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Emerging investigator series: quaternary treatment with algae-assisted oxidation for antibiotics removal and refractory organics degradation in livestock wastewater effluent†

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In this study, we investigated the combination of algal treatment and subsequent oxidation to improve the antibiotics removal and refractory organics degradation in livestock wastewater effluent. We also proposed a better combination of oxidation processes for robust treatment of recalcitrant wastewater, mainly focusing on the removal of antibiotics. Stand-alone processes of O_3, O_3/H_2O_2, ultraviolet C (UVC), and UVC/H_2O_2 as well as their combined effects were evaluated as a downstream oxidation process. Prior to those oxidation processes, the effluent was treated with microalgae under continuous light illumination, which turned it into alkaline wastewater (pH $>$ 10). The algal treatment was feasible not only to directly remove antibiotics resistant to a specific oxidation method, but also to improve the degradation of antibiotics and effluent organic matter in the alkaline wastewater upon subsequent oxidation. The alkaline wastewater was more favorable for the removal of antibiotics (especially for clopidol) with UVC treatment (UVC and UVC/H_2O_2). The algal treatment promoted ozonation to some extent for the degradation of florfenicol in the effluent. The removal of antibiotics by ozonation decreased with added H_2O_2, whereas the UVC performance was improved by adding H_2O_2. For ozonation followed by UVC treatment, the ozonation dominated the degradation of selected antibiotics.

Water impact

Since a remarkable amount of veterinary antibiotics has been detected even in public water sources, more reliable treatment of livestock wastewater beyond complying with typical discharge goals is increasingly demanded. We propose here an algae-integrated treatment strategy to successfully remove antibiotic residues. This provides further insight into the contributions and roles of microalgae in advanced oxidation of refractory wastewaters.

1. Introduction

Over the past decades, antibiotics have gained much attention as environmental pollutants because their residues are frequently found in surface water bodies and pose a potential risk to public health and aquatic environments even at vestigial concentrations.[1–3] Previous studies reported that these trace organic chemicals chronically affected the ecosystem and aquatic species and led to an explosion of antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARGs).[4–8] ARB and AGRs are emerging at alarming rates, and the World Health Organization announced that they are major threats to human life in this century.[9,10] More than half of all antibiotics around the world are used for animal husbandry, and veterinary antibiotic residues have been frequently observed in aquatic environments, including groundwater, surface water, and terrestrial matrices.[11–14]

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Wide varieties of veterinary antibiotics worldwide are used in concentrated animal feeding operations (CAFOs) to treat infectious diseases and as nutritional supplements in livestock feed. After use, a significant amount of antibiotics as parent compounds and their metabolites are excreted in the urine and feces, since they are not very well adsorbed by the animals' intestines.[15] Thus, these compounds are frequently detected in the effluents from livestock manure and CAFOs wastewater treatment facilities,[16,17] indicating that conventional technologies for livestock wastewater treatment cannot decompose them effectively.[18–20] Because the main purpose of conventional livestock manure treatment is to remove suspended solids, carbonaceous organics, and inorganic nutrients in order to comply with discharge goals, high levels of various antibiotics and the associated ARB and AGRs cannot be effectively eliminated and eventually enter the environment. Considering interrelated water resources, antibiotics-laden surface water can be a source of drinking water supplies; thereby, treatment technologies with high reliability should be applied for robust removal of antibiotic residues in livestock wastewater before it is discharged into the receiving water bodies.[21–24]

Ozonation is credited as a promising candidate for treating livestock wastewater and is increasingly combined with other techniques. For instance, O_3 and ultraviolet C (UVC) can be coupled with H_2O_2 or other chemicals (*e.g.*, persulfate) to greatly improve the performance of oxidation for the degradation of micropollutants in water and wastewater. Both ozonation and UVC irradiation are well established and operated in full-scale drinking water and water reuse applications.[25] A wide variety of advanced oxidation processes (AOPs) have been investigated for removing the emerging pollutants of concern in different types of wastewaters.[25–29] The feasibility of AOPs depends on the generation of OH radicals and on the reactivity of target compounds.[30] The reactions can be drastically affected by wastewater characteristics, such as pH, organic matter, and alkalinity. In general, degradation rates of organic compounds tend to increase with higher pH in the aqueous phase, as a result of increased generation of OH radicals.[28,31–33] Therefore, the treatment of alkaline wastewater with AOPs could be a better option to effectively remove antibiotics remaining after secondary or tertiary treatment of livestock wastewater. Unfortunately, the pH adjustment ($pH > 10$) of wastewater using caustic chemicals is cost-ineffective and thus unfeasible. In our previous studies, we found that the growth of *Scenedesmus quadricauda* in reverse osmosis (RO) concentrate significantly increased the pH, effectively reduced alkalinity,[34] and removed inorganic nutrients from a wide range of wastewaters.[35,36] We also reported the ability of *S. quadricauda* to remove pharmaceutical residues from RO concentrate in our prior work.[37] Microalgae can effectively eliminate several types of micropollutants by means of different mechanisms such as intracellular and extracellular biodegradation, bioaccumulation, and biosorption.[38,39] To date, some investigations have confirmed the efficiency of algae-mediated removal of emerging pollutants in different types of wastewaters.[40–42]

