan

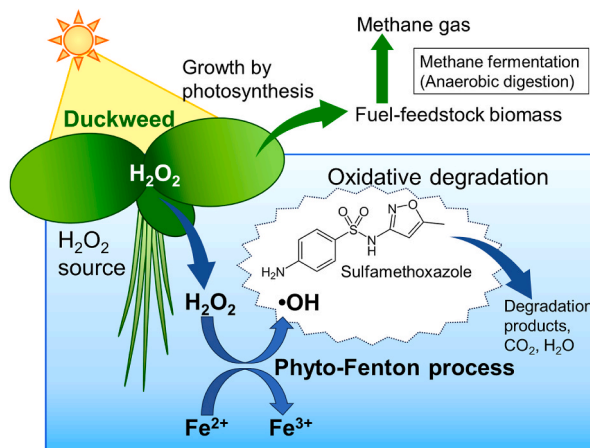
<sup>b</sup> Integrated Graduate School of Medicine, Engineering, and Agricultural Sciences, University of Yamanashi, 4-3-11 Takeda, Kofu, Yamanashi, 400-8511, Japan

<sup>c</sup> Division of Biosphere Science, Graduate School of Environmental Science, Hokkaido University, Kita-10 Nishi-5, Kita-ku, Sapporo, 060-0810, Japan

## HIGHLIGHTS

- Duckweed produced  $H_2O_2$  and released it to water phase.
- Duckweed supplied enough  $H_2O_2$  for the phyto-Fenton process.
- Sulfamethoxazole (SMX) was efficiently removed by the phyto-Fenton process.
- Duckweed biomass (7.95 g-dry weight)  $m^{-2} d^{-1}$  was produced during SMX removal.
- Duckweed biomass was converted to 353 NL methane  $kg^{-1}$  volatile solids.

## GRAPHICAL ABSTRACT



## ARTICLE INFO

Handling Editor: E. Brillas

### Keywords:

Duckweed  
Phyto-Fenton process  
Hydrogen peroxide  
Sulfamethoxazole  
Biomass production  
Wastewater

## ABSTRACT

The phyto-Fenton process, which generates hydroxyl radicals through Fenton and Fenton-like reactions using plant-derived hydrogen peroxide ( $H_2O_2$ ) and ferrous iron (Fe (II)) can degrade organic pollutants. Duckweed, an aquatic plant, is promising for a co-beneficial phytoremediation process that combines wastewater treatment and biomass production for biofuel feedstock. However, the phyto-Fenton process using duckweed has not been extensively studied. Because sulfamethoxazole (SMX), a major antibiotic, is distributed widely and is an emerging contaminant, its effective removal from contaminated water is necessary. The present study investigated the possibility of the simultaneous efficient removal of SMX from polluted water and biomass production for fuel feedstock by the phyto-Fenton process using duckweed. This is the first attempt to demonstrate the co-benefits of SMX removal and biomass production using duckweed. Intracellular  $H_2O_2$  was produced using four

**Abbreviations:**  $H_2O_2$ , Hydrogen peroxide; Fe (II), Ferrous iron; SMX, Sulfamethoxazole; PPCPs, Pharmaceuticals and personal care products; AOPs, Advanced oxidation processes; FW, Fresh weight; DW, Dry weight; VS, Volatile solids; NL, Normal liters.

\* Corresponding author.

E-mail addresses: [ttohyama@yamanashi.ac.jp](mailto:ttohyama@yamanashi.ac.jp) (T. Toyama), [kobayashi.maki@swing-w.com](mailto:kobayashi.maki@swing-w.com) (M. Kobayashi), [yubi@yamanashi.ac.jp](mailto:yubi@yamanashi.ac.jp) (Rubiy'atno), [morikawa@ees.hokudai.ac.jp](mailto:morikawa@ees.hokudai.ac.jp) (M. Morikawa), [mori@yamanashi.ac.jp](mailto:mori@yamanashi.ac.jp) (K. Mori).

<https://doi.org/10.1016/j.chemosphere.2024.142592>

Received 1 April 2024; Received in revised form 31 May 2024; Accepted 10 June 2024

Available online 10 June 2024

0045-6535/© 2024 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

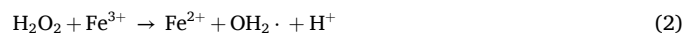
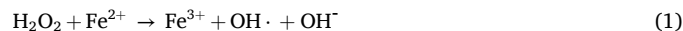
duckweeds, *Lemna aequinoctialis*, *L. minor*, *Landolina punctata*, and *Spirodela polyrhiza*, in the range of 16.7–24.6  $\mu\text{mol g}^{-1}$  fresh weight, and extracellular  $\text{H}_2\text{O}_2$  was released into the water phase. Consequently, duckweed could be used as an  $\text{H}_2\text{O}_2$  supply source for the phyto-Fenton process. Specifically, 0.5 g fresh duckweed almost completely eliminated 1  $\text{mg L}^{-1}$  SMX after 5 d in 50 mL sterile modified Hoagland solution containing 10 mM Fe (II). Fe (II)-dependent elimination of SMX indicated the occurrence of phyto-Fenton reaction. The phyto-Fenton process using duckweed effectively removed SMX. *S. polyrhiza* duckweed similarly removed 1  $\text{mg L}^{-1}$  SMX even in sewage effluent containing other organic contaminants. During this treatment, duckweed biomass was generated at 7.95 g dry weight  $\text{m}^{-2} \text{d}^{-1}$ , which was converted into methane at 353 normal liters  $\text{CH}_4 \text{kg}^{-1}$  volatile solids by anaerobic digestion. For the first time, this study clearly demonstrates the potential for simultaneous SMX removal and biomass production from SMX-contaminated wastewater using duckweed.

## 1. Introduction

Pharmaceuticals and personal care products (PPCPs) have increasingly attracted attention owing to their widespread and extensive use, frequent contamination of aquatic environments, and potential adverse impacts and risks to ecosystems, living organisms, and humans (Adeleye et al., 2022). Therefore, various technologies, including physical, chemical, or biological processes have been investigated for removing PPCPs from polluted water and wastewater (Wang and Wang, 2016). Sulfamethoxazole (SMX) is a major antibiotic released into the aquatic environment from hospital and pharmaceutical industrial effluents, domestic sewage, and veterinary fields. It is among the 30 most frequently detected water contaminants with a half-life of 85–100 d and more (Prasannamedha and Kumar, 2020). SMX exerts toxic effects on aquatic organisms, and long-term SMX exposure in aquatic environments can increase the number of resistant microbes (Prasannamedha and Kumar, 2020), leading to the emergence of multidrug-resistant bacteria. Therefore, efficiently removing SMX from contaminated water is necessary to reduce the risks to human health and aquatic environments.

Adsorption by activated carbon (Ogata et al., 2012; Bonvin et al., 2016) and advanced oxidation processes (AOPs), such as electrochemical oxidation (Dirany et al., 2010), ozone oxidation (Gao et al., 2014), and photocatalysis (Cai and Hu, 2018; Balakrishnan et al., 2023a; b, 2024a, b; Rasouli et al., 2023a, b) have been examined for the removal of SMX and other PPCPs. However, the adsorption treatment process shows limited adsorption capacity. It requires the separation of pollutants from the adsorbent, and the AOPs require continuous input of expensive chemicals and energy to keep them working and are energy intensive.

