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In-situ removal of residual antibiotics (enrofloxacin) in recirculating aquaculture system: Effect of ultraviolet photolysis plus biodegradation using immobilized microbial granules

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ABSTRACT

The misuse of antibiotics and ineffective treatment in aquaculture cause serious environmental problems. The closed-loop design of recirculating aquaculture systems (RAS) enables the effective use of antibiotics and rapid removal of antibiotic residues. Hitherto, few studies have been carried out on these. This study aims to examine the feasibility of rapid in-situ removal of residual antibiotics in RAS using integrated process and characterize the operational parameters. The fate of the selected antibiotic enrofloxacin (ENR) in different units of a laboratory-scale RAS was investigated with photolysis pretreatment followed adsorption-biodegradation of photolysis intermediates by immobilized microgranules. The degradation kinetics was also characterized. The results indicated that the ultraviolet (UV) device in RAS played an important role in ENR removal. The wavelength and light intensity were crucial for the removal efficiency of ENR. The F^- produced by defluorination could reproduce the effect of the decomposition of ENR by UV photolysis. The UV photolysis products of ENR had a positive relationship with photolysis time, indicating incomplete decomposition of ENR. Under optimal condition (UV 80 W/254 nm and flow rate of 60 L/h), the ratio of BOD_5 to COD (B/C) after photolysis increased from 0.041 to 0.28, which supported biodegradation. The photolysis products were partially adsorbed by activated carbons in the immobilized microbial granules and then biodegraded by microbes of the granules. Based on this study, a four-step process was proposed for the control of residual antibiotics in RAS.

1. Introduction

Aquaculture, with its continually increasing products, has become an important source of food worldwide (Naylor et al., 2021). It faces the challenge of disease control, especially occurring in high-density farming (Naylor et al., 2021). Antibiotics are an effective means of disease control in aquaculture. However, the environmental problems associated with antibiotic pollution due to the misuse of antibiotics (Chen et al., 2020) and ineffective treatment of residual antibiotics in aquaculture (Krkosek, 2010) have received widespread attention. Major developed countries, such as Europe (Rosa et al., 2019) and the United States (Love et al., 2020), have prohibited the use of antibiotics in aquaculture while many countries, especially developing countries, still continue to use antibiotics due to the lack of stringent control measures (Defoirdt et al., 2011). In recent years, China, which accounts for more than 60% of the total aquaculture production worldwide (FAO, 2018), has been imposing restrictions to antibiotics.

To date, antibiotics are still an effective means of disease control in high-density farming, and it is unrealistic for the traditional aquaculture industry to completely eliminate the use of antibiotics (Smith, 2012). The use of antibiotics is expected to continually increase in the aquaculture industry, following the intensive development in the developing countries (Klein et al., 2018). Hence, it is vital to stress on the scientific use of antibiotics and propose effective treatment of residual antibiotics in the aquaculture industry, rather than to ban the use of antibiotics.

The recirculating aquaculture system (RAS) is considered as a sustainable approach compared to the conventional aquaculture system (Ahmed et al., 2021). The aquaculture mainly established in ponds, lakes, reservoirs, and offshore ponds is an open system, which makes it difficult to effectively control antibiotic pollution (Ottinger et al., 2016). While RAS adopts a land-based aquaculture mode and is equipped with water treatment units, such as biological filters, ultraviolet disinfection,

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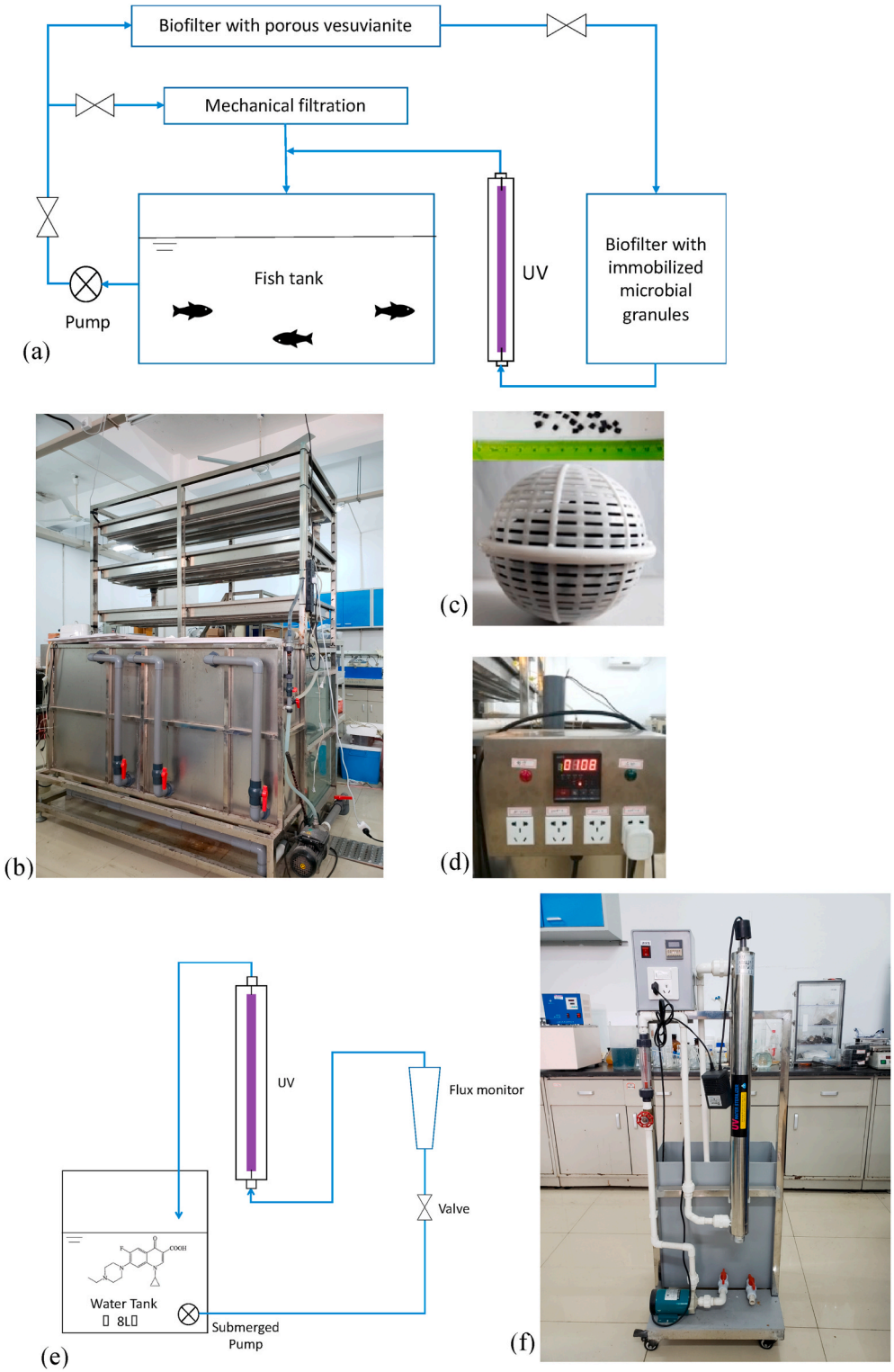


Fig. 1. Representation of recirculating aquaculture system: (a) system schematic; (b) photograph of the recirculating aquaculture system; (c) IMG in biofilter; (d) ultraviolet control equipment; (e) UV system schematic; (f) UV system.

and other equipment. This facilitates in situ removal of pollutants to achieve the desired water quality (Schreier et al., 2010). The closed-loop design of the system can enable the effective use of antibiotics and efficiently eliminate the residues (Wang et al., 2018), which are of less concern. RAS has a nominal hydraulic volume, a lower dose of antibiotics is sufficient to achieve the desired antibacterial activity, thereby efficiently utilizing antibiotics (Alexandrino et al., 2017). However, their utilization needs an appropriate approach. Moreover, the residual antibiotics should be removed rapidly by the equipped water treatment unit to avoid the negative effect on the quality and safety of the aquatic products, and the bacteria of the biofilter unit due to the accumulation of antibiotics in the water, and the generation of resistance genes.

