ANTIBIOTICS UPTAKE AND GROWTH OF DUCKWEED *LEMNA AOUKIKUSA* FOR THE TREATMENT OF PHARMACEUTICAL WASTEWATER

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1. Introduction

For several decades, hundreds of antibiotics have been discovered, synthesized and are being used to treat infections in both humans and animals. A huge amount of these pharmaceutical products gets into the environment on a day-to-day basis as human and livestock excretions through sewage disposal systems. Approximately 38% of the sewage produced in India is treated through conventional wastewater treatment plants [1], which are not designed or regulated to remove pharmaceutically active compounds. This has led to the generation of antibiotic-resistant bacteria causing a global threat to humankind. The case in Medak district, Telangana state, South India was reported as a typical case of severe aquatic environmental pollution [2]. Although several water treatment methods have been reported earlier, studies on remediation of such wastewater with high antibiotic concentration are still insufficient. Removal of pharmaceuticals from wastewater using aquatic plants have gained a lot of attention in the recent years as they were found to possess the ability of purifying and taking up pollutants from contaminated water bodies.

The aim of this study is the removal of antibiotic contaminants in the model pharmaceutical wastewater using duckweed species *Lemna aoukikusa*, an aquatic floating plant widely found around the world. The effects of antibiotics on duckweed growth was investigated and the removal of antibiotics due to its decomposition and uptake by duckweed incorporating the growth was studied.

2. Duckweed growth in aqueous solution of antibiotics

2.1. Experimental

Ciprofloxacin ($C_{17}H_{18}FN_3$, molar mass: 331.35 kg kmol^{-1}, CIP, broad spectrum, fluoroquinolone antibiotic) and Sulfamethoxazole ($C_{10}H_{11}N_3O_3S$, molar mass: 253 kg kmol^{-1}, SMX, broad spectrum, sulphonamide antibiotic) were selected as target antibiotics because they were the most widely detected in the polluted environment water in India [3]. These antibiotics were of analytical grade and were purchased from FUJIFILM Wako Pure Chemical Corporation.

Duckweed (*Lemna aoukikusa*) was purchased from the local market and was cultured for 2 weeks in a water bath maintained at 298 K and provided with continuous illumination of PPF=650±50 μmol s^{-1} m^{-2} by a metal halide lamp (Eye HID LAMP, 400W IWASAKI ELECTRIC CO. LTD.). It was then carefully cleaned thrice with deionized water and used in the following experiments.

The experimental conditions of the growth study are represented in Table 1. The addition of antibiotics in the aqueous solution and illumination were changed to study the effects of them on the growth of duckweed for 15 days. Under dark condition (without illumination), the samples were covered with a double layer of aluminum foil to avoid any light penetration. Under light condition (with illumination of PPF =650 μmol s^{-1} m^{-2}), samples were covered by transparent paraffin sheet with holes to allow light to penetrate, to avoid significant evaporation of the growth medium and for ensuring duckweed activity. The growth was measured by counting the number of fronds and the biomass was weighed at several time intervals.

2.2. Results and discussion

Since the mass of duckweed, *m*, was almost proportional to the number of fronds, *n*, under all conditions in the range of this work as,

$$m = 0.040n \quad (1)$$

the growth was studied by the number of the fronds.

Fig. 1 represents the effect of PPF (illumination) and antibiotic in the aqueous solution on the number of duckweed fronds, Vn. In all cases, at first Vn increased with time until around t =200 h and after that deceased. This may be due to the lack of nutrients and the toxic effects of the antibiotic in the solution. Vn in the cases with illumination (PPF = 650 μmol s^{-1} m^{-2}) were considerably larger than those without illumination. The illumination enhanced the growth of the duckweed. Vn with antibiotics in the solution were smaller than those without antibiotics. Antibiotics disturbed the duckweed growth and SMX was more toxic to duckweed than CIP.

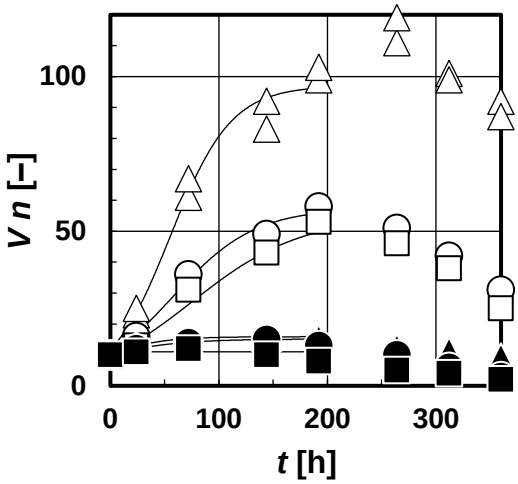
The duckweed growth was correlated using the function of logistic curve,

$$Vn = \frac{c}{1+ae^{-bt}} \quad (2)$$

where *a*, *b*, and *c* are the constants in the function and were determined in order to fit the function into the