The objective of this study was to investigate the effects of algal treatment and subsequent oxidation on the removal of selected antibiotics and organic residues in tertiary livestock wastewater effluent. We evaluated standalone processes of O_3, O_3/H_2O_2, UVC, and UVC/H_2O_2 as well as their combined effects as a downstream oxidation process. In our latest work, we demonstrated that H_2O_2 could be generated on-site through the oxygen reduction reaction during the bioelectrochemical degradation of carbonaceous organic matter.[43] Based on this previous observation, the on-site generation of H_2O_2 was supposed in this study and was integrated with the downstream oxidation processes. As shown in Fig. 1, organic-rich anaerobic digestate could be partially used as a feedstock in the bioelectrochemical system for the on-site generation of H_2O_2 that can be further used as a mediator for downstream AOPs (O_3/H_2O_2 and UVC/H_2O_2). In this study, feasible oxidation processes were applied for treatment of tertiary effluent, and the contribution of algal pretreatment to the oxidation processes was also addressed with emphasis on antibiotics removal and refractory organics degradation. A microalga *S. quadricauda* was employed to increase the pH of wastewater *via* photosynthesis prior to various oxidation processes. *Scenedesmus* is a dominant genus of algae commonly found in wastewater ponds.[44] To the best of our knowledge, no one has yet reported that microalgae can be used for alkalization of wastewater to improve the performance of AOPs.

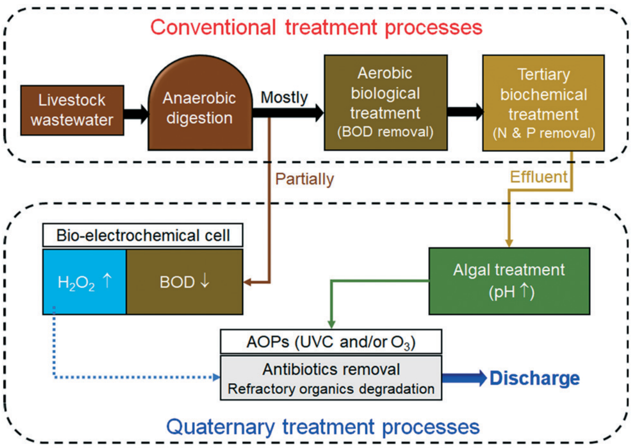

Fig. 1 Conceptual schematics for quaternary treatment of livestock wastewater using a hybrid system combining (i) bioelectrochemical treatment for on-site H_2O_2 production, (ii) multi-purpose algal treatment, and (iii) O_3 and UVC for advanced oxidation of antibiotics with H_2O_2 produced on-site. The on-site H_2O_2 production with bioelectrochemical treatment using anaerobic digestate is out of the scope of this research.

2. Materials and methods

2.1. Livestock wastewater effluent

In this study, the tertiary effluent was collected from a local livestock wastewater treatment plant that uses sequential aerobic biological treatment, chemical floatation, and biofiltration for treating farm-generated animal manure. The wastewater sample was collected in sterile plastic containers and transferred to the laboratory to add selected antibiotics. Twenty antibiotics were selected based on their occurrence in livestock wastewater and were supplied by Sigma-Aldrich (St. Louis, MO, USA). Stock solutions of the 20 antibiotics (*i.e.*, amoxicillin, ampicillin, ceftiofur, chlortetracycline, clopidol, enrofloxacin, erythromycin, florfenicol, lincomycin, oxytetracycline, penicillin-G, penicillin-V, sulfadiazine, sulfamethazine, sulfamethoxazole, sulfathiazole, tetracycline, tiamulin, trimethoprim, and tylosin) were individually prepared in HPLC-grade methanol using each of the antibiotics. A diluted mixture was used as a working solution and was spiked into the livestock wastewater sample in order to achieve a final concentration of 20 μg L^{-1} for each antibiotic. The starting concentration of antibiotics varied widely, and this is more likely due to varying extents of decay during wastewater storage since the recoveries appeared to be fairly consistent for all the antibiotics (see ESI† Table S2). The eventual concentration of total antibiotics ranged between 203 and 253 μg L^{-1}. The characteristics of livestock wastewater effluent are summarized in Table 1. The sample was designated as raw wastewater and stored in a refrigerator at 4 °C in the dark until use in batch experiments. Unless specified otherwise, all experiments addressed in this study were carried out at room temperature.