Currently, studies on the fate of antibiotics in RAS are limited. Most studies focus on the residues of antibiotics in fish bodies, sediments, and

biological filters in RAS. Meinertz et al. (2014) studied the residue of florfenicol (FFC) in fish at different concentrations and temperatures, and the effect of antibiotic input on the function of biological filters. Bebak et al. (2002) investigated the changes in oxytetracycline in water, sediment, and biological filters. Leal et al. (2018) showed that in the existing RAS system, adding antibiotics can inhibit the activity of microbial flora on biological filters, thus reducing its purification capability and deteriorating the water quality. Liu et al. (2020) concluded that the antibiotic resistance genes (ARGs) generated may penetrate the biofilter, which causes propagation, prevalence, and dissemination of ARGs. No report has been observed for the control of antibiotics by engineering approach in RAS.

The effective control of antibiotics is critical for the development of RAS. Antibiotic removal methods can be categorized into physical, physicochemical, and biological methods which have been tested or used for wastewater treatment.

Physical adsorption, with mild action and no toxic substances produced, is regarded as a fast and efficient method for treating antibiotics. Currently, activated carbon is the most common adsorbent for antibiotics, and the adsorption efficiency obtained by research is relatively satisfactory (Yang et al., 2019). Mendez-Diaz et al. (2010) studied the bulk adsorption of trimethoprim on activated carbon with imidazoles and sulfonamides, and obtained an adsorption efficiency of approximately 90%. In general, the adsorption and removal effect of enrofloxacin by activated carbon under neutral pH condition is satisfactory (Fu et al., 2017). However, physical adsorption may face the risk of secondary pollution due to desorption.

Physicochemical methods, especially photolysis, can effectively remove antibiotics via decomposition. Most antibiotics have photolysis characteristics when exposed to light, especially the ultraviolet light, such as the research by Ding et al. (2020) and Batchu et al. (2014). The efficiency of photolysis varies among antibiotics and is affected by factors such as light intensity, water quality, wavelength, and pH. Sturini et al. (2012) investigated the photolysis mechanism and dynamics of enrofloxacin. Enrofloxacin has a shorter photolysis half-life in mid-summer all year round and at low latitudes (Burhenne et al., 1997), indicating the significant effect of light intensity on photolysis efficiency. Enrofloxacin has the best UV absorption effect in a neutral pH environment, where it exists in the zwitterionic state (Snowberger et al., 2016). Ge et al. (2010) investigated the effect of wavelength on the ultraviolet absorption of the quinolone antibiotic sarafloxacin (SAR), and the best range of wavelength was 250–280 nm. The RAS is equipped with an ultraviolet disinfection unit; hence, ultraviolet photolysis can be used to oxidize and decompose antibiotics to reduce its environmental toxicity. In general, the decomposition products through UV photolysis still retain excess of active ingredients, which is harmful to the environment. For example, the photolysis of enrofloxacin produces ciprofloxacin (CIP) (Snowberger et al., 2016), which is an environmentally hazardous quinolone antibiotic. Therefore, UV photolysis of antibiotics can be considered as a pretreatment, which assists in further degradation.

Biodegradation is performed poorly for antibiotic treatment without pre-treatment because most antibiotics are persistent to biodegrade and exhibit inhibition to microbial flora in the biological treatment unit (Klaver and Matthews, 1994). For example, the biodegradation efficiency of enrofloxacin is considerably affected by the concentration of antibiotics, and has low removal efficiency (Almeida et al., 2019). Alexandrino et al. (2017) found that at increasing concentrations of enrofloxacin, its degradation rate in the culture medium decreased, with a removal rate of 40%–55%. However, antibiotics removal can be enhanced by selecting antibiotics-degrading microorganisms, such as wood-rotting fungi (Martens et al., 1996). In addition, if pretreated through chemical methods, such as photolysis, it is a cost-effective method that completely decomposes the intermediates, owing to their bio-based nature (Ding et al., 2020). Yan et al. (2013) found that after ultraviolet photolysis, biodegradation efficiency of quinolone antibiotics was significantly improved due to considerably improved microbial activity. In general, the concentration of antibiotics in aquaculture systems is low, and strategies for enriching antibiotic-degrading bacteria are a major technical concern. In this context, the use of immobilized microorganisms can effectively retain the microbial strains in the system while ensuring a high bioprocessing efficiency and reducing the effects of toxic substances on the microorganisms (Dong et al., 2011). The immobilization approach is expected to effectively improve the biodegradation efficiency of antibiotics in aquaculture systems by retaining antibiotics-degrading microbes.

To achieve rapid removal of residual antibiotics in RAS, an integrated process combining physical, chemical, and biological methods would be favorable. Antibiotics can be pretreated with photolysis to enhance biodegradability. The residual products are then removed via biodegradation and adsorption. A integrated process can significantly enhance the removal efficiency of antibiotics.

The aims of this study are to examine the feasibility of rapid in-situ removal of residual antibiotics in RAS using above proposed integrated process and characterize the operational parameters. A laboratory-scale RAS system was installed, consisting of a fish tank, a mechanical filtration unit, two biofilter units, and an ultraviolet disinfection unit. The levels of the selected antibiotic enrofloxacin (ENR) was monitored in each unit. In this regard, the effect of photolysis conditions on the efficiency and photolysis of intermediate products was characterized. After pretreatment, immobilized microbial granules with activated carbon were used to adsorb and biodegrade photolysis intermediate products with their kinetic characterization. Based on the results, an application process including UV photolysis, adsorption and biodegradation of ENR was proposed.

2. Materials and methods

2.1. Setup of recirculating aquaculture system

The setup of the RAS was shown in Fig. 1(a–d). It consists of a fish tank, a mechanical filtration unit (with porous sponge media), a biofilter with porous vesuvianite, a biofilter with immobilized microbial granules, and an ultraviolet disinfection unit.

The fish tank (196 cm length, 70 cm width, and 100 cm height) with a water volume of 823 L was stocked with 12 crucian carp (*Carassius auratus*). The average weight of the crucian carp was approximately 300 g. The mechanical filtration unit was equipped with a porous sponge made of polyester fiber (30 cm length, 20 cm width, and 2 cm height) via the internal cycle, which was used for the removal of fish sludge (faeces and residual feed). The biofilter with a three-layer filter tank (196 cm length, 70 cm width, and 10 cm height) filled with porous vesuvianite was used to intercept the residual particulate matter and remove organic matter. A biofilter (196 cm length, 32 cm width, and 100 cm height, water volume of 564 L) filled with immobilized microbial granules (IMG) aided in the attachment and growth of nitrifying bacteria that convert ammonia to nitrate (Dong et al., 2011). The IMGs, which contained microbial culture, bamboo-based powdered activated carbon and waterborne polyurethane gel (Tabassum et al., 2018), were packed into a spherical porous PVC material (Fig. 1c) and were operated as a moving-bed biofilm reactor (MBBR). The UV disinfection unit operating at a wavelength of 253.7 nm was used to kill pathogenic bacteria in water. The flow rate of water in the system was 350 L/h, and the total hydraulic retention time was approximately 4.6 h.