Table 1 Experimental conditions of duckweed growth in aqueous solution

V_0 [m^3]	250×10^{-6}
n_0 [m^{-3}]	0.04×10^{6}
Antibiotic (A)	ciprofloxacin (CIP), sulfamethoxazole (SMX)
$C_{A,0}$ [kmol m^{-3}]	0, 5×10^{-5}
PPF [μmol s^{-1} m^{-2}]	0, 650±50
Temperature [K]	298



Keys	PPF [μmol s^{-1} m^{-2}]	A, $C_{A,0}$ [kmol m^{-3}]
▲	0	0
●	0	CIP, 5×10^{-5}
■	0	SMX, 5×10^{-5}
△	650±50	0
○	650±50	CIP, 5×10^{-5}
□	650±50	SMX, 5×10^{-5}
——	(correlation)	

Fig. 1 Effects of PPF and antibiotic in aqueous solution on duckweed growth

experimental results. As this function doesn't take the death of duckweed into account, only the results under t < 200 h were correlated. The results of the correlation are also shown in Fig. 1. The function expressed the results well with empirically determined constants.

3. Antibiotic removal from aqueous solution with duckweed

3.1. Experimental

The fractional removal of antibiotics, R_A, was defined as,

$$R_A = \frac{V_0 C_{A,0} - V C_A}{V_0 C_{A,0}} \quad (3)$$

and this was used with the assumption of V=V_0.

The principle experimental conditions of antibiotic removal in aqueous solution are listed in Table 2. The effects the antibiotic uptake by duckweed on overall removal and the illumination on the uptake were examined varying the addition of duckweed and the illumination. High Performance Liquid Chromatography (SPD-10AVP, SHIMADAZU CORPORATION, HPLC) was used for determining the concentration of antibiotic in the solution and the analysis conditions were referred to the Japanese Pharmacopoeia [4].

3.2. Results and discussions

It was assumed that antibiotics in the aqueous solution decomposed and removed by hydrolysis, photolysis, and uptake by duckweed as,

$$r_T = -\frac{dC_A}{dt} = r_H + r_P + r_{DW} \quad (4)$$

Here, r_T, r_H, r_P, and r_{DW} are the total removal rate, hydrolysis rate, photolysis rate, and the rate of uptake by duckweed, respectively. It was also assumed that these phenomenon, hydrolysis, photolysis, and duckweed uptake, presented independent and did not affect one another.

Table 2 Experimental conditions of antibiotic removal with duckweed

V_0 [m^3]	250×10^{-6}
Antibiotic (A)	ciprofloxacin (CIP), sulfamethoxazole (SMX)
$C_{A,0}$ [kmol m^{-3}]	5×10^{-5}
n_0 [m^{-3}]	0, 0.04×10^6
PPF [μmol s^{-1} m^{-2}]	0, 650±50
Temperature [K]	298

The hydrolysis of antibiotic was assumed to be a reversible reaction expressed as,

$$A \underset{k_{H,b}}{\overset{k_{H,f}}{\rightleftarrows}} B_1 + B_2 \quad (5)$$

$$r_H = r_{H,f} - r_{H,b} = k_{H,f} C_A - k_{H,b} C_{B_1} C_{B_2} \quad (6)$$

where A, B_1 and B_2 are the antibiotic and products of hydrolysis. The $r_{H,f}$, $r_{H,b}$, $k_{H,f}$, and $k_{H,b}$ are forward, backward reaction rates of antibiotic removal by hydrolysis, forward, and backward hydrolysis rate constants, respectively.

The photolysis was assumed to be an irreversible first order reaction expressed as,

$$A \overset{k_P}{\rightarrow} D_1 + D_2 \quad (7)$$

$$r_P = k_P C_A \quad (8)$$

where D_1, D_2 are the products of photolysis, and k_P is the photolysis rate constant.