2.2. Batch treatment of livestock wastewater effluent

2.2.1. Algal treatment. The raw wastewater sample was divided into two aliquots, one of which was used for algal treatment with *S. quadricauda*. The stock culture of the *S. quadricauda* strain (AG 10003) was prepared as described in our prior work.[37] The algal biomass in the exponential growth phase was collected *via* centrifugation (1620*g*, 10 min), washed with mineral bottled water, and inoculated at an optical density (OD) of 0.4 cm^{-1} (680 nm) into flasks containing 800 mL of raw wastewater. The flasks were incubated without aeration at 25 °C while shaking at 130 rpm for 12 h. The incubation was conducted twice in duplicate under continuous illumination with fluorescent lamps. The incoming light intensity to the flasks was 150 μmol photons m^{-2} s^{-1}, which was measured using an MQ-500 quantum meter (Apogee Instruments, Inc., USA). The supernatant was collected from the flasks at the end of the test, filtered with 1.2 μm glass-fiber microfilters (Whatman, Grade GF/C), and mixed uniformly in a single container. The algal-treated wastewater (pH 10.2) was characterized as described in section 2.3 and stored in a refrigerator at 4 °C pending further oxidation treatments.

Table 1 Characteristics of livestock wastewater effluent used in this study

Item	Value
pH	7.1–8.2
Conductivity (mS cm^{-1})	0.78–2.24
DOC (mg L^{-1})	69–86
COD (mg L^{-1})	262–285
UV_{254} (cm^{-1})	0.33–0.35
SUVA (L mg^{-1} m^{-1})	0.39–0.51
Color (mg Pt–Co per L)	21–25
TP (mg L^{-1})	0.04–0.06
TN (mg L^{-1})	5–16
Alkalinity (mg $CaCO_3$ per L)	25–29
Total antibiotics[a] (μg L^{-1})	203–253

[a] Spiked intentionally.

2.2.2. UVC treatment. The UVC treatment of raw and algal-treated wastewaters was carried out at 254 nm using a collimated beam apparatus with a 20 W low-pressure monochromatic mercury lamp (UVP XX-20S, Analytik Jena Co., Germany). The apparatus and method of UVC treatment have been reported in detail in our prior work.[43] Briefly, the batch experiments were conducted in an open crystalline Petri dish containing 40 mL of water samples. The Petri factor (0.863), reflection factor (0.975), and water factors (0.786–0.868 depending on the UV absorbance of the wastewater samples) were applied to correct the UVC intensity, which was measured at the same height as the water level in the Petri dish using a calibrated UVX radiometer (Analytik Jena Co., Germany). The UVC treatment was performed in duplicate at doses of approximately 0, 500, 1000, and 2000 mJ cm^{-2}, which was calculated using the average intensity of 0.404–0.446 mW cm^{-2} and exposure time.[45]

We carried out separate UVC treatments to investigate the effects of H_2O_2 addition on the degradation of antibiotics upon UVC irradiation. Commercially available H_2O_2 was added to raw and algal-treated wastewaters in a final concentration of 0.5 mM and the UVC irradiation was performed in duplicate as described above. The concentration of H_2O_2 remaining after the UVC irradiation was measured using the vanadate method.[46] If needed, H_2O_2 remaining in the samples was quenched using bovine liver catalase for further characterizations described in section 2.3.

2.2.3. Ozonation. Ozonation of raw and algal-treated wastewaters was carried out with an O_3 generator (Lab-I Ozone Tech Co. Ltd., Korea) using >99.9% O_2 and continued for up to 10 min. Tests were performed twice in a glass cylindrical reactor containing 900 mL of wastewater samples. The gaseous ozone exhausted from the reactor was detected and quantified by measuring the absorption of electromagnetic radiation at a wavelength of 254 nm using an ozone analyzer (Model H1, IN USA Inc.). O_3 was injected at a rate of 11.4 mg min^{-1} through a gas diffuser placed at the bottom of the reactor. O_3 doses normalized by the dissolved organic carbon (DOC) of the wastewater samples were reported. For some experiments, commercially available

H_2O_2 was added into raw and algal-treated wastewaters in final concentrations of 0.5 and 1.0 mM and subsequent ozonation was performed as described above, in order to investigate the effects of the H_2O_2 addition on the degradation of antibiotics upon ozonation. After ozonation under the given conditions, residual H_2O_2 was measured and then quenched as described in the above section prior to the analyses of the water samples.

