REMOVAL OF ANTIBIOTICS CONTAMINATING ENVIRONMENTAL WATER USING DUCKWEED (*LEMNA AOUKIKUSA*)

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1. Introduction

For these decades, great varieties of antibiotics have been widely used for both human and veterinary to prevent infectious diseases extensively and effectively. On the other hand, inappropriate discharge of wastewater from pharmaceutical or farming industry should lead to serious contamination of environmental water by antibiotics. These contaminations would first annihilate microorganisms around the polluted areas, and then destroy the local ecosystem. Then, antibiotic-resistant bacteria would be generated, causing propagation of further environmental damage even seriously threatening human health [1]. In these occasions, the pollution due to the pharmaceutical wastewater was the most serious because of higher concentration of antibiotics in the wastewater, extremely deteriorating the surrounding environment. The case in Medak district, Telangana state, South India was reported as a typical case of the most severe aquatic environmental pollution [2]. Therefore, in order to remediate those contaminated areas, a treatment technology should be necessary to be developed. Although several treatments have been reported as possible methods to treat the environmental water, the studies focusing on the treatment of wastewater of high antibiotic concentration are still insufficient. On the other hand, treatment of water environment contaminated by organic matters by using aquatic plant has been studied as a promising method to improve the water environment. Duckweed (*Lemna aoukikusa*) has been reported to effectively remove nutrients and organic compounds in the wastewater [3].

This study aimed the treatment of aqueous solution contaminated by antibiotics using duckweed. The removal of antibiotics in the aqueous solution using duckweed was measured and the effects of the operating conditions on the antibiotics removal was studied to analyze the removal mechanism.

2. Experimental

2.1. Materials

Two antibiotics, ciprofloxacin ($C_{17}H_{18}FN_3$, molar mass: 331.35kg kmol^{-1}, CIP) and sulfamethoxazole ($C_{10}H_{11}N_3O_3S$, molar mass: 253kg kmol^{-1}, SMX) were selected as target antibiotics because they were numerously detected in the polluted environment water in India [4]. CIP is belonging to new quinolone group, having a bicycle core structure related to the 4-quinolone and used for a wide variety of infections. SMX is a member of sulfonamide group, widely used in aquaculture and animal husbandry. These antibiotics were of analytical grade and purchased from FUJIFILM Wako Pure Chemical Corporation.

Duckweed (*Lemna aoukikusa*) were purchased from local market and grown under a specific condition. Before each experiment, duckweed was cultured in the water bath in which the temperature was controlled at 298K. Illumination was continuously supplied by a metal halide lamp (Eye HID LAMP, 400W IWASAKI ELECTRIC CO. LTD.), with 650μmol s^{-1}m^{-2} photosynthetic photon flux density. Air was also continuously provided in the bath by an air pump (4.5W NISSO CORPORATION). After the duckweed was cultured for 4 weeks, it was carefully cleaned by deionized water three times and used in the experiments.

2.2. Conditions and methods

The principle experimental conditions of antibiotic removal in aqueous solution are listed in Table 1. All the experiments were carried out with a constant temperature shaker set at the specified temperature for 240 hours (10 days). Under light condition, the experiment was carried out with Erlenmeyer flask, and for dark condition, the shading bottle was used. The initial antibiotic concentration, light condition, load of duckweed, and temperature were changed to measure the removal of antibiotic. In the cases of the experiments without duckweed, the flask was sealed up by cup to prevent water evaporation. In the cases with duckweed, the flack was unsealed for ensuring duckweed activity, and the mass of each sample solution was periodically adjusted.

Table 1 Experiment conditions for Experiment A~D

Antibiotics in feed solution	CIP/SMX
Initial Conc. of feed [kmol m^{-3}]	1.00×10^{-5}/5.00×10^{-5}
Volume of feed [m^3]	8×10^{-5}
Exp. Temperature [k]	293/298/303
Exp. period [h]	240
Mass of duckweed [kg]	0/0.003(Wet mass)
Illumination period [h]	0/240
Illumination density [μmol s^{-1} m^{-2}]	0/650±50

Generally, the organic compounds should be decomposed due to hydrolysis and photolysis in the aqueous solution, and then the respective effects of the hydrolysis, photolysis and duckweed uptake on the antibiotic removal were able to measure through Experiments A~D. In Experiment A, the operations were conducted under dark condition without duckweed to measure the effects of only hydrolysis on antibiotics removal. In Experiment B, the operations were carried out under continuous illumination conditions without duckweed to measure the effects of the hydrolysis and photolysis simultaneously. In Experiment C, the operations were conducted under dark conditions with

duckweed to measure the effects of hydrolysis and uptake without illumination on antibiotics removal. In Experiment D, the experiments were operated under illumination condition with duckweed to measure the effects of hydrolysis, photolysis and uptake with illumination. High Performance Liquid Chromatography (SPD-10A*VP*, SHIMADAZU CORPORATION, HPLC) was used for determining the concentration of antibiotic in the solution and the analysis conditions were referred to the Japanese Pharmacopoeia [5].