In contrast, duckweed, an aquatic plant-based wastewater treatment, is considered a low-cost, low-energy, easily operable, and environmentally friendly option with high potential for valuable biomass production for biofuels, biomaterials, and feeds (Xu et al., 2012; Zeller et al., 2013; Toyama et al., 2018; Lai et al., 2023). Using the duckweed-based treatment process, some researchers have demonstrated the removal of xenobiotic chemicals such as phenols (Toyama et al., 2006) and PPCPs Li et al. (2017); Hu et al. (2019); dos Reis et al. (2013); Inagaki et al. (2016) proposed a highly efficient phyto-Fenton oxidation process, in which highly reactive OH radicals could be produced through Fenton and Fenton-like reactions using intracellular hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) in aquatic plants (Eq. (1) and (2), respectively). This is termed the phyto-Fenton process because the  $\text{H}_2\text{O}_2$  produced by plants is converted to strong oxidizing OH radicals in the presence of an iron catalyst and used to oxidize various organic pollutants to  $\text{CO}_2$ , water, and minerals. The phyto-Fenton process can overcome the disadvantages of the conventional Fenton process because it does not require a continuous supply of  $\text{H}_2\text{O}_2$ . The phyto-Fenton process is a low-cost and highly efficient technique for wastewater treatment. Pentachlorophenol (dos Reis et al., 2013), bisphenol-A, 2,4-dichlorophenol, 4-tert-octylphenol (Reis et al., 2014), 17 $\alpha$ -ethinylestradiol (Inagaki et al., 2016), and organochlorine pesticides, including *p,p'*-dichlorodiphenyltrichloroethane (Tran et al., 2020), were effectively removed from water or soil by phyto-Fenton processes using aquatic or terrestrial plants.



We hypothesized that the phyto-Fenton process, which utilizes duckweed, might be a cost-effective alternative treatment for producing valuable biomass from SMX-polluted water. The phyto-Fenton process using duckweed has not been extensively examined. Duckweed (Lemnaceae) have five genera and 36 or more species (Tippery et al., 2015; Bog et al., 2019) but the specific species suited for phyto-Fenton process are unknown. No study has demonstrated simultaneous SMX removal and production of valuable feedstock biomass through the phyto-Fenton process using duckweed.

Therefore, the objective of this study was to assess the possibility of using duckweed in the phyto-Fenton process for efficient oxidation and SMX removal from polluted water while simultaneously producing biomass for biofuel feedstock. In this study, (i) the intracellular and extracellular  $\text{H}_2\text{O}_2$  productivity of four duckweed species, *Lemna aequinoctialis*, *L. minor*, *Landolina punctata*, and *Spirodela polyrhiza*, and (ii) the ability of the phyto-Fenton process using the four duckweed species were examined for SMX removal capacity from water. (iii) Demonstration experiments were conducted for simultaneous SMX removal from contaminated wastewater using the phyto-Fenton process and biomass production using *S. polyrhiza* cultures. This study, for the first time, provides scientific knowledge of the phyto-Fenton process using various duckweed species and the co-benefits of SMX removal and biomass production using duckweed.

## 2. Materials and methods

### 2.1. Plant materials

Bacteria-free *L. aequinoctialis*, *L. minor*, *L. punctata*, and *S. polyrhiza* were repeatedly and aseptically sub-cultured for a long time in flasks containing the sterile modified Hoagland (mH) solution (36.1  $\text{mg L}^{-1}$   $\text{KNO}_3$ , 293  $\text{mg L}^{-1}$   $\text{K}_2\text{SO}_4$ , 3.87  $\text{mg L}^{-1}$   $\text{NaH}_2\text{PO}_4$ , 103  $\text{mg L}^{-1}$   $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 147  $\text{mg L}^{-1}$   $\text{CaCl}_2 \cdot \text{H}_2\text{O}$ , 3.33  $\text{mg L}^{-1}$   $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.95  $\text{mg L}^{-1}$   $\text{H}_3\text{BO}_3$ , 0.39  $\text{mg L}^{-1}$   $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ , 0.03  $\text{mg L}^{-1}$   $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , 0.08  $\text{mg L}^{-1}$   $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ , and 0.254  $\text{mg L}^{-1}$   $\text{H}_2\text{MoO}_4 \cdot 4\text{H}_2\text{O}$ ; pH 7.0) in a growth chamber ( $28 \pm 1^\circ\text{C}$ , fluorescent lamps at a photosynthetic photon flux density of 80  $\mu\text{mol m}^{-2}\text{s}^{-1}$ , 16L:8D photoperiod). Bacteria-free conditions for the four duckweed species were periodically confirmed by bacterial culture on R2A agar plates.

### 2.2. Chemicals

SMX was purchased from Tokyo Chemical Industry Co. Ltd. (Tokyo, Japan). Ferrous sulfate heptahydrate ( $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ ) was purchased from Kanto Chemical Co., Inc. (Tokyo, Japan).

### 2.3. Determination of intracellular and extracellular $\text{H}_2\text{O}_2$ productivity of duckweeds

To determine the intracellular and extracellular  $\text{H}_2\text{O}_2$  productivities

of the four duckweeds, each duckweed plant (0.5 g fresh weight [FW]) was planted in a 100-mL capacity flask containing 50 mL of sterilized mH solution. The flasks were incubated in a growth chamber for 3 d, following which the duckweed plant samples (0.5 g FW) were collected from the flask and immediately crushed into powder using a BioMasher disposal homogenizer (Nippi; Ibaraki, Japan) and liquid nitrogen, and then mixed with 1 mL of 0.1 M phosphate buffer (pH 6.8). The mixture was centrifuged ( $12,000\times g$ , 4 °C, 15 min), and then the supernatant was analyzed for intracellular  $H_2O_2$  produced by the duckweed samples. In addition, the extracellular  $H_2O_2$  released from duckweed tissues in the mH solution was measured. The  $H_2O_2$  concentration was measured using the OxiSelect Hydrogen Peroxide/Peroxidase Assay Kit (Cell Biolabs, Inc., San Diego, CA, USA) according to the manufacturer's protocol. The intracellular  $H_2O_2$  concentration was calculated as  $\mu\text{mol } H_2O_2 \text{ g}^{-1}$  FW of duckweed, and the extracellular  $H_2O_2$  concentration was calculated as  $\mu\text{mol } H_2O_2 \text{ L}^{-1}$  of mH solution.