Separate ozonation was conducted in duplicate to evaluate the pre-ozonation combined with UVC and UVC/H_2O_2, mainly focusing on the degradation of antibiotics in raw and algal-treated wastewaters. The ozonation was carried out as described above; aliquots were further subjected to UVC treatment (UVC and UVC/H_2O_2) performed as described in section 2.2.2.

2.3. Analytical methods

The water samples were manually filtered with 0.45 μm microfilters prior to measuring the water quality parameters. Each measurement was carried out at least twice, and the average and standard deviation values were reported. Dichromate, persulfate digestion, and acid persulfate digestion methods were employed to measure the chemical oxygen demand (COD), total nitrogen (TN), and total phosphorus (TP) in the water samples using a DR/5000 spectrophotometer. An identical spectrophotometer was also used to measure the chromaticity, UV_{254}, and OD680. The DOC was determined (TOC-V CPN, Shimadzu, Japan) to obtain the specific UV absorbance (SUVA) value, which is calculated from the UV_{254} divided by the DOC of the water sample. The pH and conductivity were also measured using a pH meter (Orion Star A215, Thermo Scientific). The alkalinity was measured by titrating 100 mL of liquid sample with diluted sulfuric acid to pH 4.5. The fluorescence spectra were obtained using a Shimadzu RF-5301PC fluorescence spectrometer with a 150 W xenon lamp source. Three-dimensional spectra were obtained by repeatedly measuring the emission spectra within a range of 280–600 nm, with excitation wavelengths from 220 to 400 nm at 10 nm intervals in the excitation domain. The spectra were then concatenated into an excitation–emission matrix. The analytical conditions were reported in detail elsewhere.[36]

The selected antibiotics were extracted (see ESI† Fig. S1) and analyzed using an Agilent 1200 high-performance liquid chromatograph. The quantification of antibiotics was performed by internal standard calibration using amoxicillin-d4, florfenicol-d3, and sulfamethoxazole-d4. The chromatographic separation was carried out on a Zorbax Eclipse Plus C18 column (2.1 × 150 mm, 3.5 μm particle size) with mobile phase A (0.1% formic acid and 10 mM ammonium formate in water) and mobile phase B (acetonitrile). The elution rate was set at 0.35 mL min^{-1}, and the injection volume of samples was 3 μL. Mass spectrometry detection was performed on an Agilent 6460 triple-quadrupole mass spectrometer equipped with a dual jet stream electrospray ionization source. Quantitative and qualitative analysis for the selected antibiotics were performed by multiple reaction monitoring (MRM). The MRM precursor and product ion pairs were selected at the highest peak intensities with different fragment and collision energies (see ESI† Table S1). Analytical performance was assessed in terms of the limit of detection (LOD), limit of quantification (LOQ), relative recoveries, relative standard deviation (RSD) of recoveries, and correlation coefficients (R^2) of calibration curves (see ESI Table† S2). Based on the chromatographic response, the LOD and LOQ for each analyte were determined at signal-to-noise ratio (S/N) of 3 and 10, respectively. The linear regression analysis was performed by plotting the peak area *versus* the analyte concentration and showed linear responses ($R^2 \geq 0.99$) to the calibration curves for all detected analytes. Recovery experiments were conducted by spiking tertiary effluent with standard solutions. The average and RSD of relative recoveries during extraction were calculated from triplicate spiking experiments. Spiked recoveries were obtained in the range of 78–127% for the selected antibiotics, with the exception of erythromycin (149 ± 6%). The matrix profiles can be affected by advanced oxidation, and thus a limitation of this study was that the relative recoveries of antibiotics were not evaluated in treated wastewaters.

3. Results and discussion

3.1. Removal of antibiotics by microalgae in livestock wastewater effluent

The algal treatment of livestock wastewater effluent not only increased the pH of wastewater as discussed later, but also removed 29% of total antibiotics for the first 12 h cultivation of *S. quadricauda* in the light without supplemented CO_2 (see Fig. 2). Among the twenty antibiotics examined in this study, nine were removed at over 75% (ceftiofur, penicillin-V, penicillin-G, tetracycline, tiamulin, erythromycin, chlortetracycline, tylosin, and oxytetracycline), eight decreased in concentration but by less than 10% (florfenicol, lincomycin, sulfamethazine, sulfamethoxazole, clopidol, trimethoprim, sulfathiazole, and sulfadiazine), and enrofloxacin was not reduced.