The uptake by duckweed was also represented by an irreversible first-order reaction as,

$$A(\text{solution}) \overset{k_{DW}}{\longrightarrow} A(\text{duckweed}) \quad (9)$$

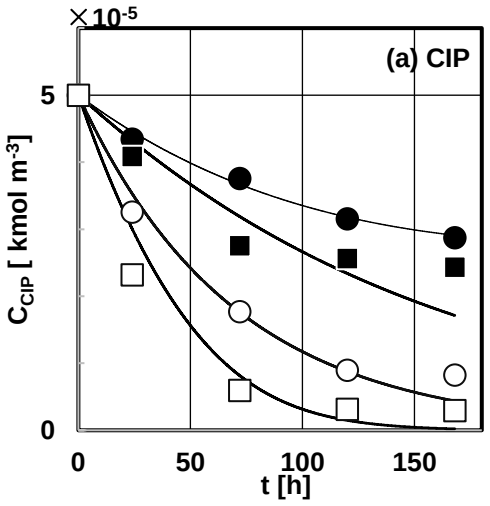
$$r_{DW} = k_{DW} V n C_A \quad (10)$$

where k_{DW} is uptake rate constant per unit volume.

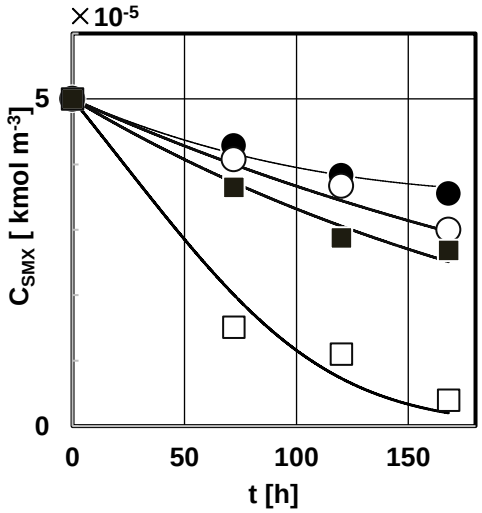
Fig. 2 shows the experimental results of the concentration changes of antibiotics over time. In all runs, even without illumination or duckweed, C_A decreased with time. C_A in the cases with illumination were lower than those without illumination. Duckweed in the solution further lowered C_A. These results confirmed that antibiotics in the solution could be removed by uptake by duckweed as well as hydrolysis and photolysis and the uptake was enhanced by illumination. C_{SMX} was higher than C_{CIP} in the case without illumination and duckweed as SMX was more stable because of the sulfonic acid structure in the molecule which is stable against hydrolysis [5]. At the end of the run, R_{CIP} was 0.43 and R_{SMX} 0.29. In the case with illumination and without duckweed, C_{SMX} was higher than C_{CIP} as it was more difficult to decompose or remove SMX than CIP as SMX was more stable against photolysis. At the end of the experiment, R_{CIP} reached 0.83, while R_{SMX} was 0.4.

When the experiment was conducted with duckweed under dark conditions the metabolism of the duckweed species has been affected, and hence decrease in the antibiotic concentration was very small after the initial few days. At the end of the run, R_{CIP} was 0.51 and R_{SMX} was 0.46.

When both illumination and duckweed was present,



(a)



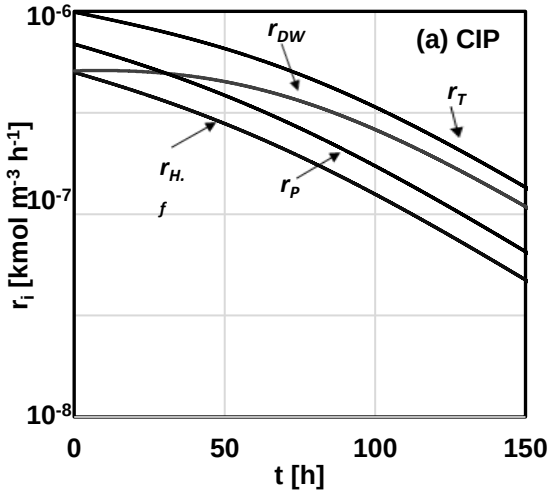
(b)

Keys	PPF [μmol s^{-1} m^{-2}]	n_0 [m^{-3}]
●	0	0
■	0	0.04×10^6
○	650±50	0
□	650±50	0.04×10^6
——	estimation using model	

