Phytoremediation of aquatic environment polluted by antibiotics

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1. Introduction

Various kinds of antibiotics have been widely used for both human beings and animals to get rid of the bacteria. However, in India, pharmaceutical company discharged wastewater produced during production process with high concentration antibiotics [1-4]. This may cause 2 main problems: the increase of antibiotic-resistant bacteria and the sediment in animals (especially in aquatic animals) and plants through irrigation and soil [5]. Antibiotics being accumulated in animals and plants will enter human body and will lead to the ineffectiveness during a surgery or cure.

Since the last decades, ponds of duckweed(Lemna minor) has been utilized in the removal of nutrients and organic compounds successfully. Then, the utilization of duckweed has been studied in the removal of the organic compounds in water.

This study aimed the treatment of aqueous solution contaminated by antibiotics with duckweed. First, removal of antibiotics in the aqueous solution by the duckweed was measured. Then, the effects of operating conditions on the antibiotics removal was studied to discuss the mechanism of antibiotics removal.

2. Experimental

2.1 Experiment materials

Two kinds of antibiotics were selected, ciprofloxacin (CIP), and sulfamethoxazole (SMX), which are detected in the polluted water in India. Ciprofloxacin is a member of fluoroquinolone that share a bicycle core structure related to the 4-quinolone. Sulfamethoxazole belongs to sulfonamide group. In this research, antibiotics are purchased from Wako Pure Chemical Industries. Ltd. The structure and other chemical data are shown as below in Table 1.

Table 1 Structure and molar mess for each antibiotic

	Ciprofloxacin	Sulfamethoxazole
Formula	$C_{17}H_{18}FN_3 \cdot HCl \cdot H_2O$	$C_{10}H_{11}N_3O_3S$
Molar mass	385.82	253.28

Duckweed were purchased and grown under a specific condition. Temperature of water was monitored and controlled at 24 ± 0.5℃(297.15 ± 0.5K). Illumination was supplied by a metal halide lamp (Eye HID LAMP, 400W IWASAKI ELECTRIC CO. LTD.), which could provide 680μmol/m^2s. Illumination was provided 16 hours per day. In order to reproduce duckweed, aeration was provided by an air pump (4.5W NISSO CORPORATION) for 24 hours per day. Duckweed cultures were grown for 4 weeks before experiments.

2.2 Methods

The wet mass of duckweed was measured after wipe off the surface of random amount of duckweed. Then the duckweed was rdied in a dryer at 110℃(383.15K) for 24 hours, and the dried mass was measured, too.

Experiment A~D were set to investigate the efficiency of each mechanism and the efficiency of duckweed in the removal process. Experiment conditions were set as described in Table 2. Hydrolysis was conducted in experiment A, hydrolysis and photo-degradation were conducted in experiment B. Sorption, uptake, hydrolysis and uptake were conducted. Under a condition with light, mechanisms conducted in the removal will be hydrolysis, photo-degradation and sorption with uptake by plant. This condition was considered as Condition 1.

Experiment D was set to investigate the effectiveness of hydrolysis and plant under the condition without light. In this case, the mechanisms related to the removal will be hydrolysis, sorption and uptake under no-light condition(Condition 2).

All experiments were set triplicate to avoid accidental mistake, and calculation were based on the average of each kind of data. Relationship between each experiment and the mechanism conducted was shown in Table 3.

2.3 Experiments and analytic methods for the removal mechanisms investigation

Table 2 Experiment conditions for experiment A~D

	A	B	C	D
Weight of duckweed(g)	×	×	3.00	3.00
Illumination	×	○	○	×
Antibiotics in feed	CIP/SUL	CIP/SUL	CIP/SUL	CIP/SUL
Concentration of feed (C_0 mol/L)	5.00×10^{-5} /1.00×10^{-5}	5.00×10^{-5} /1.00×10^{-5}	5.00×10^{-5} /1.00×10^{-5}	5.00×10^{-5} /1.00×10^{-5}
Amount of feed (mL)	80	80	80	80
Growth medium (amount mL)	4.00×10^{-3}	4.00×10^{-3}	4.00×10^{-3}	4.00×10^{-3}
Time(hrs)	168	168	168	168

Table 3 Mechanism involved in each experiment and the calculation method for their effectiveness

	A	B	C	D
Hydrolysis	○	○	○	○
Photo-degradation	×	○	○	×
Uptake by plant	×	×	○	○*

C_0 was the concentration of feed solution. Based on the calibration curve and the formula prepared before experiments, by scanning the sample from each flask, C_A ~C_C (concentration of antibiotic in sample) could be calculated. Therefore, C_0 - C_A (mol/L) is the amount that hydrolysis worked. The rest can be done as the same: C_A–C_B is the work by photo-degradation, and C_B –C_C is the work by duckweed.C_A-C_D is the work by plant under no-light condition.

As a hypothesis of these experiments and the calculation, we consider there's no relation between mechanisms, which means the effect of each mechanism only based on the experimental condition being set. And the so-called 'efficiency' for hydrolysis, photo-degradation and plant(uptake) will be calculated by the ratio of the concentration gap and the concentration of feed (C_0).

3. Results and Discussion

3.1 Moisture content of Duckweed

The moisture content of duckweed was estimated with the

following equation:

$$Moisture\ content = 1 - \frac{Dried\ mass}{\text{Wet mass}}$$

Experiment were conducted 9 times. Data is shown below in Table 4.