The algal treatment almost completely removed ceftiofur (>99%), penicillin-V (>98%), penicillin-G (>98%), and tetracycline (>96%). Amoxicillin and ampicillin are also antibiotics of the penicillin class like penicillin-V and penicillin-G, but they were not detected even in the raw wastewater without added microalgae, likely due to varying extents of decay during wastewater storage. An effective removal was also observed for tiamulin (88%), erythromycin (85%), chlortetracycline (84%), tylosin (79%), and oxytetracycline (77%). The high removal of macrolide antibiotics (erythromycin and tylosin) using microalgae was consistent with previous observations.[38,42] The significant removal ($p < 0.05$) of tetracycline antibiotics (chlortetracycline, tetracycline, and oxytetracycline) also

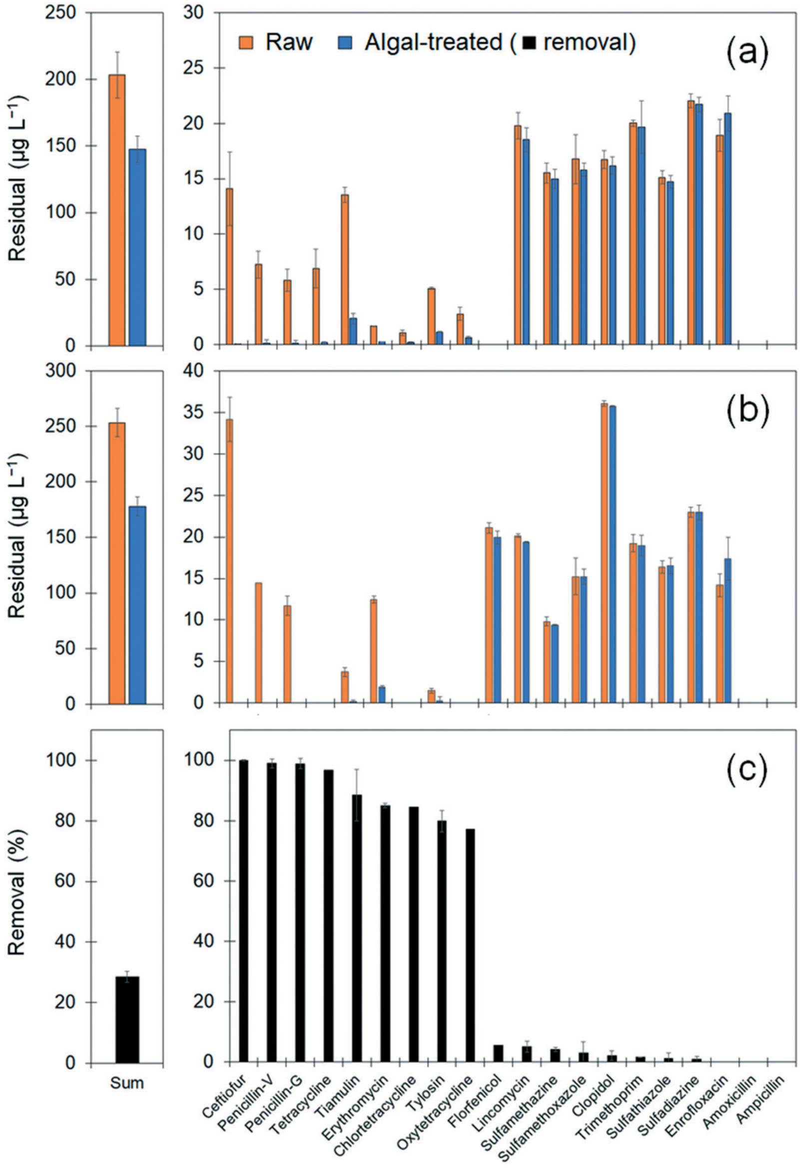

Fig. 2 The levels of selected antibiotics in raw nominally neutral and algal-treated alkaline livestock wastewaters. Twenty antibiotics were spiked in the tertiary effluent (which was collected twice on two different days (a) 1st and (b) 2nd from a livestock wastewater treatment plant), and the mixture was designated as raw wastewater. That sample was divided into two aliquots, and algal treatment was conducted using one of those for which *S. quadricauda* was cultivated in the light for 12 h. The antibiotics are displayed in decreasing order of their removal (c).