#### 2.4. SMX removal from mH solution by phyto-Fenton using four duckweed species under sterile conditions

To determine the SMX removal efficiency of the phyto-Fenton process using the four duckweed species, five different test samples were prepared in 100-mL capacity flasks as follows. Test 1 consisted of 50 mL of sterilized mH solution supplemented with SMX ( $1 \text{ mg L}^{-1}$ ), duckweed plant (0.5 g-FW), and 10 mM Fe (II) ( $10 \text{ mM FeSO}_4\cdot 7H_2O$ ). Test 2 consisted of 50 mL of a sterilized mH solution supplemented with SMX ( $1 \text{ mg L}^{-1}$ ) and duckweed plant (0.5 g-FW). Test 3 consisted of 50 mL of sterilized mH solution supplemented with SMX ( $1 \text{ mg L}^{-1}$ ) and 10 mM Fe (II). Test 4 consisted of 50 mL of sterilized mH solution supplemented with SMX ( $1 \text{ mg L}^{-1}$ ). Test 5 consisted of 50 mL of sterilized mH solution without SMX but with duckweed plant (0.5 g-FW). In Tests 1, 2, and 5, 12 flasks were replicated and incubated in a plant growth chamber for 5 d. Three flasks in each test were then collected for SMX measurement and/or intracellular  $H_2O_2$  measurement at 0, 1, 3, and 5 d after incubation. On each sampling day, duckweed plant materials (0.5 g-FW) were collected from a flask, and intracellular  $H_2O_2$  was measured as described above, and 1 mL of solution was collected from each flask for SMX concentration measurement. In Tests 3 and 4, three flasks were replicated and incubated for 5 d in the chamber, and 1 mL of solution was sampled from each flask for SMX measurement. The SMX content in the collected samples was analyzed by high-performance liquid chromatography (HPLC) using a Shimadzu HPLC system with a Shimpack VP-ODS column ( $150 \text{ mm} \times 4.6 \text{ mm i.d.}$ , particle size  $5 \mu\text{m}$ ; Shimadzu, Kyoto, Japan). For HPLC analysis, acetonitrile and 10 mM phosphate buffer (pH 2.5) (20:80, v/v) were used as the mobile phase at a flow rate of  $1.0 \text{ mL min}^{-1}$ , and SMX was detected at a wavelength of 262 nm.

#### 2.5. Demonstration of simultaneous removal of SMX from wastewater and biomass production by phyto-Fenton process using the duckweed, *S. polyrrhiza*

*S. polyrrhiza* was used in the demonstration experiment. Municipal wastewater effluent (Table S1) was collected from the final sedimentation process of a conventional activated sludge municipal wastewater treatment plant in Kofu City, Yamanashi, Japan, and used for the demonstration experiment. SMX was dissolved at  $1 \text{ mg L}^{-1}$  in the wastewater effluent, and the effluent was used as a model SMX-polluted wastewater. *S. polyrrhiza* was precultured in the SMX polluted wastewater for 7 d, and the *S. polyrrhiza* was planted at 100% surface coverage into 1 L of the SMX polluted wastewater with 10 mM Fe (II) in three plastic containers ( $160 \text{ mm} \times 125 \text{ mm} \times 80 \text{ mm}$ ). Control SMX wastewater containers with duckweed and without 10 mM Fe (II) were prepared in triplicate. The duckweed phyto-Fenton systems were incubated for 5 d in a growth chamber. Over the 5 d, wastewater samples were collected from three containers for each test, centrifuged ( $10,000\times g$ , 5 min), and the SMX concentration was determined using HPLC (as

described in Section 2.4). After 5 d, all duckweed samples were harvested from all the test containers, dried ( $70^\circ\text{C}$  for 6 h), and their dry weight was measured. The dried biomass was subjected to methane fermentation in batch anaerobic digestors in triplicate. Anaerobic digestion was performed in a 100-mL glass vessel containing 30 mL of anaerobic digestion sludge and 1.0 g of dried duckweed powder. The volatile solids (VS) content of the duckweed samples was  $67.7\%$  ( $0.677 \text{ g g}^{-1}$ ). The inoculum was collected from a mesophilic anaerobic digestion reactor at a night-soil treatment plant in Koshu City, Yamanashi, Japan. Each vessel was sealed and flushed with  $N_2$  gas to maintain the anaerobic condition. Methane fermentation was conducted statically at  $38^\circ\text{C}$  for 20 d. Methane fermentation with inoculum sludge alone or without duckweed biomass was conducted as a blank experiment. The biogas produced during anaerobic digestion by the sludge was collected in a gas collection bag, and its volume was determined. After 20 d, the biogas composition, including methane and carbon dioxide, was determined using a Shimadzu gas chromatography system with a thermal conductivity detector and a SHINCARBON ST column ( $2 \text{ m} \times 3 \text{ mm}$ ; Shimadzu GLC Ltd., Tokyo, Japan). The net volume of biogas produced from duckweed biomass was corrected based on the blank experiments and normalized to standard conditions (standard atmospheric pressure, 1013 hPa;  $0^\circ\text{C}$ ). Finally, the biogas productivity was standardized to the amount of duckweed biomass VS and expressed as normal liters (NL) of  $CH_4 \text{ kg}^{-1}$  VS.

#### 2.6. Statistical analysis

All experiments were performed in triplicate. Statistical significance ( $p < 0.05$ ) was analyzed using the Student's t-test in R version 3.5.1 (<http://www.r-project.org>).

### 3. Results and discussion

#### 3.1. Intracellular and extracellular $H_2O_2$ production abilities of duckweed

The four duckweed species (*L. aequinoctialis*, *L. minor*, *L. punctata*, and *S. polyrrhiza*) were cultured in mH solution under natural conditions without chemical stress for 3 d. The concentrations of intracellular  $H_2O_2$  in duckweed and extracellular  $H_2O_2$  in mH solution after 3 d of culture are summarized in Table 1.

The four duckweed species produced intracellular  $H_2O_2$  in their cells and released extracellular  $H_2O_2$  into the surrounding water (Table 1).  $H_2O_2$  is generated in the chloroplasts, mitochondria, plasma membranes, apoplasts, and cell walls of plant cells (Foyer and Noctor, 2016; Waszczak et al., 2018; Smirnoff and Arnaud, 2019) and plays several key roles in cell expansion, development, defense, and cell signaling in response to stresses, plant hormones, and physical or chemical changes outside the cells (Foyer and Noctor, 2016; Waszczak et al., 2018; Smirnoff and Arnaud, 2019).  $H_2O_2$  concentrations range from  $0.67$  to  $3.6 \mu\text{mol g}^{-1}$  FW in the leaf tissue of mangrove plants (Cheeseman, 2006) and  $10$ – $50 \mu\text{mol g}^{-1}$  FW in the leaf tissue of riparian plants (Asaeda et al., 2022) under natural conditions. Gomes and Juneau (2016); Ishizawa et al. (2017) reported the  $H_2O_2$  concentration of approximately  $3$ – $5 \mu\text{mol g}^{-1}$  FW of duckweed *L. minor* under non-stress

**Table 1**

Concentration of intracellular and extracellular  $H_2O_2$  produced by the four duckweed test species, *L. aequinoctialis*, *L. minor*, *L. punctata*, and *S. polyrrhiza*, grown in mH solution under natural conditions.

|                          | Intracellular $H_2O_2$ ( $\mu\text{mol } H_2O_2 \text{ g-FW}^{-1}$ ) | Extracellular $H_2O_2$ ( $\mu\text{mol } H_2O_2 \text{ L}^{-1}$ ) |
|--------------------------|----------------------------------------------------------------------|-------------------------------------------------------------------|
| <i>L. aequinoctialis</i> | $20.41 \pm 2.89$                                                     | $0.95 \pm 0.096$                                                  |
| <i>L. minor</i>          | $17.07 \pm 4.96$                                                     | $0.784 \pm 0.185$                                                 |
| <i>L. punctata</i>       | $22.04 \pm 5.49$                                                     | $0.952 \pm 0.096$                                                 |
| <i>S. polyrrhiza</i>     | $24.56 \pm 5.35$                                                     | $0.833 \pm 0.163$                                                 |