Table 4 Experiment result for moisture content

Group	Wet mass (g)	Dried mass (g)	Moisture content
1	5.61	0.63	0.89
2	4.15	0.43	0.90
3	3.88	0.41	0.89
4	4.20	0.42	0.90
5	4.01	0.43	0.89
6	2.94	0.33	0.89
7	3.51	0.40	0.89
8	2.66	0.33	0.88
9	3.20	0.37	0.88

By calculating the average of all the moisture content, the average moisture content is around 89%.

3.2 Removal Mechanisms

All results of efficiency is based on the formula described below.

Table 5 Formula for the calculation of efficiency

	$E_{hydrolysis}$	$E_{photo-degradation}$	E_{plant}	$E_{plant(NL)}$
Formula	$\frac{C_0 - C_A}{C_0 - C_C}$	$\frac{C_A - C_B}{C_0 - C_C}$	$\frac{C_B - C_C}{C_0 - C_C}$	$\frac{C_B - C_C}{C_0 - C_D}$

When the concentration of feed (C_0) is 5×10^{-5}mol/L, the result is shown in Table 6.

Table 6 Experiment result (C_0=5×10^{-5}mol/L)

Condition 1	C_{cip} (10^{-5}mol/L)	E_{cip}	C_{sul} (10^{-5}mol/L)	E_{sul}
Experiment A	4.49	0.15	4.69	0*
Experiment B	3.00	0.45	4.94	0.05*
Experiment C	1.70	0.40	3.77	0.95
Condition 2	C_{cip} (10^{-5}mol/L)	E_{cip}	C_{sul} (10^{-5}mol/L)	E_{sul}
Experiment A	4.49	0.17	4.69	0.13
Experiment D	1.97	0.83	2.57*	0.87

At this concentration, both the ciprofloxacin and the sulfamethoxazole group, part of the duckweed died after 2 weeks. At this concentration, duckweed's metabolism was influenced.

In the case of ciprofloxacin, hydrolysis and photo-degradation showed their effectiveness in the removal process. Together, hydrolysis and photo-degradation occupied 60% of the ciprofloxacin being removed. Uptake and sorption by plant did benefit the removal by taking up 40% of the work.

On the other hand, in the case of sulfamethoxazole, duckweed showed its effectiveness in the process. Uptake and sorption by plant maintained 95% of the decrease in concentration. Sulfamethoxazole is stable towards hydrolysis and photo-degradation but could be removed by plants during a period, mainly because sulfamethoxazole has sulfonic acid as a part of it, which can make sulfamethoxazole stable towards hydrolysis.

In condition 2, we could find out no matter what the antibiotics is in the feed, plant could still remove antibiotics from water. Though the effect is somehow weaker than under condition 1.

When the concentration of feed is 1×10^{-5}mol/L for both antibiotic, the result is as described in Table 5.The calculation is based on the formula described in Table 5, results are showed as the ratio of work done by each mechanism (D-value) and the concentration of feed.

Table 7 Experiment result (C_0=1×10^{-5}mol/L)

Condition 1	C_{cip} (10^{-5}mol/L)	E_{cip}	C_{sul} (10^{-5}mol/L)	E_{sul}
Experiment A	0.77	0.31	1.01	0
Experiment B	0.45	0.43	0.79	0.21*
Experiment C	0.26	0.26	1.24	*
Condition 2	C_{cip} (10^{-5}mol/L)	E_{cip}	C_{sul} (10^{-5}mol/L)	E_{sul}
Experiment A	0.77	0.50	1.01	0
Experiment D	0.54	0.50	0.67	1.00

Ciprofloxacin tends to collapse under hydrolysis and photo-degradation, and could be removed by duckweed. Compared to former experiment, the antibiotic remained in the solution is less, decreased from 0.34 to 0.26. In condition 2, hydrolysis share the same efficiency with sorption and uptake. But obviously, plant showed a low efficiency under no-light condition.

The result of sulfamethoxazole at this concentration is complex. Again, sulfamethoxazole was stable towards hydrolysis. Sulfamethoxazole collapsed under photo-degradation, account for 21% of the sulfamethoxazole been removed. Data for E_{uptake} could not be calculated, because the concentration 'increased', which is normally impossible. The reason is that in condition 2, sulfamethoxazole decreased from 1×10^{-5}mol/L to 0.67×10^{-5}mol/L . Based on experience in this research, the 'increase' may be considered as no change in concentration.

There are several reasons for this result. The first reason is about the metabolite from duckweed during the experiment. As mentioned in 2.5, to avoid the influence from the metabolite, we have set a control group. And in the calculation, data from this group were being subtracted from the absorbance of group C. But still, individual difference of plant may exist. Furthermore, the spectrometer could only measure the absorbance of sample but not distinguish compounds in the sample as a Liquid Chromatography, which means is the sample is a mixture, the absorbance may lead to a 'increase' in the signal and in the data.

4. Conclusion

In this research, we observed and calculated the effectiveness for each mechanism in the removal of antibiotics from water. Ciprofloxacin could be removed by hydrolysis, photo-degradation and uptake by duckweed in both concentration. On the other hand, remove of sulfamethoxazole is difficult. Because it showed stability towards these 3 mechanisms. Among all the mechanisms, the participation of duckweed displayed its potential in removing sulfamethoxazole. However, at feed concentration 1×10^{-5}mol/L, effect is still questionable, which may need new analytical methods or different analyze machine based on other theory like LC(liquid chromatography).

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