2.2. Preparation of the bait with ENR

In this study, ENR was added as a mixed fish bait. ENR, a specialized veterinary drug, is currently one of the few fluoroquinolone drugs approved for use in animal feed, with molecular formula $C_{19}H_{22}FN_3O_3$. The fluorine atom, located at the sixth position of ENR effectively enhances its antibacterial effect, whereas the cyclopropyl located at the

Table 1
The kinetic parameters under different reaction conditions.

Photolysis Conditions	C_0 (mg/L)	k_1 (min^{-1})	half-life period (min)	R^2
40 W/254 nm/40 L/h	9.91	0.134	5.17	0.93
	9.62	0.149	4.66	0.89
40 W/254 nm/60 L/h	9.86	0.145	4.79	0.91
	9.62	0.137	5.06	0.80
40 W/254 nm/80 L/h	10.18	0.120	5.76	0.67
40 W/254nm/100 L/h				
40 W/254nm/160 L/h				
40 W/254 nm/60 L/h	9.62	0.149	4.66	0.89
	9.90	0.164	4.21	0.99
80 W/254 nm/60 L/h	10.29	0.117	6.15	0.99
40 W/185 nm/60 L/h				

The results indicated that UV photolysis significantly affected the degradation of ENR. Among the factors of circulating flow rates, wavelengths, and light intensity, the wavelength and light intensity played a crucial role for the removal efficiency of ENR. In this study, the optimal parameters for the UV photolysis of ENR was 80 W/254 nm under the flow rate of 60 L/h.

first position, enhances its penetration ability (see Table 3-J). ENR has a broad antibacterial spectrum and a low price; hence, it is widely used in the treatment and prevention of infectious diseases in the aquaculture industry (Quesada et al., 2013). The traditional sewage treatment process cannot completely remove ENR, leading to its high concentration in the environment.

ENR powder was purchased from Henan Huikang Animal Pharmaceutical Co., Ltd. The effective ENR content was 10%. According to the instruction of ENR for aquaculture disease control, the dosage of ENR powder was 0.2% of the weight of the fish, i.e., 7.0 g per time, and the fish feed was 2% of the weight of the fish per day, i.e., 70 g per time. Sodium alginate was used as the adhesive to bind the ENR and bait. Firstly, 0.14 g and 0.7 g of sodium alginate was separately added to ENR powder (7.0 g) and bait (70 g), respectively. They were subsequently combined with a small amount of water to create the bait with ENR.

2.3. Variation of ENR in RAS

A 5-day ENR dosing experiment was conducted to investigate the variation in ENR in each unit of the RAS. Approximately, 77.84 g of the mixed bait (with 7 g ENR) was added to the fish tank at 10 a.m. every day. The concentration of ENR in the fish tank and water treatment unit was measured at 3, 12, and 24 h. After the 5-day dosing period, the concentration of ENR in the system was monitored at intervals of 24 h for 3 days (without dosing the bait with ENR) in order to determine the decomposition efficiency of residual ENR.

The static experiment was conducted by suspending the recirculation to investigate the variation of ENR in each unit of the RAS. First, the recirculation was suspended, and 77.84 g of the mixed bait (with 7 g ENR) was added to the fish tank. The concentration of ENR was detected every hour, which imitated the utilization of ENR by the fish. After 6 h, recirculation resumed, and the concentration of ENR in each unit was detected within 7 h. The recirculation was suspended again, and the concentration of ENR in the fish tank, biofilter unit with IMG, and the UV disinfection unit were monitored at 0, 3, 6, and 16 h.

2.4. Ultraviolet photolysis kinetics of ENR

A recirculating UV photolysis device was designed to investigate the kinetics of the UV photolysis of ENR (Fig. 1 e and f). It consisted of a water tank (20 L), a flow meter, a submersible pump, and a UV lamp (diameter 2.5 cm), which was equipped with a stainless-steel sleeve (diameter 6 cm). The UV lamp was preheated for 10 min prior to the experiment. The photolysis conditions, including the flow rate, wavelength, and light intensity, were listed in Table 1.

Three UV lamp tubes were used as the light source, that is, UV-1 (40 W/254 nm), UV-2 (40 W/185 nm), and UV-3 (80 W/254 nm). ENR solution (10 L of 10 mg/L) was prepared for the experiment, which operated every 30 min. The pH and ENR content of the solution were measured at 5-min intervals. The samples for ENR were stored at −4 °C, and filtered through a 0.45 μm filter membrane before measurement. Furthermore, flow rates of 40, 60, 80, 100, 120, and 160 L/h were investigated.

To characterize the photolysis products, 10 L of 10 mg/L ENR solution was photolyzed with UV-1 (40 W/254 nm) for 90 min at 60 L/h. The intermediate photolysis products were analyzed using LC-MS/MS. The concentrations of chemical oxygen demand (COD), biochemical oxygen demand (BOD_5), total organic carbon (TOC), total ammonia nitrogen (TAN), nitrate nitrogen (NO_3^--N), and fluoride ion (F^-) were measured at 15, 30, 60, and 90 min.

2.5. Biodegradation kinetics of ENR photolysis products

The immobilized microbial granules (IMG) can improve the biodegradation efficiency of photolysis products of ENR in aquaculture systems via activated carbon adsorption together with microbial biodegradation. The microbes retained in IMGs ensured high bioprocessing efficiency for photolysis products of ENR. While the activated carbon in IMGs enriched the concentration of photolysis products of ENR and reduced the negative effects of toxic substances (such as the non-degradable fraction of photolysis products) on the microorganisms. The biodegradation of ENR photolysis products by IMGs was investigated under two types of carbon sources: ENR photolysis products with sodium acetate (NaAc) and only individual ENR photolysis products.

First, IMG was cultured in a solution containing ENR or ENR photolysis products and sodium acetate (NaAc). The solution with photolysis products was prepared through a recirculating UV device, in which 10 mg/L ENR was added to the water tank (10 L) and was circulated at a flow rate of 60 L/h using UV-1 (40 W/254 nm). The samples (500 mL solution) were withdrawn at 0, 15, 30, and 60 min, which were then transferred into four Erlenmeyer flasks with IMG (20 g) and the nutrient containing CH_3COONa (80 mg/L), $(NH_4)_2SO_4$ (40 mg/L), $Na_2HPO_4{\cdot}2H_2O$ (35 mg/L), $MgSO_4$ (20 mg/L), and trace elements (10 mL/L) (Alexandrino et al., 2017). The Erlenmeyer flasks were placed in a shaker and oscillated (80 rpm) at a constant temperature (25 °C) for 7 days. The shaker was kept in dark during the experiment. The pH of the solution was adjusted to 7.0, and the DO was approximately 4.0 mg/L. TOC was measured daily.

Second, the IMG, which was used in the experiment above, was transferred into the solution containing ENR individually or the ENR photolysis products to investigate the degradation kinetics. In this experiment, 20 mg/L of the ENR solution and the nutrient solution containing N, P, and trace elements were added to the water tank for UV photolysis. The photolysis conditions were the same as those in the previous step. The experiment lasted 4 days, and the TOC was measured every day.

2.6. Analytical method

The concentration of ENR was determined by LC-MS/MS (TSQ Quantum Access Max triple quadrupole LC/MS, Thermo, USA) with a C18 column at a column temperature of 30 °C, with a total injection volume of 10 μL, an injection speed of 2 μL/s. Furthermore, 0.1% formic acid solution was used as mobile phase A and acetonitrile as mobile phase B. The flow rate of the mobile phase was 0.3 mL/min, with a linear gradient that changed gradually.