Values represent mean  $\pm$  SD.

conditions. In this study, the intracellular  $\text{H}_2\text{O}_2$  concentrations in the four duckweed plants ranged from  $16.7 \mu\text{mol g}^{-1}$  FW in *L. minor* to  $24.6 \mu\text{mol g}^{-1}$  FW in *S. polyrrhiza*; however,  $\text{H}_2\text{O}_2$  production was statistically insignificant among the four duckweeds (Table 1), and the intracellular  $\text{H}_2\text{O}_2$  concentrations were relatively higher than those previously reported for duckweeds and comparable to those of riparian plants. Our results showed that these four duckweed species produced intracellular significant amount of  $\text{H}_2\text{O}_2$  under natural conditions and released extracellular  $\text{H}_2\text{O}_2$  into the aqueous phase. Therefore, these duckweed species are useful as an “ $\text{H}_2\text{O}_2$  supply source” to advance the phyto-Fenton process for wastewater treatment. Because  $\text{H}_2\text{O}_2$  is a byproduct of photosynthetic reaction, duckweed can be considered a low-cost and low-energy-consuming sustainable supplier of  $\text{H}_2\text{O}_2$ .

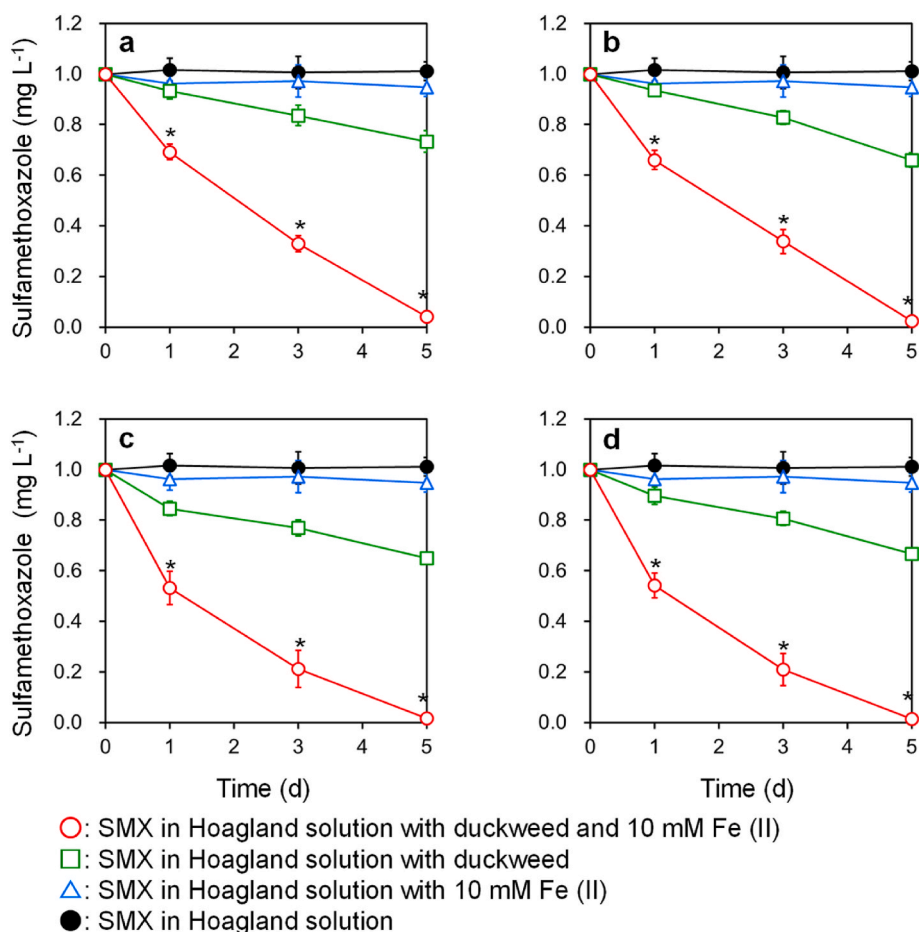
### 3.2. SMX removal from mH solution by phyto-Fenton process using duckweeds under sterilized conditions

Duckweed species were cultured in SMX containing mH solution with and without 10 mM Fe (II) for 5 d to examine the potential of the phyto-Fenton reaction using duckweed for SMX removal. The time courses of the SMX concentration during SMX removal tests in mH solutions for each duckweed species are shown in Fig. 1. There was no change in SMX concentration in the control mH solution without duckweed and 10 mM Fe (II). A slight decline was found over 5 d in the mH solution containing 10 mM Fe (II) (Fig. 1). In contrast, in the mH solution with sterilized duckweed, the SMX concentration decreased considerably over 5 d for all four duckweed species and 26.7%–39.1% of the SMX was removed from the mH solution within 5 d by sterilized

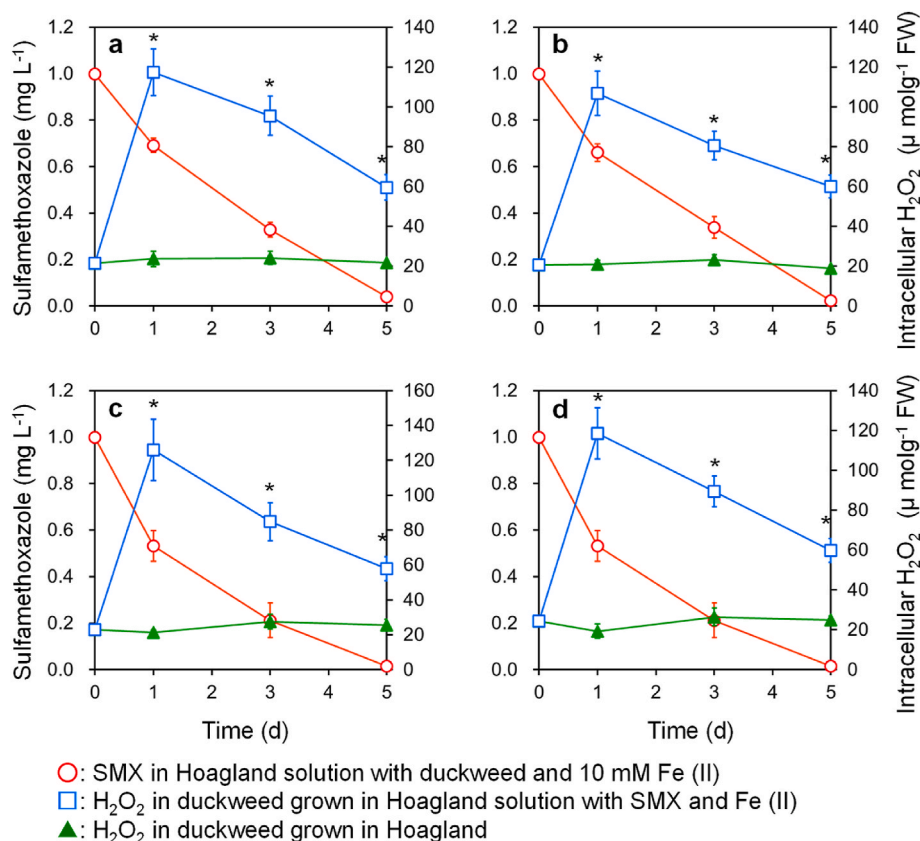
duckweed (Fig. 1).