3. Results and Discussion

3.1. Basic equations for antibiotic removal models

The fractional removal of antibiotic A by experiment j, $Y_{A,j}$ was defined as,

$$Y_{A,j} = \frac{C_A}{C_{A,0}} \tag{1}$$

Here, $C_{A,0}$ and C_A indicate the initial concentration of antibiotic and concentration of antibiotic at t of each experiment j (j=A, B, C or D).

The antibiotics removal model was suggested as follows. In the case of hydrolysis, the hydrolysis reaction of antibiotic was assumed to be expressed as,

$$A \underset{k_{H,b}}{\overset{k_{H,f}}{\rightleftarrows}} B+C \tag{2}$$

Here A, B and C stand for the contaminant antibiotic and products due to the hydrolysis. This reaction was assumed to be reversible and followed the first-order one relative to the concentrations of respective compounds.

The photolysis reaction was assumed to be expressed as,

$$A \xrightarrow{k_P} D+E \tag{3}$$

Here D and E stand for the products due to the photolysis. This reaction was assumed to be irreversible and follow the first-order one as same as reaction (2).

In the cases of uptake by duckweed under dark and illumination conditions, it was assumed to be expressed as,

$$A(\text{aqueous solution}) \xrightarrow{k_{DW,D}\,/\,k_{DW,L}} A(\text{duckweed}) \tag{4}$$

It was also assumed that the uptake should be irreversible and follow the first-order reaction.

The total removal rate for antibiotic A, r_T, was calculated as,

$$r_T = -\frac{dC_A}{dt} = r_H + r_P + r_{DW} \tag{5}$$

Here, C_A, t, r_H, r_P and r_{DW} indicate the concentration of antibiotic at time t, the antibiotics removal rate by hydrolysis, photolysis and duckweed uptake, respectively. In the case of Experiment A, r_P=0 and r_{DW}=0 because only hydrolysis should occur for antibiotics removal. For Experiment B, r_{DW}=0. For Experiment C, r_{DW}=$r_{DW,D}$ and r_P=0 because uptake by duckweed should be conducted under dark condition. On the other hand, for Experiment D, r_{DW}=$r_{DW,L}$ because it should be carried out under light condition.

The antibiotics removal rate by hydrolysis, r_H was defined as,

$$r_H = r_{H,f} - r_{H,b} = k_{H,f}C_A - k_{H,b}C_B C_C \tag{6}$$

Here, $r_{H,f}$ and $r_{H,b}$ were the forward and backward reaction rates of antibiotic by hydrolysis, respectively, where $k_{H,f}$ and $k_{H,b}$ were forward and backward hydrolysis reaction rate constants, respectively. And C_B and C_C are the concentrations of B and C.

The antibiotic removal rate by photolysis, r_P, was defined as,

$$r_P = k_P C_A \tag{7}$$

Here, k_P was photolysis reaction rate constant.

The antibiotic removal rate by duckweed uptake under dark and illumination conditions, $r_{DW,D}$ and $r_{DW,L}$, were defined as,

$$r_{DW,D} = k_{DW,D}C_A \tag{8}$$

$$r_{DW,L} = k_{DW,L}C_A \tag{9}$$

Here, $k_{DW,D}$ and $k_{DW,L}$ were uptake rate constants under dark and illumination conditions, respectively. Illumination should be essential to maintain plant activity and the different rates under dark and illumination conditions were expected. Furthermore, it was assumed that each removal factor, such as hydrolysis, photolysis or duckweed uptake, was independent of each other.

3.2. Removal of antibiotics in aqueous solution.

Figures 1 shows the experimental results of the concentration changes of CIP and SMX along the operation time at T=303K and $C_{A,0}$=5.00×10^{-5}kmol m^{-3}, as shown by the plots.