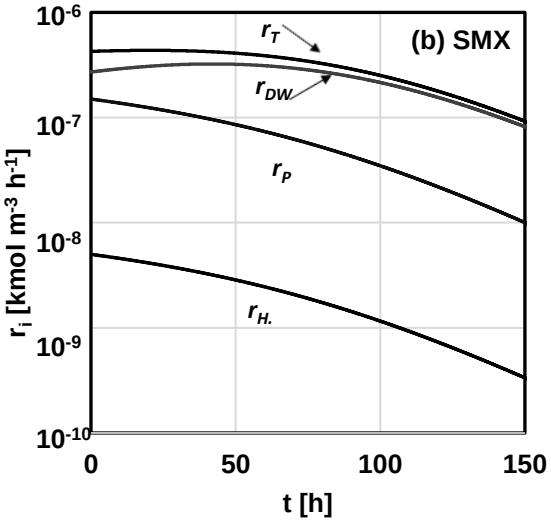
Fig.2 Effects of PPF and duckweed in aqueous solution on time courses of C_A: (a) A=CIP (ciprofloxacin); (b) A=SMX (sulfamethoxazole)

decrease in the antibiotic concentration was the highest. At the end of run, both R_{CIP} and R_{SMX} reached 0.94 and 0.92, respectively. SMX was found to be more toxic to the duckweed species, difficult to hydrolyze and photolyze. Duckweed could uptake CIP and SMX effectively under illumination condition showing that light is essential for duckweed uptake to occur.

The relation represented by Eqs (2), (4), (6), (8), and (10) were fit into the experimental results in Fig. 2. The $k_{H,f}$ and $k_{H,f}$ could be estimated with the results under PPF=0 μmol s^{-1} m^{-2} and n_0=0, *i.e.*, r_P=0 and r_{DW}=0 in Eq.(4). k_P was estimated with $k_{H,f}$, $k_{H,f}$, and the results under PPF=650 and n_0=0, *i.e.*, r_{DW}=0. Similarly, k_{DW} without illumination, $k_{DW,0}$ could be estimated with $k_{H,f}$,



(a)



(b)

Fig. 3 The time courses of removal rates where n_0 =0.04×10^6 m^{-3} and PPF=650±50 μmol s^{-1} m^{-2}

$k_{H,f}$, and the results under PPF=0 μmol s^{-1} m^{-2} and n_0 =0.04×10^6 m^{-3}. Finally, k_{DW} with illumination, $k_{DW,650}$, was estimated using the results under PPF=650 μmol s^{-1} m^{-2} and n_0 =0.04×10^6 m^{-3}. The estimated rate constants are summarized in Table 3.

Table. 3 Reaction rate constants

A	CIP	SMX
$k_{H,f,A}$ [h^{-1}]	0.005	0.003
$k_{H,b,A}$ [h^{-1} m^3 kmol $^{-1}$]	0.0035	0.004
$k_{P,A}$ [h^{-1}]	0.0095	0.0001
$k_{DW,A,0}$ [h^{-1} kg-DW^{-1}]	2.5	2.5
$k_{DW,A,650}$ [h^{-1} kg-DW^{-1}]	12.5	13.8

The estimated concentration changes along the operation time under each experiment are also shown in Fig. 2 as lines. The removal models with the obtained constants could fully estimate the experimental results. $k_{H,f}$ for SMX was smaller than CIP, showing SMX had

stronger stability against hydrolysis. SMX had stronger stability against photolysis which is understood by a much smaller k_P.

Fig. 3 shows the time courses of removal rates where $n_0 = 0.04 \times 10^6$ m^{-3} and PPF=650 μmol s^{-1} m^{-2}. The respective removal rates were estimated with Eqs. (4), (6), (8) and (10). In both cases of CIP and SMX, r_{DW} under light conditions showed the highest removal rate, which meant that duckweed uptake was the most effective in removal of CIP and SMX at the given concentration. Moreover, for SMX, having higher hydrolysis and photolysis stability, duckweed uptake can be considered as an effective method for remediation.

4. Conclusion

The growth of duckweed was enhanced by illumination. While, antibiotics in the aqueous solution hindered the growth and SMX hindered more than CIP, the duckweed could alive for around 8 days. The antibiotics in the aqueous solution could be removed by uptake by duckweed, as well as hydrolysis. The rate of ciprofloxacin uptake was higher than that of sulfamethoxazole. The antibiotic uptake by duckweed was enhanced by illumination. The uptake by duckweed governed the removal of antibiotics. Accordingly, the treatment using duckweed *Lemna aoukikusa* was proposed as a potential technique to remove antibiotics from the contaminated water.

Nomenclatures

C_A = Concentration of antibiotic in aqueous solution [kmol m^{-3}]
k = Reaction rate constant [h^{-1}]
n = Number of duckweed fronds per unit volume of aqueous solution [m^{-3}]
PPF= Photosynthetic photo flux [μmol s^{-1} m^{-2}]
r = Removal rate of antibiotics [kmol m^{-3} h^{-1}]
V = Volume of aqueous solution [m^3]
<Subscript>
CIP= Ciprofloxacin
DW= Duckweed uptake
H= Hydrolysis
P= Photolysis
SMX= Sulfamethoxazole

References

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