SMX removal from water through plant uptake (Liu et al., 2021) and adsorption on plant roots (Adesanya et al., 2020) has been reported. Thus, SMX removal from the mH solution with sterilized duckweed material might be due to uptake and adsorption on duckweed surfaces. In the mH solution with duckweed and 10 mM Fe (II), the SMX concentration declined rapidly and almost completely; that is, >96.1% of the SMX was removed from the mH solution after 5 d (Fig. 1). This rapid removal of SMX from the mH solution with duckweed and 10 mM Fe (II) can be attributed to the phyto-Fenton process reactions using  $\text{H}_2\text{O}_2$  produced by duckweed. The phyto-Fenton process occurs under these conditions to generate OH radicals (Inagaki et al., 2016). Removal ratios were greater than those using duckweed alone (Fig. 1). Therefore, the results clearly indicate that the phyto-Fenton process using each of the four duckweed species (*L. aequinoctialis*, *L. minor*, *L. punctata*, and *S. polyrrhiza*) can efficiently remove SMX from contaminated solutions under sterile conditions. Similarly, the efficient removal of pentachlorophenol (dos Reis et al., 2013) by phyto-Fenton reaction using duckweeds (*L. aoukikusa* and *S. polyrrhiza*) and 17 $\alpha$ -ethinylestradiol (EE2) (Inagaki et al., 2016) by phyto-Fenton reaction using *S. polyrrhiza* was reported. This is the first study to demonstrate an enhanced phyto-Fenton process by four duckweed species.

During the SMX removal test in the mH solution containing duckweed and 10 mM Fe (II), the intracellular  $\text{H}_2\text{O}_2$  concentration in duckweed tissue was monitored. Intracellular  $\text{H}_2\text{O}_2$  concentrations in duckweed in the presence and absence of SMX and 10 mM Fe (II) and the absence of them (natural conditions) are shown in Fig. 2 for each duckweed species. The intracellular  $\text{H}_2\text{O}_2$  in duckweed grown under



**Fig. 1.** Decrease in sulfamethoxazole concentrations in mH solution by the four duckweed species under sterile conditions. a: *L. aequinoctialis*, b: *L. minor*, c: *L. punctata*, and d: *S. polyrrhiza*. Values represent mean  $\pm$  SD. Asterisks indicate a significant difference ( $p < 0.05$ ) between the mH solution with duckweed/Fe (II) and mH solution with duckweed.



**Fig. 2.** Decrease in sulfamethoxazole concentrations in mH solution with duckweed and 10 mM Fe (II), and changes in intracellular H<sub>2</sub>O<sub>2</sub> in duckweed during its growth in mH solution with duckweed and 10 mM Fe (II) and mH solution without SMX/Fe (II). a: *L. aequinoctialis*, b: *L. minor*, c: *L. punctata*, and d: *S. polyrhiza*. Values represent mean  $\pm$  SD. Asterisks indicate a significant difference ( $p < 0.05$ ) in intracellular H<sub>2</sub>O<sub>2</sub> between duckweed grown in the mH solution with SMX/Fe (II) and mH solution.

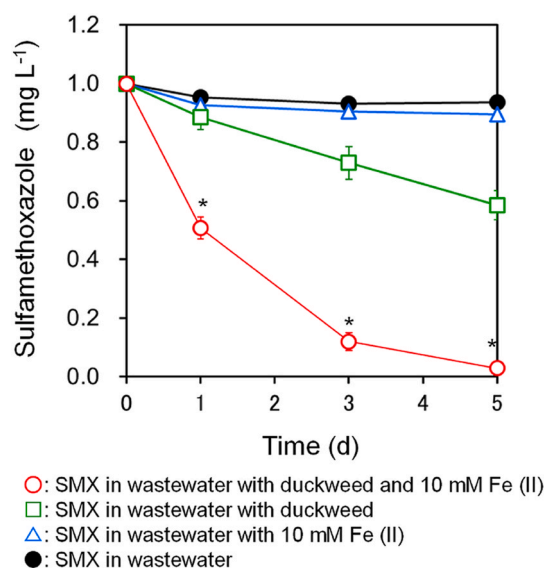
natural conditions without SMX and 10 mM Fe (II) was maintained in the range of 19.1–27.5  $\mu\text{mol g}^{-1}$  FW of duckweed (Fig. 2). In contrast, the intracellular H<sub>2</sub>O<sub>2</sub> concentration in duckweed in the presence of SMX and 10 mM Fe (II) increased rapidly to over 105  $\mu\text{mol g}^{-1}$  FW in the first 24 h (Fig. 2). Subsequently, from 1 d to 5 d, H<sub>2</sub>O<sub>2</sub> decreased gradually to about 60  $\mu\text{mol g}^{-1}$  FW in line with the decrease in the SMX concentration in mH solution (Fig. 2). Plants produce H<sub>2</sub>O<sub>2</sub> intracellularly and extracellularly in response to various stressors (Foyer and Noctor, 2016; Waszczak et al., 2018; Smirnov and Arnaud, 2019), such as salts (Hernandez et al., 2010), herbicides (Gomes and Juneau, 2016), copper (Panda, 2008), and selenium (Ríos et al., 2009). In this study, the production of H<sub>2</sub>O<sub>2</sub> in duckweeds might have been induced in response to SMX in mH solution, and the elevated intracellular and extracellular H<sub>2</sub>O<sub>2</sub> concentrations contributed effectively and efficiently to the phyto-Fenton reaction to degrade SMX around duckweeds. In previous studies, the dynamics of the H<sub>2</sub>O<sub>2</sub> levels produced by plants in the phyto-Fenton process for treating polluted water were unclear; however, this study clarifies some of these aspects.

### 3.3. Simultaneous SMX removal and biofuel-feedstock biomass production from SMX contaminated wastewater by using a duckweed *S. polyrhiza* under non-sterile conditions

*S. polyrhiza* was cultured in model SMX (1 mg L<sup>-1</sup>)-contaminated wastewater with and without 10 mM Fe (II) to evaluate the potential for SMX removal by the phyto-Fenton process using *S. polyrhiza* and duckweed biomass production under non-sterile conditions mimic actual SMX-contaminated water. The concentrations of SMX in the contaminated wastewater without *S. polyrhiza* and 10 mM Fe (II), and contaminated wastewater with 10 mM Fe (II) but without *S. polyrhiza* did not

decline over the 5 d (Fig. 3).