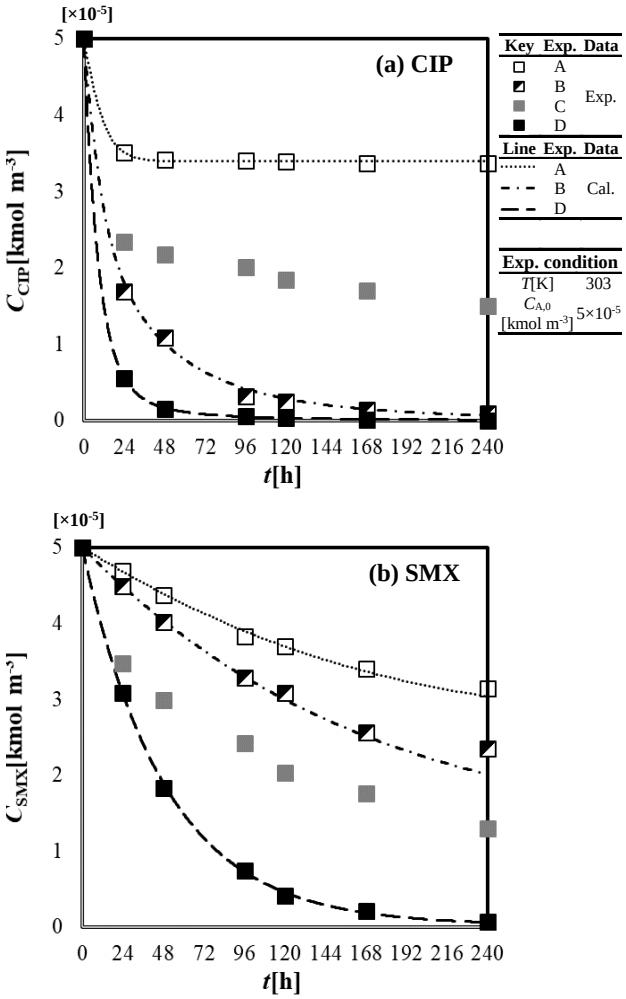

Fig. 1 Antibiotics concentration change along the operation time

For Experiments C and D, duckweed could uptake both antibiotics even in higher concentration ranges. For all cases, the concentration of both antibiotics decreased as the operation time passed, and the removal rates of both antibiotics in Experiment D were the highest. Also, the removal rates of CIP were larger than those of SMX, except in Experiment C.

In the cases of Experiment A, the concentration of CIP, became constant after 48 hours to attain reaction equilibrium, and on the other hand, the concentration of SMX was still kept decreasing after 240 hours, The hydrolysis rate of CIP was larger than that of SMX, and SMX was more stable because of the sulfonic acid structure in the molecular, stable to hydrolyze [6].

In the cases of Experiment B, for both antibiotics, the removal rates were higher than those in Experiment A. Moreover, the removal rate of CIP was significantly higher than that of SMX and SMX was more stable against photolysis. Then, $Y_{CIP,B}$ attained more than 0.97 while $Y_{SMX,B}$ was only around 0.55.

For Experiment C, the effects of hydrolysis and uptake by duckweed under dark condition on antibiotics removal were measured. For both antibiotics, although the concentrations for antibiotics decreased with time continually, the decrease rates of concentration obviously became smaller after the specified period. In the case of CIP, the removal rate in Experiment C was smaller than in Experiment B, which meant photolysis was more effective for CIP removal. On the other hand, while the removal rate of SMX in Experiment C was greater than that in Experiment A and B, $Y_{SMX,C}$ was 0.26 and SMX still remained in a relative high concentration.

For Experiment D, the effects of hydrolysis, photolysis and uptake by duckweed under illumination conditions on antibiotics removal were measured. The concentrations of both antibiotics successively decreased with time, and $Y_{A,D}$ achieved 1.00, showing the antibiotic in the sample solution was unable to detect. In the cases of $C_{A,0}$=1.00×10^{-5}kmol m^{-3}, $Y_{CIP,D}$ and $Y_{SMX,,D}$ achieved 1.00 at 298K and 303K. Even in $C_{A,0}$=5.00×10^{-5}kmol m^{-3}, $Y_{CIP,D}$ attained to 1.00. Duckweed could uptake CIP and SMX effectively under continuously illumination condition. Furthermore, illumination was one of essential point for keep duckweed uptake effectively.