Chemical oxygen demand (COD), biochemical oxygen demand (BOD_5), total nitrogen (TN), nitrate nitrogen (NO_3^--N), nitrite nitrogen

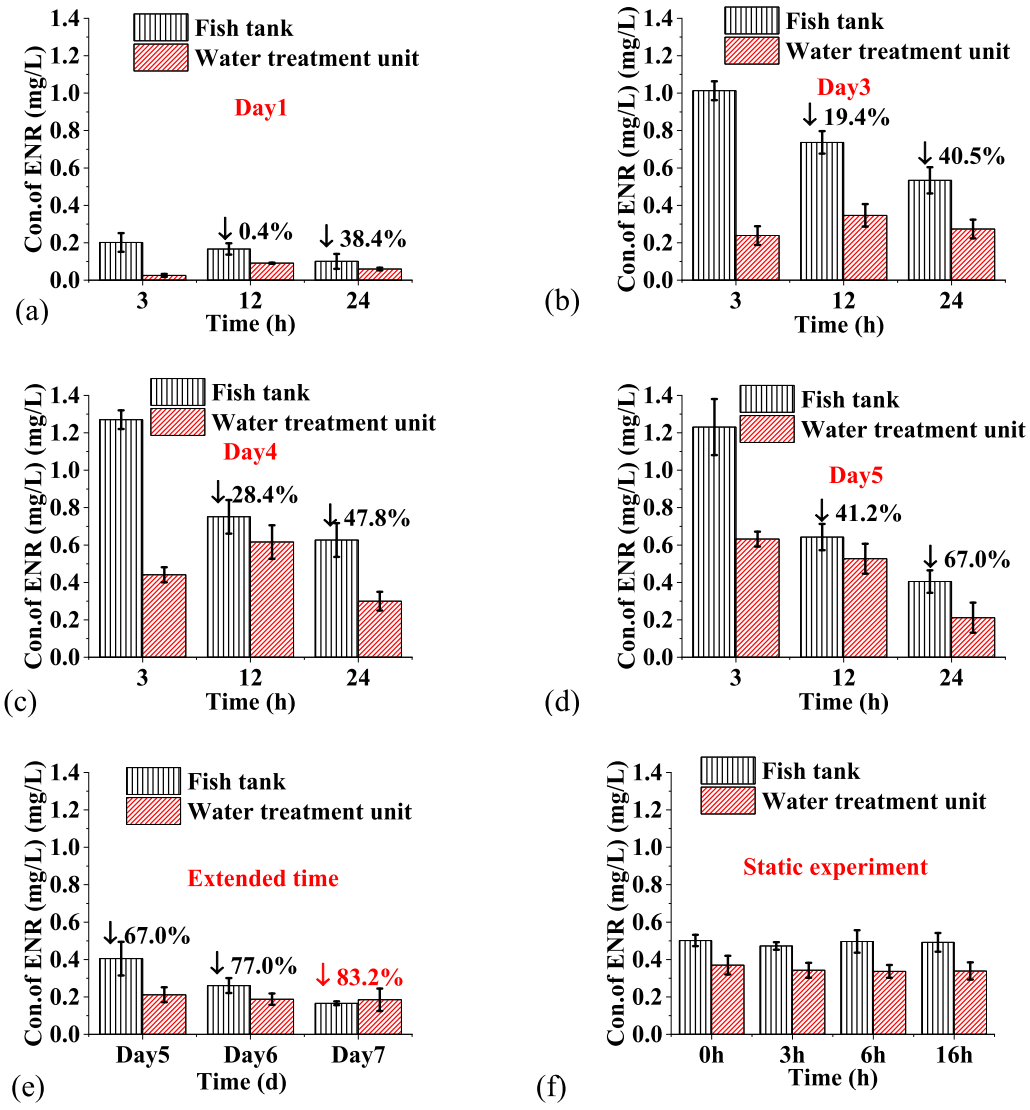


Fig. 2. Fate of ENR concentration in each unit: (a)–(d) Changes in ENR concentration in each unit of the dosing system in 5 days under the recirculating condition, (e) The change of ENR concentration in each unit when suspending the recirculation, (f) The change of ENR concentration in each unit in the static experiment.

(NO_2^--N), total ammonia nitrogen (TAN), total phosphorus (TP), and dissolved total phosphorus (DTP) were determined according to standard methods (NEPA, 2002). TOC was measured using a TOC analyzer (TOC-L, Shimadzu, Japan).

3. Results and discussion

3.1. The fate of ENR in each unit of RAS

The changes in the ENR concentration in each unit on days 1, 3, 4, and 5 were shown in Fig. 2(a–d).

Overall, the accumulation of ENR in the fish tank was observed for over 5-days. The ENR concentration in each unit was relatively low on day-1, which increased significantly on day-3. The highest concentration of 1.0 mg/L reached in 3 h in the fish tank, which gradually decreased, by 19.4% at 12 h and 40.5% at 24 h. Meanwhile, the ENR concentration following the water treatment unit (two biofilter units and UV unit) also increased compared to that on day-1, reaching the highest value at 12 h and then decreasing at 24 h.

The trend on day-4 was similar to that on day-3. The ENR concentration in the fish tank increased further to 1.2 mg/L at 3 h, indicating a significant accumulation, which then decreased by 28.4% at 12 h and 47.8% at 24 h. Correspondingly, the ENR concentration in the water treatment unit also reached its highest value at 12 h, and then decreased to its lowest value at 24 h.

The ENR concentration on day-5 in the fish tank at 3 h was close to the value on day-4, indicating that it was stable. It subsequently decreased by 41.2% at 12 h and 67.0% at 24 h. In contrast, the ENR concentration in the water treatment unit reached its highest value at 3 h, and then decreased to its lowest value at 24 h.

Fig. 2a-d (the arrow in the figures) showed the increase in removal efficiency from 0.4% on day-1 to 67% on day-5, despite ENR being accumulated in the fish tank due to the daily dosage.

The ENR concentration was measured continuously on day-6 and day-7, when bait with ENR was not added (Fig. 2e). During the extended period, the ENR concentration in the fish tank continuously decreased by 77.0% on day-6 and by 83.2% on day-7. In contrast, the ENR concentration after being treated in the water treatment unit (i.e., after two biofilter units and a UV disinfection unit) remained stable. On day-7, the ENR concentration in the fish tank was close to the value obtained from the water treatment unit.

When the recirculation was suspended, the removal ability of the fish tank and water treatment units (in fact, only the biofilter unit with IMG worked) was investigated (Fig. 2f). The results showed that the ENR