The SMX concentration declined by approximately 42% in the contaminated wastewater containing *S. polyrhiza* after 5 d (Fig. 3). This SMX removal may be due to uptake by *S. polyrhiza* (Liu et al., 2021) and



**Fig. 3.** Decrease in sulfamethoxazole concentrations during wastewater treatment under non-sterile conditions using *S. polyrhiza*. Values represent mean  $\pm$  SD. Asterisks indicate a significant difference ( $p < 0.05$ ) between the duckweed-containing wastewater with and without Fe (II).

adsorption onto the roots of *S. polyrhiza* (Adesanya et al., 2020), similar to the SMX removal obtained under sterile experimental conditions (Fig. 1). Duckweed can recruit and enrich various organic pollutant-degrading bacteria around its roots and fronds (Toyama et al., 2009). Biodegradation of organic pollutants such as aromatic compounds can be promoted around duckweed by the synergy of duckweed and pollutant-degrading bacteria (Toyama et al., 2006). In this study, SMX was efficiently degraded by bacteria associated with *S. polyrhiza* in contaminated wastewater. In fact, the reduction of SMX in non-sterile wastewater was more than in sterile mH conditions. In contrast, SMX was removed rapidly and considerably from the contaminated wastewater in the presence of *S. polyrhiza* and 10 mM Fe (II), where the conditions are appropriate to the phyto-Fenton process reactions (Fig. 3). The results indicate that the phyto-Fenton process using *S. polyrhiza* was effective in removing SMX from wastewater containing several organic and inorganic compounds and various microorganisms.

Simultaneously, 0.795 g-DW of biomass of *S. polyrhiza* was produced from 1 L of the SMX-contaminated wastewater with 10 mM Fe (II) during the 5 d; the biomass production rate per unit area was estimated to be 7.95 g-DW m<sup>-2</sup> d<sup>-1</sup> (Table 2). *S. polyrhiza* can proliferate and produce biomass during wastewater treatment at about 4–12 g-DW m<sup>-2</sup> d<sup>-1</sup> (Xu et al., 2011; Zhao et al., 2015; Iwano et al., 2020). The biomass production rate per unit area of *S. polyrhiza* during SMX wastewater treatment by the phyto-Fenton process in this study was equivalent to that reported previously.

Methane gas was produced from the dried biomass powder of the collected samples of *S. polyrhiza* (Fig. S1). The methane production potential (methane yield per unit of organic matter of duckweed, NL-CH<sub>4</sub> kg<sup>-1</sup> VS) was 353 NL-CH<sub>4</sub> kg<sup>-1</sup> VS (Table 2). A significant amount of methane gas can be produced from the biomass of *S. polyrhiza* grown in a variety of wastewaters at 340–413 NL-CH<sub>4</sub> kg<sup>-1</sup> VS (Toyama et al., 2018). The methane production potential in this study was within the above range of methane production potential for *S. polyrhiza* biomass. Thus, the *S. polyrhiza* biomass material produced during SMX removal by the phyto-Fenton process has a high potential value as biogas feed. Therefore, the results of this study demonstrate an excellent advantage of simultaneous energy-saving SMX removal and fuel-feedstock biomass production from SMX-contaminated wastewater using duckweed *S. polyrhiza* under non-sterile conditions close to actual SMX-contaminated water. Duckweed can be used as “H<sub>2</sub>O<sub>2</sub> supply source” for the phyto-Fenton process. For this system’s practical application of this phyto-Fenton process, efficient and inexpensive Fe (II) supply methods need to be studied in the future. Photocatalysts process is an effective technique to degrade pollutants in wastewaters (Cai and Hu, 2018; Balakrishnan et al., 2023a, 2023b, 2024a, 2023b, b; Rasouli et al., 2023a, 2023b). The phyto-Fenton process can degrade pollutants in wastewater and produce biomass simultaneously.

#### 4. Conclusions

This study investigated the possibility of efficient SMX removal and simultaneous biomass production for biofuel feedstock from polluted wastewater using the phyto-Fenton process with duckweed. Four duckweed species, *L. aequinoctialis*, *L. minor*, *L. punctata*, and *S. polyrhiza*, produced intracellular H<sub>2</sub>O<sub>2</sub> in the range of 16.7–24.6 μmol g<sup>-1</sup> fresh weight and released extracellular H<sub>2</sub>O<sub>2</sub> into the surrounding water phase. All four duckweed species efficiently removed SMX from contaminated mH solution in the presence of 10 mM Fe (II), which strongly indicated the occurrence of the phyto-Fenton reaction. The results indicate that various duckweed species can efficiently remove SMX by the phyto-Fenton process. *S. polyrhiza* removed SMX and produced biomass at 7.95 g dry weight m<sup>-2</sup> d<sup>-1</sup> in the model SMX polluted wastewater containing other contaminants. The produced biomass was successfully converted into methane with a high yield (353 normal liters CH<sub>4</sub> kg<sup>-1</sup> volatile solids). The duckweed is an excellent sunlight-driven tool, which enables us to couple efficient SMX removal and biofuel

**Table 2**

Biomass and methane production capacity associated with phyto-Fenton treatment of SMX polluted water by *S. polyrhiza*.

| Biomass production                                                                                  |               |
|-----------------------------------------------------------------------------------------------------|---------------|
| Initial biomass in the SMX polluted wastewater (g-DW)                                               | 0.68 ± 0.01   |
| Final biomass in the SMX polluted wastewater (g-DW)                                                 | 1.48 ± 0.07   |
| Biomass production over the 5 d phyto-Fenton process treating SMX polluted wastewater (g-DW)        | 0.795 ± 0.072 |
| Biomass production rate per unit area (g-DW m <sup>-2</sup> d <sup>-1</sup> )                       | 7.95 ± 0.72   |
| Methane production                                                                                  |               |
| Methane concentration in the produced biogas (%)                                                    | 65.3 ± 1.7    |
| Methane yield per unit organic matter of duckweed biomass (NL CH <sub>4</sub> kg VS <sup>-1</sup> ) | 353 ± 9       |

Values represent mean ± SD.

feedstock production. The system uses photosynthesis as its power source. Thus, it will be an effective strategy for energy savings and energy generation during wastewater treatment.

#### Funding

This research was supported in part by the Advanced Low Carbon Technology Research and Development Program (ALCA) of the Japan Science and Technology Agency (JST) (Grant Number: JPMJAL1108) and the Science and Technology Research Partnership for Sustainable Development (SATREPS) joint project of JST (Grant Number: JPMJSA2004) and the Japan International Cooperation Agency (JICA).

#### CRediT authorship contribution statement

**Tadashi Toyama:** Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Maki Kobayashi:** Writing – review & editing, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Rubiy'atno:** Writing – review & editing, Data curation, Writing – review & editing, Data curation. **Masaaki Morikawa:** Writing – review & editing, Funding acquisition. **Kazuhiro Mori:** Writing – review & editing.

#### Declaration of competing interest

The authors declare that they have no competing financial interests or personal relationships that may have influenced the work reported in this study.

#### Data availability

No data was used for the research described in the article.

#### Acknowledgement

Keita Yamamoto helped the SMX removal experiments.

#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.chemosphere.2024.142592>.