Then, the antibiotic removal model was fitted to the experimental results to obtain the respective reaction rate constants and the obtained rate constants of hydrolysis, $k_{H,f}$ and $k_{H,b}$, photolysis, k_P, and duckweed uptake under light condition, $k_{DW,L}$ are summarized in Table 2, respectively. The estimated concentration changes along the operation time under each experiment are shown in Fig. 1 as lines.

From the results of Experiment A, it was confirmed that the hydrolysis reaction of each antibiotic followed the first-order reaction, as Eq. (6). The $k_{H,f}$ and $k_{H,b}$ of both antibiotics were independent of the initial antibiotics concentration and increased as the operation temperature was elevated Moreover, $k_{H,f}$ for SMX were obviously smaller than CIP, showing SMX had stronger stability for hydrolysis.

In the cases of photolysis, it was confirmed that the photolysis reaction of each antibiotic followed the first-order reaction, as Eq. (7). For both CIP and SMX, k_P was independent of the initial antibiotic concentrations and increased by elevating experimental temperature. SMX also had stronger stability for photolysis due to much smaller k_P of SMX.

In the cases of Experiment C, the model was unable to be fitted to the experiment results and $k_{DW,D}$s were unable to obtain. The suggested model must have been too simple to express the uptake of antibiotics by duckweed under dark condition. On the other hand, the duckweed uptake rate constants under light condition, $k_{DW,L}$s were able to be obtained, and it was confirmed that the duckweed uptake of each antibiotic followed the first-order reaction, as Eq. (9). For both CIP and SMX, $k_{DW,L}$ increased with experimental temperature increasing and decreased with antibiotics initial concentration increasing.

Table 2 Reaction rate constants

CIP					
$C_{A,0}$ [kmol m^{-3}]	**1×10^{-5}/5×10^{-5}**			**1×10^{-5}**	**5×10^{-5}**
T [K]	$k_{H,f}$ [h^{-1}]	$k_{H,b}$ [kmol^{-1}m^{3}h^{-1}]	k_P [h^{-1}]	$k_{DW,L}$ [h^{-1}]	
293	0.55	1.25	0.45	0.99	0.68
298	0.71	1.28	0.68	1.96	1.81
303	0.99	1.30	1.15	2.50	2.06
SMX					
$C_{A,0}$ [kmol m^{-3}]	**1×10^{-5}/5×10^{-5}**			**1×10^{-5}**	**5×10^{-5}**
T [K]	$k_{H,f}$ [h^{-1}]	$k_{H,b}$ [kmol^{-1}m^{3}h^{-1}]	k_P [h^{-1}]	$k_{DW,L}$ [h^{-1}]	
293	0.04	0.02	0.04	0.71	0.30
298	0.05	0.03	0.05	0.90	0.60
303	0.11	0.05	0.06	1.09	0.64

Figure 2 shows the reaction rate constants for each antibiotics removal mechanism under different temperatures. The obtained rate constants of hydrolysis, $k_{H,f}$ and $k_{H,b}$, photolysis, k_P, and duckweed uptake under light condition with different initial concentrations, $k_{DW,L}$ all increased as the the opreating temperature was elevated. On the other hand, $k_{DW,L}$ decreased as initial antibiotics concentration increased because duckweed uptake might be hindered due to the high ratio of antibiotic concentration to duckweed load in the solution. For CIP, all the reaction rate constant were higher than in the cases of SMX, especially for hydrolysis and photolysis. In both cases, $k_{DW,L}$ were relatively higher than $k_{H,f}$ and k_P, which meant duckweed uptake could remove CIP and SMX effectively even in the higher concentrations. Moreover, for SMX, having higher hydrolysis and photolysis stability, duckweed was expected as an effective plant to remediate the water environment.