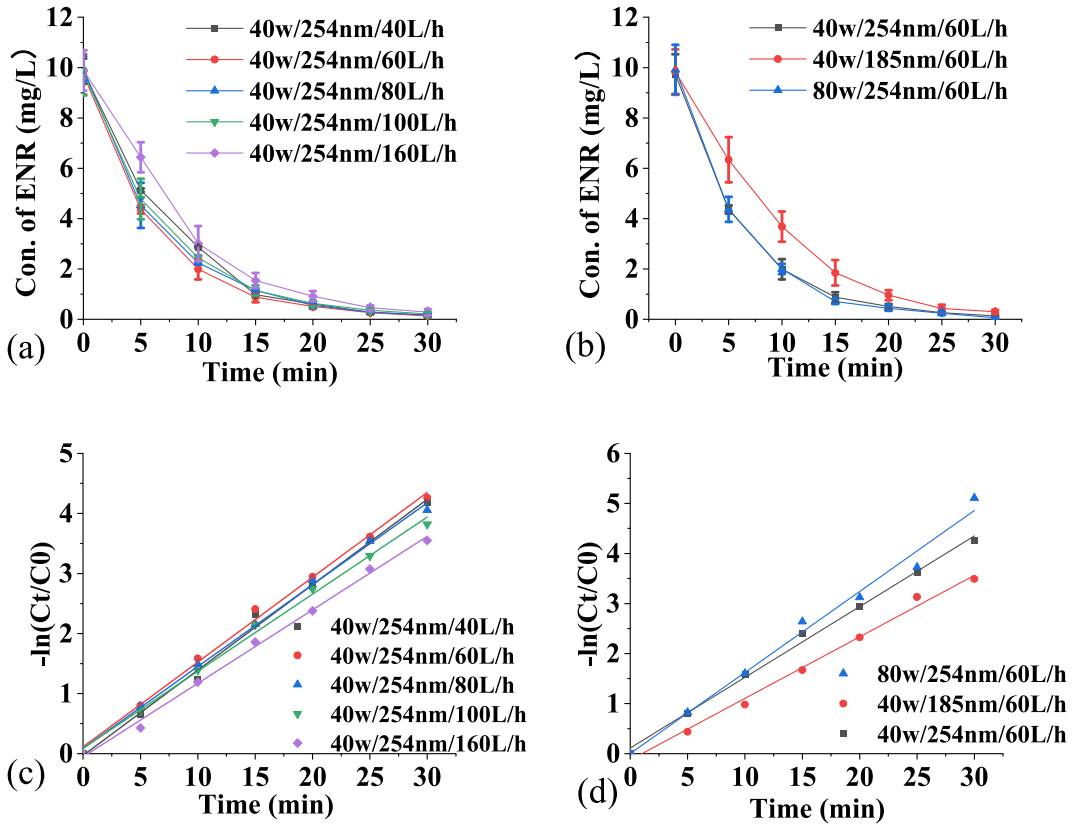


Fig. 3. UV photolysis kinetics of ENR under different condition (a) effect of circulating flow rate used, (b) effect of wavelength and light intensity, (c) and (d) The first order kinetic fitting of (a) and (b).

concentration in both the fish tank and biofilter unit with IMG was stable, indicating minimal effect on the removal of ENR. In fact, in this case, the UV disinfection device had less effect on ENR removal because of the absence of water flow through the UV device. The removal of ENR in Fig. 2e was likely ascribed to ultraviolet photolysis rather than biodegradation. Guo et al. (2020) studied the migration and transformation of florfenicol in RAS and concluded that the UV disinfection device was the key unit in removing florfenicol in systems. In this study, the UV disinfection device in the RAS was attributed to ENR removal, which depended on the operation time.

From above, after a 5-day dosage of ENR, the accumulated ENR was decomposed by UV photolysis; however, the residual ENR concentration after 7 days was still 137 μg/L, indicating a limited removal ability, which needs to be further improved.

In this study, ENR was added as a mixed fish bait, and sodium alginate was used as adhesive to bind the ENR and bait. During the static experiment (section 2.3), when the recirculation was suspended, 77.84 g of the mixed bait (with 7 g ENR) was added to the fish tank. The concentration of ENR in fish tank, which was detected hourly, increased quickly within 2 h and reached a peak value of 1173.3 μg/L after 3 h (the average value was 1086.7 μg/L from 3 h to 6 h) before the recirculation resumed after 6 h. Therefore, the ENR released into the fish tank (the water volume of 823 L) was estimated to be 0.89 g. The remained ENR (7 − 0.89 = 6.11 g) were in the faeces of fish (solid waste) and the fish body. In this study, the distribution of remaining ENR was not determined since it did not occur in aqueous phase, and further research could be focused on the ENR mass balance.

3.2. UV photolysis kinetics of ENR under different condition

The UV photolysis kinetics of ENR were investigated using a recirculating UV device. The effects of photolysis conditions on the removal efficiency of ENR investigated included circulating flow rates, UV wavelengths, and light intensity. The results were shown in Fig. 3, and

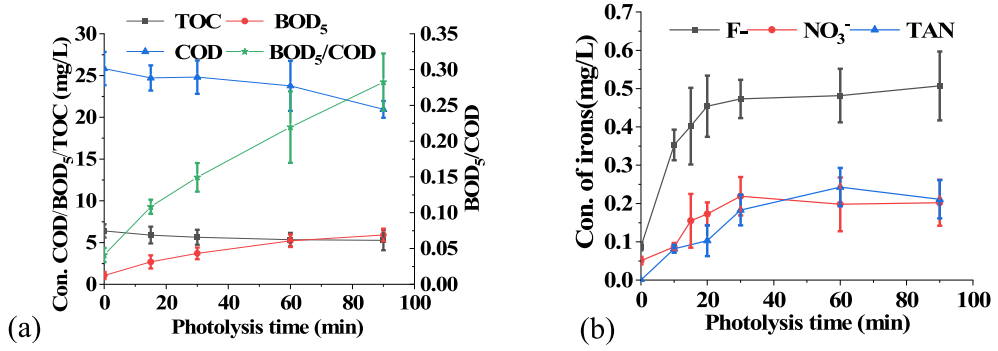


Fig. 4. Change in water quality during UV photolysis: (a) COD, TOC, BOD_5, and BOD_5/COD, (b) F^-, NO_3^- and TAN.

Table 2
The fitting formula and the kinetic parameters for UV photolysis of ENR.

photolysis Product	Fitting formula	Ce (mg/L)	k1 (min-1)	R2
F	y = 0.49*[1-exp(-0.12t)]	0.49	0.12	0.92
NO_3	y = 0.21*[1-exp(-0.08t)]	0.21	0.08	0.78
TAN	y = 0.24*[1-exp(-0.04t)]	0.24	0.04	0.93

the kinetic parameters were listed in Table 1.

As shown in Fig. 3, the photolysis kinetics of ENR conformed to first-order reaction kinetics. This demonstrated that an optimal circulating flow rate was 60 L/h for UV photolysis, above or below which the photolysis efficiency was reduced owing to the short contact time. The kinetic parameter, k was 0.149 min^{-1} for a flow rate of 60 L/h under the same light intensity (40 W) and wavelength (254 nm).

Under the same wavelength (254 nm) and circulating flow rate (60 L/h), a higher light intensity (80 W) displayed a higher photolysis kinetics with k = 0.164 min^{-1} compared with k = 0.149 min^{-1} for 40 W. The stronger the light intensity, the more photons were produced, resulting in the effective degradation of the targeted substrates as described previously (Knapp et al., 2005).

At a fixed light intensity (40 W) and circulating flow rate (60 L/h), the kinetic parameter k at a wavelength of 185 nm was 0.117 min^{-1}, which was significantly lower than that at 254 nm (0.149 min^{-1}). According to the study by Zoschke et al. (2014), UV with shorter wavelengths had weaker permeability in water (in the range of 1–5 mm), resulting in a lower removal efficiency. This study confirmed similar results.

3.3. Water quality during UV photolysis of ENR

Under the optimal condition (80 W/254 nm and 60 L/h), the changes in COD, TOC, and BOD_5 during the photolysis process were shown in Fig. 4 (a). After 90 min of photolysis, the COD decreased from 25.84 mg/L to 20.95 mg/L and the TOC decreased from 6.33 mg/L to 5.27 mg/L, whereas the BOD_5 increased from 1.06 mg/L to 5.92 mg/L. The ratio of BOD_5 to COD (B/C) represents the biodegradability of the organic matters in water. The B/C value of the water after photolysis increased from 0.041 to 0.28, indicating improvement of biodegradability of residual organics.

The changes in the concentrations of F^-, TAN, and NO_3^- during the photolysis process were shown in Fig. 4 (b). The fitting formula and kinetic parameters of the decomposition of F^-, NO_3^-, and TAN were listed in Table 2.