coincided with results reported in previous studies[47–49] and could be attributed to photo-degradation, hydrolysis, and bio-sorption during the algal treatment. Zhao *et al.* (2013)[50] reported that the photolytic degradation of oxytetracycline was accelerated with increasing pH even under visible light due to a red shift of the absorption spectrum at high pH. In contrast to the macrolide and tetracycline antibiotics, none of sulfonamide antibiotics (sulfathiazole, sulfamethoxazole, sulfamethazine, sulfadiazine, and trimethoprim) was removed by more than 5%. The tetracycline antibiotics are amphoteric and unstable under strong alkaline and acidic conditions. In this study, the removal of antibiotics was independent of their hydrophobicity during the algal treatment. Sulfamethoxazole and trimethoprim are hydrophilic chemicals like tetracycline, but their removal was much lower than that of tetracycline. Some studies showed a 14–20% reduction of sulfamethoxazole, mostly because of photolysis for a long incubation time.[47,49] Similarly, trimethoprim was highly resistant to biodegradation by algae.[40] The removal could also differ with algal species, water matrix, initial antibiotics concentration, photolysis, hydrolysis, and operational parameters (*e.g.*, temperature, retention time, and light intensity). This study showed the feasibility of using microalgae to remove certain chemicals during refractory wastewater treatment, although there is little information in the literature about algae-mediated removal of ceftiofur, penicillin-V, or penicillin-G.

3.2. The effects of algal pretreatment on the removal of antibiotics with UV and UV/H_2O_2

Phototrophs use CO_2 and simultaneously release hydroxide ions (OH^-) in aqueous medium.[51,52] The pH of the livestock wastewater effluent increased from 7.1 to 10.4 for the first 12 h cultivation of *S. quadricauda* in the light without aeration (see ESI† Fig. S2). We investigated the pH effects on the degradation of selected antibiotics upon various oxidation treatments of the effluent with two different pH values (*i.e.*, raw nominally-neutral and algal-treated alkaline). The UVC removed the selected antibiotics better for algal-treated wastewater (see Fig. 3b). The removal of total antibiotics increased from 51% to 72% as the UVC dose increased (500 to 2000 mJ cm^{-2}), and further increased up to 81% with added H_2O_2. The highest removal difference between raw neutral and algal-treated alkaline wastewaters for the examined antibiotics was recorded for clopidol (see Fig. 4a). Alkaline UVC treatment achieved >95% removal of clopidol regardless of H_2O_2 addition, which was much greater than that achieved by neutral UVC treatment (71–74%). Unlike clopidol, enrofloxacin, sulfadiazine, sulfamethoxazole, and sulfathiazole showed a pH-independent removal tendency in both UVC and UVC/H_2O_2 treatments (see ESI† Fig. S3).

Fig. 3 also shows that the extent of antibiotics removal was proportional to UVC dose (up to 2000 mJ cm^2) applied to the wastewater effluent. When using an identical dose of UVC, the addition of 0.5 mM H_2O_2 to alkaline wastewater resulted in synergistic effects on the removal of some antibiotics (*e.g.*, lincomycin, sulfamethazine, and trimethoprim). For example, the removal of sulfamethazine ranged from 75 to 79% with a UVC dose of 2000 mJ cm^2 regardless of pH values examined (see Fig. 4b). This increased up to 88% when UVC was applied to alkaline wastewater with added 0.5 mM H_2O_2, but did not increase for neutral UVC/H_2O_2 treatment. Likewise, the removal of lincomycin (see Fig. 4c) and trimethoprim (see Fig. 4d) was much greater when UVC/H_2O_2 was applied to alkaline wastewater. These results were consistent with the measurements of H_2O_2 consumed during UVC treatment. The consumption of H_2O_2 was higher in alkaline UVC treatment than in neutral UVC treatment (see Fig. 5), suggesting a higher photolytic generation of OH radicals. This result was attributed to a partial dissociation of H_2O_2 ($pK_a \approx 11.6$) into hydroperoxide ions (HO_2^-) at alkaline pH conditions. Some investigators have previously reported similar results.[28,31,53,54] In the UVC/H_2O_2 process, HO_2^- has about 10 times the UV molar absorptivity at 254 nm that H_2O_2 has (17.9–19.6 L mol^{-1} cm^{-1}).[30] Therefore, UVC/H_2O_2 could be more effective at higher pH, especially if the background wastewater matrix absorbs a lot of UVC light.