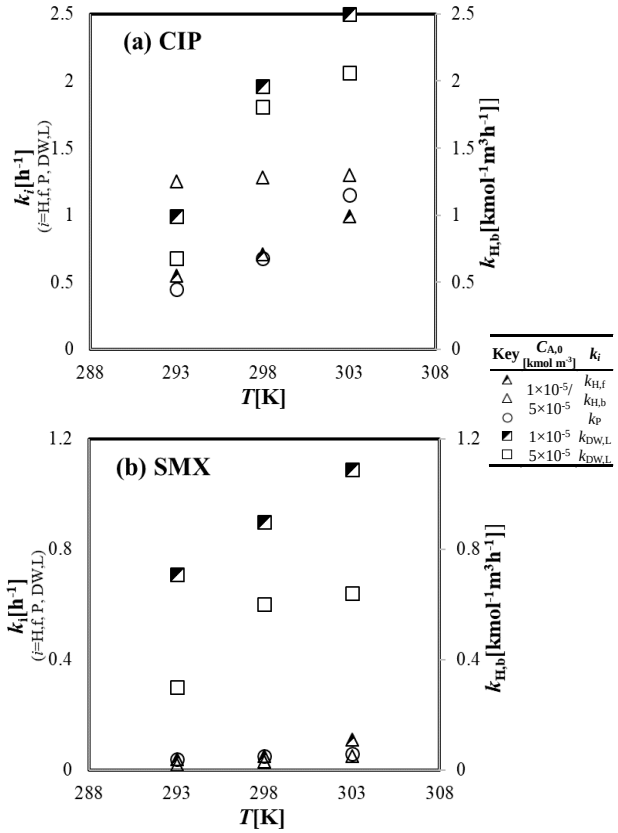

Fig. 2 The reaction rate constants for each antibiotics removal mechanism under different temperatures

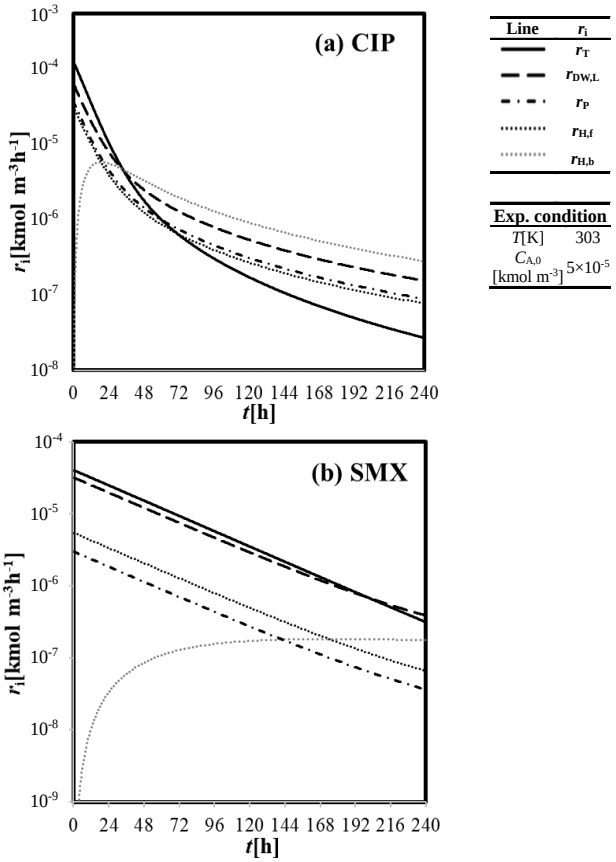

Fig. 3 The contribution of respective mechanisms to total antibiotics removal in Experiment D

Figure 3 shows the contribution of respective mechanisms to total antibiotics removal in Experiment D at T=303K and $C_{A,0}$=5.00×10^{-5}kmol m^{-3}, in which the respective removal rates were estimated with Eqs. (6), (7) and (9) and the reaction rate constants were shown in Table 2. In both cases, $r_{DW,L}$ showed the highest removal rate, which meant duckweed uptake under illumination condition contributed the most to CIP and SMX removal. Moreover, in the case of SMX, $r_{DW,L}$ was much more greater than $r_{H,f}$ and r_P, which meant duckweed uptake was relatively effective in removal of SMX, stable antibiotic relative to hydrolysis and photolysis.

4. Conclusion

In this research, hydrolysis, photolysis and duckweed uptake in the removal of antibiotics were observed. Duckweed uptake shown high possibility in the ciprofloxacin and sulfamethoxazole removal under highly concentration. Moreover, illumination condition is one key issue for duckweed uptake. Higher temperature also could enhance antibiotics removal effective. Furthermore, assumed antibiotics removal models for each mechanism fitted with experiment data greatly. Which shown the possibility on the removal of antibiotics contaminating environmental water using duckweed (*Lemna aoukikusa*).

Nomenclature and Subscript

C [kmol m^{-3}]=concentration of antibiotics
Y [-]= the fractional removal of antibiotics
r [kmol m^{-3}h^{-1}]=removal rate of antibiotics
T [K]=experiment temperature
t [h]=experiment time
k [h^{-1} or kmol^{-1}m^{3}h^{-1}]=reaction rate constant
CIP=ciprofloxacin,
SMX= sulfamethoxazole,
H=hydrolysis,
P=photolysis
DW=duckweed uptake

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