Fluorine atoms in ENR are crucial for antibacterial activity and spectrum. Therefore, defluorination can imitate the effect of the decomposition of ENR by UV photolysis (Alexandrino et al., 2017). Theoretically, 0.528 mg/L of F^- can be produced when 10 mg/L of ENR is decomposed. As shown in Fig. 4 (b), the concentration of F^- increased significantly in the first 30 min to 0.473 mg/L and then stabilized (0.507 mg/L at 90 min). Correspondingly, the efficiency of defluorination at 30 min was 89.58% and exceeded 96% over 90 min. Fitting formula for F^- matched the first order kinetical model (Table 2). The rise rate constant k_1 for F^- was 0.12 min^{-1}.

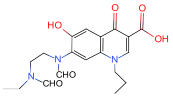
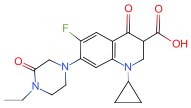
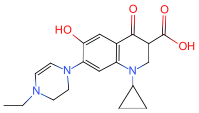
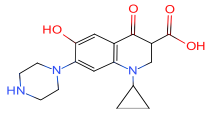
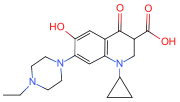
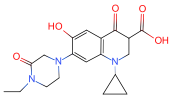
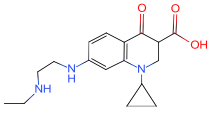
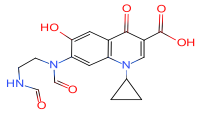
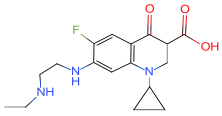
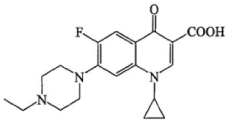
As shown in Fig. 4 (b), a small amount of NO_3^- and TAN were produced (NO_2^- was not detected), which was approximately 0.2 mg/L of both NO_3^- and TAN at 90 min, accounting for only 1.7% of the TN (11.7 mg/L) theoretically. Fitting formula for TAN, and NO_3^- matched the first order kinetical model (Table 2). The rise rate constant k_1 for TAN and NO_3^- were only 0.04 min^{-1} and 0.08 min^{-1}, respectively. This demonstrated that UV photolysis had a poor effect on the nitrogen conversion of ENR; therefore, the photolysis intermediate products were in the form of nitrogen compounds (Table 3).

In summary, UV photolysis of ENR improved the ratio of BOD_5 to COD (B/C = 0.28) and thus enhanced biodegradation. The concentrations of F^- could be selected as an indicator to reflect the degree of the decomposition of ENR by UV photolysis.

3.4. Characteristics of UV photolysis products of ENR

The intermediate products of UV photolysis for ENR were further characterized by LC-MS/MS (TSQ Quantum Access Max triple quadrupole LC/MS, Thermo, USA) using sample taken under the optimal condition (80 W/254 nm and 60 L/h). The chromatograms of the initial ENR and photolysis products at 15, 30, 60, and 90 min were shown in Fig. 5 (a–e). The molecular formulae of the main products were shown in Table 3.

Table 3
Formulas of the main photolysis products.

Num.	Molecular formulas	m/z	Num.	Molecular formulas	m/z
A		390	F		374
B		356	G		330
C		358	H		372
D		316	I		344
E		334	J (ENR)		360

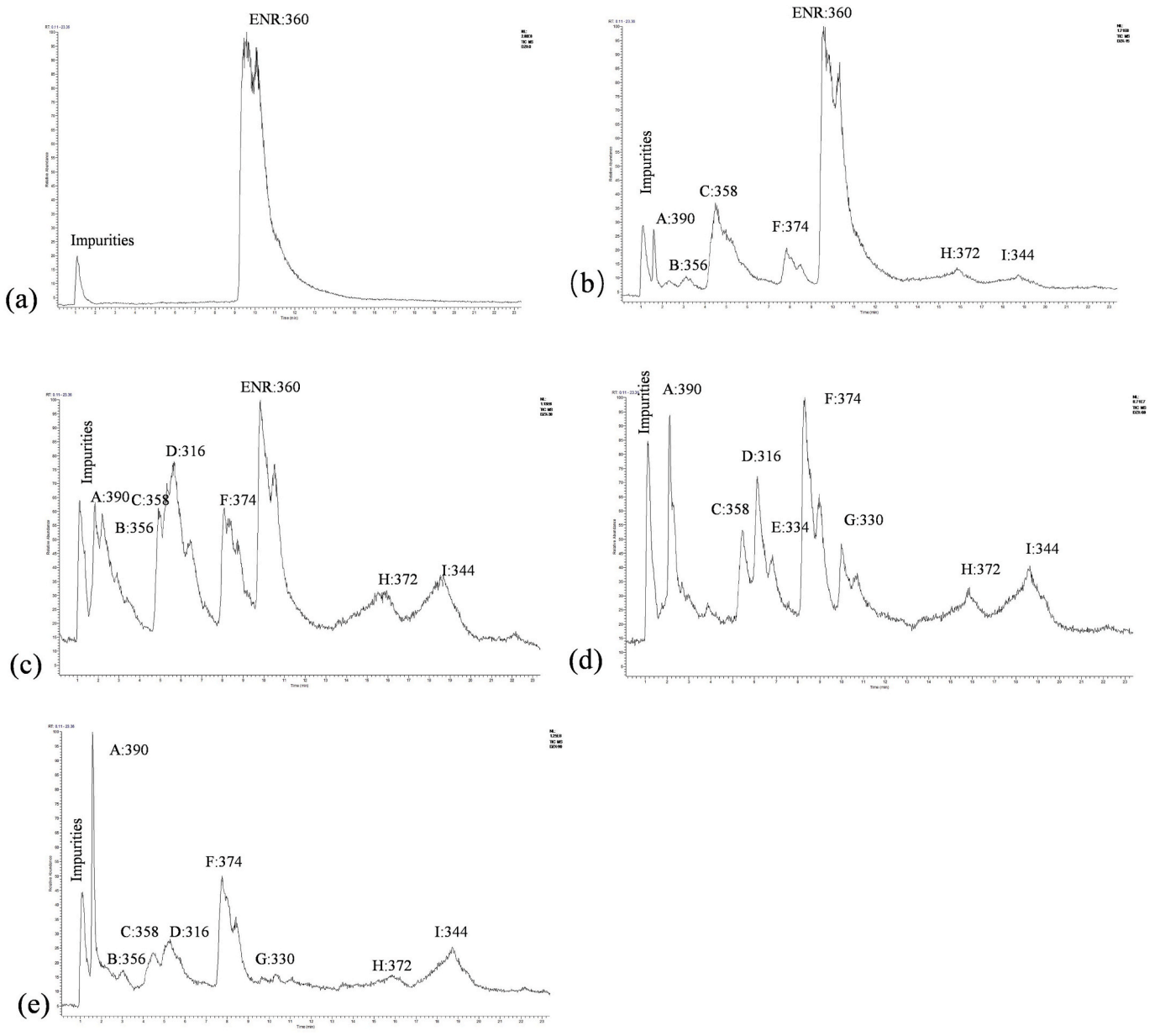


Fig. 5. The chromatograms of enrofloxacin and the photolysis products at (a) 0 min, (b) 15 min, (c) 30 min, (d) 60 min, and (e) 90 min.

As shown in Fig. 5a, the chromatographic peak of ENR (m/z 360) was observed before the photolysis reaction, in addition to the impurity peaks (m/z 208). With an increase in the photolysis time (Fig. 5b–e), more intermediate products appeared.

After 15 min of photolysis (Fig. 5b), multiple peaks appeared, including the peaks of ENR, and photolysis products A (m/z 390), B (m/z 356), C (m/z 358), F (m/z 374), H (m/z 372), and I (m/z 344). According to the molecular formulas in Table 3, the produced photolysis products were all free of the fluorine atom, which was deduced to be replaced by the hydroxyl group.