#### References

- Adeleye, A.S., Xue, J., Zhao, Y., Taylor, A.A., Zenobio, J.E., Sun, Y., Han, Z., Salawu, O. A., Zhu, Y., 2022. Abundance, fate, and effects of pharmaceuticals and personal care products in aquatic environments. *J. Hazard.* 424, 127284 <https://doi.org/10.1016/j.jhazmat.2021.127284>.
- Adesanya, T., Zvomuya, F., Farenhorst, A., 2020. Sulfamethoxazole sorption by cattail and switchgrass roots. *J. Environ. Sci. Health B* 55, 1021–1031. <https://doi.org/10.1080/03601234.2020.1807263>.
- Asaeda, T., Rahman, M., Vamsi-Krishna, L., Schoelynck, J., Rashid, M.H., 2022. Measurement of foliar H<sub>2</sub>O<sub>2</sub> concentration can be an indicator of riparian vegetation management. *Sci. Rep.* 12, 13803 <https://doi.org/10.1038/s41598-022-17658-2>.

- Balakrishnan, A., Chinthala, M., Polagani, R.K., Vo, D.-V.N., 2023a. Removal of tetracycline from wastewater using g-C<sub>3</sub>N<sub>4</sub> based photocatalysts: a review. *Environ. Res.* 216, 114660 <https://doi.org/10.1016/j.envres.2022.114660>.
- Balakrishnan, A., Kunnel, E.S., Sasidharan, R., Chinthala, M., Kumar, A., 2023b. 3D black g-C<sub>3</sub>N<sub>4</sub> isotype heterojunction hydrogels as a sustainable photocatalyst for tetracycline degradation and H<sub>2</sub>O<sub>2</sub> production. *Chem. Eng. J.* 475, 146163 <https://doi.org/10.1016/j.cej.2023.146163>.
- Balakrishnan, A., Kunnel, E.S., Sasidharan, R., Chinthala, M., Kumar, A., 2024a. Tailored citric acid-Functionalized carbon nitride homojunction-immobilized carboxymethyl cellulose 3D photocatalytic hydrogels: a multifaceted approach toward environmental remediation. *ACS Sustainable Chem. Eng.* 12, 5169–5185. <https://doi.org/10.1021/acssuschemeng.3c07961>.
- Balakrishnan, A., Vijaya Suryaa, K., Tripathy, H., Trivedi, S., Kumar, A., Chinthala, M., 2024b. Phosphorylated g-C<sub>3</sub>N<sub>4</sub>/sulfur self-doped g-C<sub>3</sub>N<sub>4</sub> homojunction carboxymethyl cellulose beads: an efficient photocatalyst for H<sub>2</sub>O<sub>2</sub> production. *J. Colloid Interface Sci.* 663, 1087–1098. <https://doi.org/10.1016/j.jcis.2024.02.110>.
- Bog, M., Appenroth, K.-J., Sree, K.S., 2019. Duckweed (Lemnaceae): its molecular taxonomy. *Front. Sustain. Food Syst.* 3 <https://doi.org/10.3389/fsufs.2019.00117>.
- Bonvin, F., Jost, L., Randin, L., Bonvin, E., Kohn, T., 2016. Super-fine powdered activated carbon (SPAC) for efficient removal of micropollutants from wastewater treatment plant effluent. *Water Res.* 90, 90–99. <https://doi.org/10.1016/j.watres.2015.12.001>.
- Cai, Q., Hu, J., 2018. Effect of UVA/LED/TiO<sub>2</sub> photocatalysis treated sulfamethoxazole and trimethoprim containing wastewater on antibiotic resistance development in sequencing batch reactors. *Water Res.* 140, 251–260. <https://doi.org/10.1016/j.watres.2018.04.053>.
- Cheeseman, J.M., 2006. Hydrogen peroxide concentrations in leaves under natural conditions. *J. Exp. Bot.* 57, 2435–2444. <https://doi.org/10.1093/jxb/erl004>.
- Dirany, A., Sirés, I., Oturan, N., Oturan, M.A., 2010. Electrochemical abatement of the antibiotic sulfamethoxazole from water. *Chemosphere* 81, 594–602. <https://doi.org/10.1016/j.chemosphere.2010.08.032>.
- dos Reis, A.R., Kyuma, Y., Sakakibara, Y., 2013. Biological Fenton's oxidation of pentachlorophenol by aquatic plants. *Bull. Environ. Contam. Toxicol.* 91, 718–723. <https://doi.org/10.1007/s00128-013-1106-4>.
- Foyer, C.H., Noctor, G., 2016. Stress-triggered redox signalling: what's in pROSpect? *Plant Cell Environ.* 39, 951–964. <https://doi.org/10.1111/pce.12621>.
- Gao, S., Zhao, Z., Xu, Y., Tian, J., Qi, H., Lin, W., Cui, F., 2014. Oxidation of sulfamethoxazole (SMX) by chlorine, ozone and permanganate—a comparative study. *J. Hazard Mater.* 274, 258–269. <https://doi.org/10.1016/j.jhazmat.2014.04.024>.
- Gomes, M.P., Juneau, P., 2016. Oxidative stress in duckweed (*Lemna minor* L.) induced by glyphosate: is the mitochondrial electron transport chain a target of this herbicide? *Environ. Pollut.* 218, 402–409. <https://doi.org/10.1016/j.envpol.2016.07.019>.
- Hernandez, M., Fernandez-Garcia, N., Diaz-Vivancos, P., Olmos, E., 2010. A different role for hydrogen peroxide and the antioxidative system under short and long salt stress in *Brassica oleracea* roots. *J. Exp. Bot.* 61, 521–535. <https://doi.org/10.1093/jxb/erp321>.
- Hu, H., Zhou, Q., Li, X., Lou, W., Du, C., Teng, Q., Zhang, D., Liu, H., Zhong, Y., Yang, C., 2019. Phytoremediation of anaerobically digested swine wastewater contaminated by oxytetracycline via *Lemna aeruquinotialis*: nutrient removal, growth characteristics and degradation pathways. *Bioresour. Technol.* 291, 121853 <https://doi.org/10.1016/j.biortech.2019.121853>.
- Inagaki, Y., Cong, V.H., Sakakibara, Y., 2016. Identification and application of Phyto-Fenton reactions. *Chemosphere* 144, 1443–1450. <https://doi.org/10.1016/j.chemosphere.2015.10.039>.
- Ishizawa, H., Kuroda, M., Morikawa, M., Ike, M., 2017. Differential oxidative and antioxidative response of duckweed *Lemna minor* toward plant growth promoting/inhibiting bacteria. *Plant Physiol. Biochem.* 118, 667–673. <https://doi.org/10.1016/j.plaphy.2017.08.006>.
- Iwano, H., Hatohara, S., Tagawa, T., Tamaki, H., Li, Y.Y., Kubota, K., 2020. Effect of treated sewage characteristics on duckweed biomass production and microbial communities. *Water Sci. Technol.* 82, 292–302. <https://doi.org/10.2166/wst.2020.168>.
- Lai, F., Jin, Y., Tan, L., He, K., Guo, L., Tian, X., Li, J., Du, A., Huang, Y., Zhao, H., Fang, Y., 2023. Bioconversion of wastewater-derived duckweed to lactic acid through fed-batch fermentation at high-biomass loading. *Biomass Convers. Biorefin.* 13, 2745–2756. <https://doi.org/10.1007/s13399-021-01274-7>.