Ceftiofur, penicillin-V, and tylosin were easily degraded when raw wastewater was treated with either UVC or UVC/H_2O_2 (see ESI† Fig. S4), and most of them could also be removed by algal treatment, as discussed in section 3.1. Regarding the necessity of pretreatment with microalgae, it is worthwhile noting that some chemicals (*e.g.*, chlortetracycline, erythromycin, oxytetracycline, penicillin-G, tetracycline, and tiamulin), which showed a moderate to strong resistance toward both UVC and UVC/H_2O_2, were effectively removed by algal treatment, as discussed earlier (see ESI† Fig. S5). Although the addition of 0.5 mM H_2O_2 to raw wastewater improved the performance of UVC treatment under the given experimental conditions, about half of those antibiotics still remained. That is, the treatment of livestock wastewater effluent with both UVC and UVC/H_2O_2 achieved 41–61% removal of chlortetracycline, 15–17% removal of erythromycin, 50–64% removal of oxytetracycline, 25–49% removal of penicillin-G, 57–58% removal of tetracycline, and 40–55% removal of tiamulin; thus, they would mostly remain even after the UVC treatment, unless algal treatment was applied.

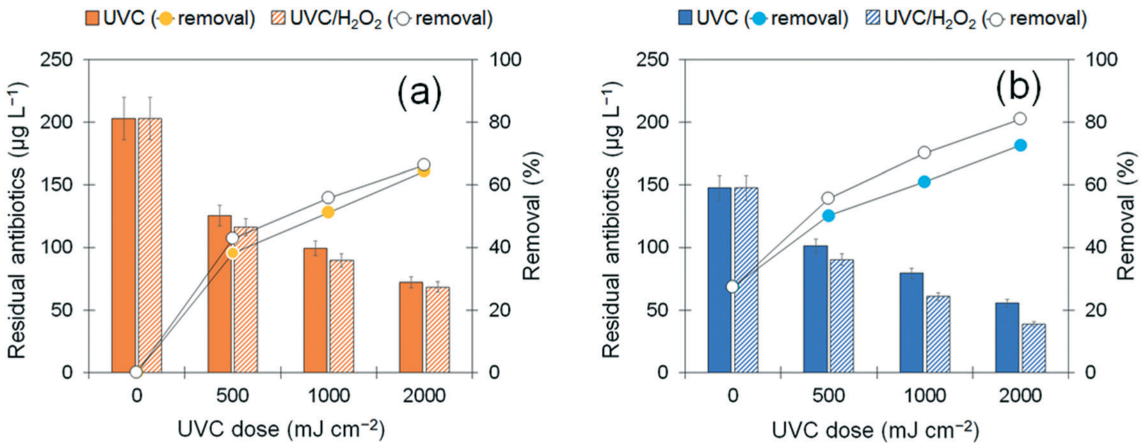

Fig. 3 Treatment of raw neutral (a) and algal-treated alkaline (b) livestock wastewater using two different methods (UVC *vs.* UVC/H_2O_2). For UVC/H_2O_2, H_2O_2 was added to raw and algal-treated wastewater in a final concentration of 0.5 mM just before UVC irradiation (0, 500, 1000, and 2000 mJ cm^{-2}). The percent removals in panel (b) include the removal (27%) achieved by algal-treatment prior to UVC-based treatment of livestock wastewater.