After 30 min (Fig. 5c), the peak of ENR was still observed, and photolysis product D (m/z 316) was simultaneously produced. In addition, the peak value of the previous photolysis product increased significantly. In particular, the peak values of products C and E considerably increased, indicating an increase in the amount of photolysis products.

After 60 min (Fig. 5d), additional photolysis products were produced. The peaks of ENR and photolysis product B disappeared, but photolysis products E and G appeared. The photolysis product F (m/z 374) was the main product after 60 min of photolysis.

After 90 min (Fig. 5e), the peak value of most of the photolysis products decreased significantly, except photolysis product A, which significantly increased and become the most concentrated product. It could be deduced that most of the photolysis products were further converted.

In this experiment, the chromatogram peaks of ciprofloxacin (m/z of 330) were observed at 15 min and 30 min (Fig. 5b and c). According to the research by Knapp et al. (2005) and Burhenne et al. (1997), ciprofloxacin is an important photolysis product of ENR. However, the peak response weakened with the progress in photolysis (Fig. 5d and e), which indicated that ciprofloxacin might be decomposed during UV photolysis. The results confirmed that ENR could be decomposed incompletely by UV photolysis, and a large number of intermediate products with molecular weights greater than 300 Da were still present. According to the results obtained in section 3.3, the ratio of BOD_5 to COD after photolysis increased from 0.041 to 0.28, indicating the biodegradability was improved. Moreover, fluorine atoms in ENR that are crucial for antibacterial activity were defluorinated by 96% over 90

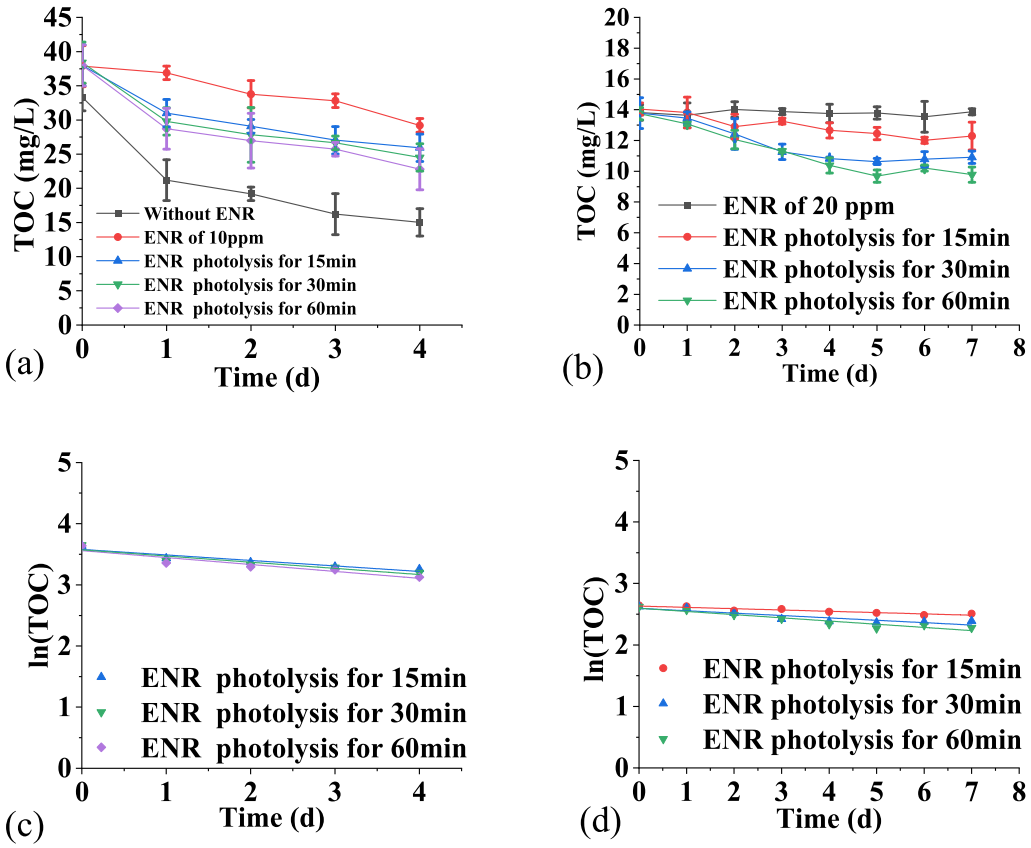


Fig. 6. Biodegradation of ENR and different UV photolysis products of ENR (a) ENR photolysis products with sodium acetate, (b) ENR photolysis products without sodium acetate, (c) and (d) the first order kinetic fitting.

min. The residual substances were mainly organic nitrogen matter. Therefore, further research should be performed to treat the intermediate products through biodegradation.

3.5. Biodegradation of photolysis products of ENR by the IMG

The use of immobilized microbial granules (IMGs) is expected to effectively improve the biodegradation efficiency of antibiotics in aquaculture systems by retaining antibiotics-degrading microbes in them. IMG were first cultivated in a nutrient solution containing photolysis products of ENR at different times (equal initial concentration of ENR) and sodium acetate for 4 days, and were simultaneously compared with nutrient solutions containing individual sodium acetate or ENR.

As shown in Fig. 6a, the group with sodium acetate individually showed the highest TOC, followed by the groups with photolysis products of ENR, while the group with ENR (10 mg/L) showed the least TOC. After 4 days, the TOC removal efficiency of the sodium acetate group was 55.0%, while that of the ENR group was 23%. The TOC removal efficiency increased with the photolysis time, with 31.7%, 36.2%, and 40.0% for 15, 30, and 60 min of photolysis, respectively.

The IMG used in the above experiment were further used to degrade the ENR photolysis products without sodium acetate. The change in TOC during the 7 days of degradation was shown in Fig. 6 (b). The group with ENR (20 mg/L) individually showed a significant inhibitory effect on the IMG bacteria, which were hardly removed after 7 days. However, all groups with photolysis products of ENR showed an apparent removal efficiency, which increased with the photolysis time, reaching 12.5%, 20.8%, and 28.8% after 15, 30, and 60 min of photolysis, respectively.

The biodegradation of the photolysis products of ENR (with or without NaAc) fit the first-order kinetics (Fig. 6 c and d), and the kinetic parameters were summarized in Table 4.

For the group with NaAc as co-substrate (10 ppm ENR and 10 ppm NaAc), the reaction rate constant k for 15, 30, and 60 min of product photolysis was significantly higher than those of groups without NaAc. The reaction rate constant k for the photolysis products of ENR (20 ppm ENR) increased significantly with an increase in photolysis time, that is, 2.5 times increase from 15 min to 60 min.

Table 4
Parameters of the first order kinetic fitting for the biodegradation of ENR photolysis products, with and without NaAc as co-substrate.