- Li, J., Zhou, Q., Campos, L.C., 2017. Removal of selected emerging PPCP compounds using greater duckweed (*Spirodela polyrrhiza*) based lab-scale free water constructed wetland. *Water Res.* 126, 252–261. <https://doi.org/10.1016/j.watres.2017.09.002>.
- Liu, X., Lu, S., Liu, Y., Wang, Y., Guo, X., Chen, Y., Zhang, J., Wu, F., 2021. Performance and mechanism of sulfamethoxazole removal in different bioelectrochemical technology-integrated constructed wetlands. *Water Res.* 207, 117814 <https://doi.org/10.1016/j.watres.2021.117814>.
- Ogata, F., Tominaga, H., Kangawa, M., Inoue, K., Kawasaki, N., 2012. Removal of sulfa drugs by sewage treatment in aqueous solution systems: activated carbon treatment and ozone oxidation. *J. Oleo Sci.* 61, 217–225. <https://doi.org/10.5650/jos.61.217>.
- Panda, S.K., 2008. Impact of copper on reactive oxygen species, lipid peroxidation and antioxidants in *Lemna minor*. *Biol. Plant.* 52, 561–564. <https://doi.org/10.1007/s10535-008-0111-7>.
- Prasannamedha, G., Kumar, P.S., 2020. A review on contamination and removal of sulfamethoxazole from aqueous solution using cleaner techniques: present and future perspective. *J. Cleaner Prod.* 250, 119553 <https://doi.org/10.1016/j.jclepro.2019.119553>.
- Rasouli, K., Alamdari, A., Sabbaghi, S., 2023a. Ultrasonic-assisted synthesis of α-Fe<sub>2</sub>O<sub>3</sub>@TiO<sub>2</sub> photocatalyst: optimization of effective factors in the fabrication of photocatalyst and removal of non-biodegradable cefixime via response surface methodology-central composite design. *Sep. Purif. Technol.* 307, 122799 <https://doi.org/10.1016/j.seppur.2022.122799>.
- Rasouli, K., Rasouli, J., Mohtaram, M.S., Sabbaghi, S., Kamyab, H., Moradi, H., Chelliapan, S., 2023b. Biomass-derived activated carbon nanocomposites for cleaner production: a review on aspects of photocatalytic pollutant degradation. *J. Clean. Prod.* 419, 138181 <https://doi.org/10.1016/j.jclepro.2023.138181>.
- Reis, A.R., Tabai, K., Sakakibara, Y., 2014. Oxidation mechanism and overall removal rates of endocrine disrupting chemicals by aquatic plants. *J. Hazard Mater.* 265, 79–88. <https://doi.org/10.1016/j.jhazmat.2013.11.042>.
- Ríos, J.J., Blasco, B., Cervilla, L.M., Rosales, M.A., Sanchez-Rodriguez, E., Romero, L., Ruiz, J.M., 2009. Production and detoxification of H<sub>2</sub>O<sub>2</sub> in lettuce plants exposed to selenium. *Ann. Appl. Biol.* 154, 107–116. <https://doi.org/10.1111/j.1744-7348.2008.00276.x>.
- Smirnov, N., Arnaud, D., 2019. Hydrogen peroxide metabolism and functions in plants. *New Phytol.* 221, 1197–1214. <https://doi.org/10.1111/nph.15488>.
- Tippery, N.P., Les, D.H., Crawford, D.J., 2015. Evaluation of phylogenetic relationships in Lemnaceae using nuclear ribosomal data. *Plant Biol. (Stuttg.)* 17 (Suppl. 1), 50–58. <https://doi.org/10.1111/plb.12203>.
- Toyama, T., Hanaoka, T., Tanaka, Y., Morikawa, M., Mori, K., 2018. Comprehensive evaluation of nitrogen removal rate and biomass, ethanol, and methane production yields by combination of four major duckweeds and three types of wastewater effluent. *Bioresour. Technol.* 250, 464–473. <https://doi.org/10.1016/j.biortech.2017.11.054>.
- Toyama, T., Sei, K., Yu, N., Kumada, H., Inoue, D., Hoang, H., Soda, S., Chang, Y.C., Kikuchi, S., Fujita, M., Ike, M., 2009. Enrichment of bacteria possessing catechol dioxygenase genes in the rhizosphere of *Spirodela polyrrhiza*: a mechanism of accelerated biodegradation of phenol. *Water Res.* 43, 3765–3776. <https://doi.org/10.1016/j.watres.2009.05.045>.
- Toyama, T., Yu, N., Kumada, H., Sei, K., Ike, M., Fujita, M., 2006. Accelerated aromatic compounds degradation in aquatic environment by use of interaction between *Spirodela polyrrhiza* and bacteria in its rhizosphere. *J. Biosci. Bioeng.* 101, 346–353. <https://doi.org/10.1263/jbb.101.346>.
- Tran, T.D., Dao, N.T., Sasaki, R., Tu, M.B., Dang, G.H.M., Nguyen, H.G., Dang, H.M., Vo, C.H., Inigaki, Y., Van Nguyen, N., Sakakibara, Y., 2020. Accelerated remediation of organochlorine pesticide-contaminated soils with phyto-Fenton approach: a field study. *Environ. Geochem. Health* 42, 3597–3608. <https://doi.org/10.1007/s10653-020-00588-1>.
- Wang, J., Wang, S., 2016. Removal of pharmaceuticals and personal care products (PPCPs) from wastewater: a review. *J. Environ. Manage.* 182, 620–640. <https://doi.org/10.1016/j.jenvman.2016.07.049>.
- Waszczak, C., Carmody, M., Kangasjärvi, J., 2018. Reactive oxygen species in plant signaling. *Annu. Rev. Plant Biol.* 69, 209–236. <https://doi.org/10.1146/annurev-arplant-042817-040322>.
- Xu, J., Cheng, J.J., Stomp, A.-M., 2012. Growing *Spirodela polyrrhiza* in swine wastewater for the production of animal feed and fuel ethanol: a pilot study. *Clean: Soil, Air, Water* 40, 760–765. <https://doi.org/10.1002/clen.201100108>.
- Xu, J., Cui, W., Cheng, J.J., Stomp, A.-M., 2011. Production of high-starch duckweed and its conversion to bioethanol. *Biosyst. Eng.* 110, 67–72. <https://doi.org/10.1016/j.biosystemseng.2011.06.007>.
- Zeller, M.A., Hunt, R., Sharma, S., 2013. Sustainable bioderived polymeric materials and thermoplastic blends made from floating aquatic macrophytes such as “duckweed”. *J. Appl. Polym. Sci.* 127, 375–386. <https://doi.org/10.1002/app.37555>.
- Zhao, Y., Fang, Y., Jin, Y., Huang, J., Bao, S., Fu, T., He, Z., Wang, F., Wang, M., Zhao, H., 2015. Pilot-scale comparison of four duckweed strains from different genera for potential application in nutrient recovery from wastewater and valuable biomass production. *Plant Biol. (Stuttg.)* 17 (Suppl. 1), 82–90. <https://doi.org/10.1111/plb.12204>.