Carbon source	Time of photolysis	Fitting formula	C_0 (mg/L)	k (day^{-1})	R^2
Photolysis products+NaAc	15 min	lnC(t) = 3.58–0.09t	35.84	0.09	0.87
	30 min	lnC(t) = 3.57–0.10t	35.59	0.10	0.83
	60 min	lnC(t) = 3.56–0.11t	35.13	0.11	0.85
Photolysis products	15 min	lnC(t) = 2.63–0.02t	13.91	0.02	0.85
	30 min	lnC(t) = 2.59–0.04t	13.40	0.04	0.77
	60 min	lnC(t) = 2.60–0.05t	13.42	0.05	0.89

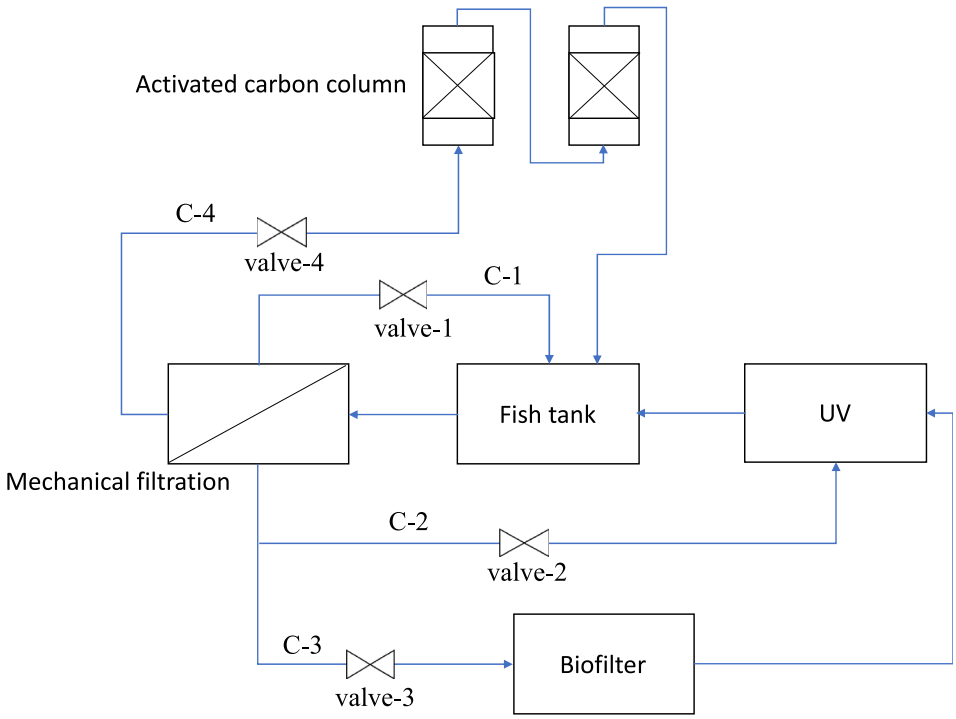


Fig. 7. Four-step process for antibiotics application and its residual control in RAS.

The above results indicated that the photolysis products could be partially degraded by IMGs. The extent of degradation was positively correlated with the photolysis time. The results indicated that the IMGs can effectively improve the biodegradation efficiency of photolysis products of ENR in aquaculture systems via activated carbon adsorption together with microbial biodegradation. The research by Dong et al. (2011) indicated that microbes were retained in IMGs for long-term and ensured high bioprocessing efficiency and the activated carbon reduced the negative effects of toxic substances on the microorganisms. Further research will be focused on the community change of antibiotics-degrading microbes during biotreatment of ENR.

The treated water still contained trace level of non-degradable fraction of photolysis products. Further study is needed to remove them by other technological methods, such as post treatment with activated carbon adsorption, which can effectively remove residual antibiotics. Based on the results of this study, in situ UV photolysis and biodegradation significantly reduced the work load for the adsorption by activated carbon, thus extending the working period if further activated carbon post treatment is needed.

3.6. *Proper use of antibiotics and control of the residues in RAS*

Although the use of antibiotics is strictly restricted due to their misuse and ineffective treatment in aquaculture, antibiotics continue to be the most effective means for disease control in farming animals. In contrast to aquaculture in open water bodies, the antibiotics used in RAS can be more accurate, and the residues can be removed effectively according to the closed-loop recirculating system. The proper use of antibiotics and the rapid removal of the residues are important in RAS, thus, avoiding the mishandling of antibiotics and eliminating the environmental pollution caused by residual antibiotics.

In general, the utilization of antibiotics could be lasted for several days (such as 5 days in this research). To achieve rapid removal of residual antibiotics in RAS, a four-step process for antibiotic application and residual control in RAS was proposed based on the UV photolysis and biodegradation characteristics of ENR in this study (Fig. 7).

As shown in Fig. 7, for each day, the first step was the utilization of antibiotics (C-1), which lasted for 3 h. Only valve-1 was opened to filter the fish sludge using a mechanical filtration unit, with all other valves closed. The bait with antibiotics was added to the fish tank. Based on the results of this study, the bait with antibiotics could be metabolized within 3 h, and the residual antibiotics accumulated in water should be removed by the subsequent steps.

The second step was in-situ UV photolysis (C-2) using a UV device equipped in the RAS. Only valve-2 was opened. The residual antibiotics produced were decomposed by the UV device, which lasted for 3 h. The photolysis time can also be extended according to the photolysis characteristics of different antibiotics.

The third step was biodegradation by the biofilter equipped in the RAS (C-3). Only valve-3 was opened. The residual photolysis intermediate products were degraded by the IMG filled in the biofilter, which lasted for approximately 16 h according to the results of the experiment. In this step, the UV device also acted as a photolysis unit.

The fourth step was adsorption by the activated carbon column (C-4). Only valve-4 was opened, and the residual antibiotics or photolysis products that could not be biodegraded in the previous stage were quickly adsorbed by the granular activated carbon in the column within 2 h.

The above steps were repeated for several days, following which the RAS resumes normal operation. This process not only provides the method for proper utilization of the antibiotics in RAS, but also enables the UV disinfection device to perform the in-situ photolysis of antibiotics, and the biofilter with immobilized microbial granules to perform in-situ degradation of residual photolysis products, and the activated carbon column to rapidly adsorb the residual non-degradable substances. Photolysis and biodegradation will reduce the load rate on the activated carbon column, prolonging its life cycle and reducing costs. This process is expected to evolve into a cleaner production technology with high potential for future development and advancement.

4. Conclusion

To achieve rapid in situ removal of residual antibiotics in RAS by integrating process, the fate of the selected antibiotic enrofloxacin

(ENR) in different units of a laboratory-scale RAS was investigated, the photolysis conditions and photolysis products of ENR by ultraviolet (UV) disinfection were characterized, and the degradation of photolysis intermediate products by immobilized microbial granules were explored. It concluded that the UV device in RAS played an important role in ENR removal. The photolysis kinetics of ENR fitted the first-order reaction kinetics. The wavelength and light intensity were crucial in the removal efficiency of ENR. The F^- produced by defluorination could be selected as the indicator to reflect the decomposition efficiency of ENR by UV photolysis. The UV photolysis products of ENR were influenced by the photolysis time and results indicated the decomposition of ENR was incomplete. Under selected optimal condition (UV 80 W/254 nm and flow rate of 60 L/h), the ratio of BOD_5 to COD (B/C) after photolysis increased from 0.041 to 0.28, indicating improvement of biodegradability. The photolysis products were partially degraded by immobilized microbial granules (IMG). The degradability was positively correlated with the photolysis time and fit first-order reaction kinetics. Based on the results, an in-situ four-step process that included the appropriate usage of antibiotics, UV photolysis, and biodegradation using the biofilter, and adsorption by the activated carbon column was proposed to achieve antibiotic application and residual control in RAS. Further research should be focused on the distribution of ENR in the fish and faeces, and microbial community of antibiotics-degrading microbes in immobilized microbial granules (IMGs).

CRediT authorship contribution statement

Sha Sha: Data curation, Investigation, Methodology, Writing – original draft. **Zhengxuan Dong:** Data curation, Investigation, Methodology. **Yueshu Gao:** Investigation, Writing – review & editing. **Haslenda Hashim:** Investigation, Validation, Writing – review & editing. **Chew Tin Lee:** Investigation, Writing – review & editing, Validation. **Chunjie Li:** Conceptualization, Funding acquisition, Project administration, Investigation, Validation, Writing – review & editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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