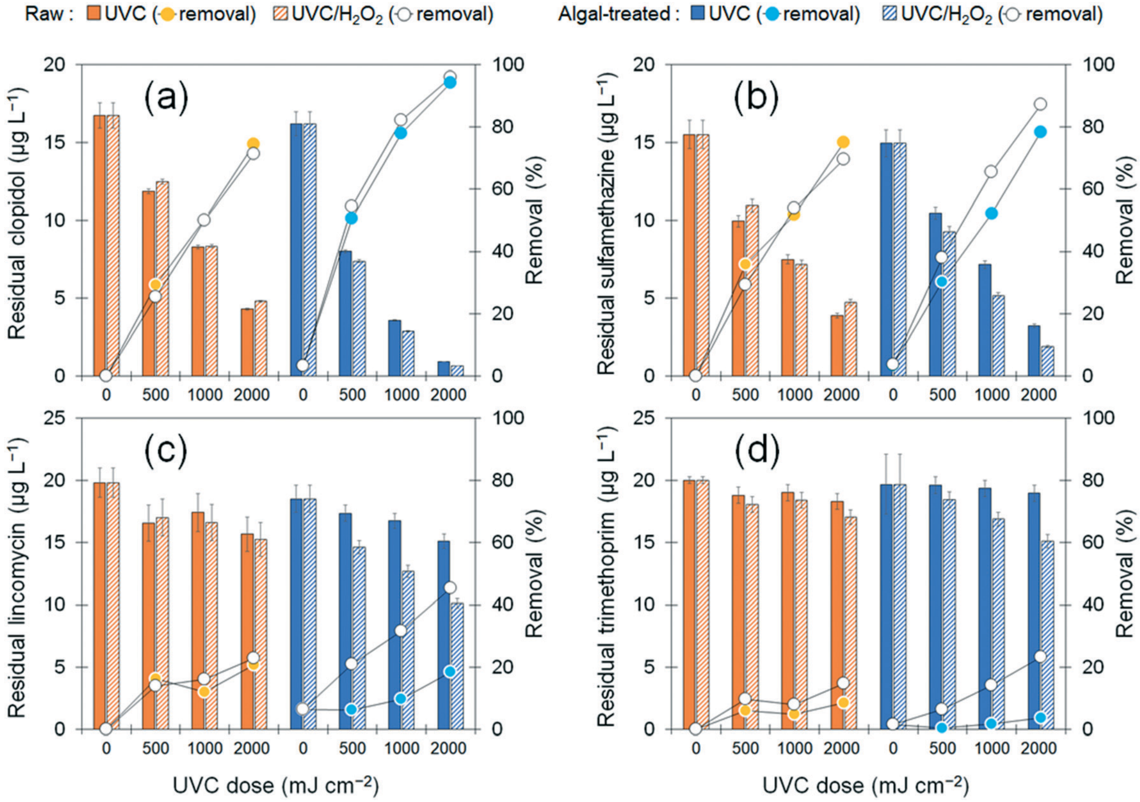

Fig. 4 Treatment of livestock wastewater using two different methods (UVC *vs.* UVC/H_2O_2) and the effects of algal pretreatment on the removal of clopidol (a), sulfamethazine (b), lincomycin (c), and trimethoprim (d). For UVC/H_2O_2, H_2O_2 was added to raw and algal-treated wastewater in a final concentration of 0.5 mM just before UVC irradiation (0, 500, 1000, and 2000 mJ cm^{-2}). For UVC-based treatment of algal-treated wastewater, the percent removals of the selected antibiotics include the removal achieved by algal-treatment.

3.3. Improved removal of antibiotics using ozonation prior to UVC and UVC/H_2O_2

In this study, we applied ozonation to the oxidation of livestock wastewater effluent ahead of UVC treatment. UVC treatment was carried out after dissolved O_3 was consumed completely; thus, photolytic O_3 decomposition was not discussed in this section. The ozonation followed by UVC treatment (O_3 + UVC and O_3 + UVC/H_2O_2) removed >99% of total antibiotics in the effluent and the pH effect was negligible (see Fig. 6). Both UVC and UVC/H_2O_2 further removed antibiotic residues in the ozonated wastewater, but less than 1% of the initial antibiotics. Although the degradation of selected antibiotics was dominated by ozonation, this should be complemented for further removal of accumulated antibiotics resistant to ozone oxidation considering the total mass of antibiotics released to the water environment. For that purpose, subsequent UVC treatment is still considered valid, and O_3/H_2O_2 could be applied as AOPs for process simplicity. However, the removal of antibiotics by ozonation was considerably decreased by addition of H_2O_2. Fig. 7 shows that the addition of H_2O_2 (up to 1 mM) to the effluent resulted in decreased removal of antibiotics upon ozonation regardless of pH values examined. The residual concentration of total antibiotics was always higher when ozonating the wastewater effluent with added H_2O_2; especially, clopidol was highly resistant to the oxidation with O_3/H_2O_2, probably because there were enough organic promoters in the effluent to promote O_3 chain reactions. Therefore, H_2O_2 added as an external promoter could reversely scavenge OH radicals produced in the chain reactions.[55,56]

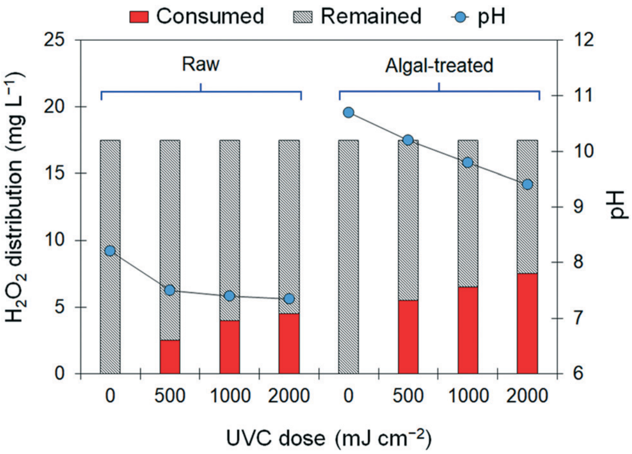

Fig. 5 The H_2O_2 consumption and the pH change from UVC irradiation (0, 500, 1000, and 2000 mJ cm^{-2}) of raw and algal-treated livestock wastewater with added 0.5 mM H_2O_2.

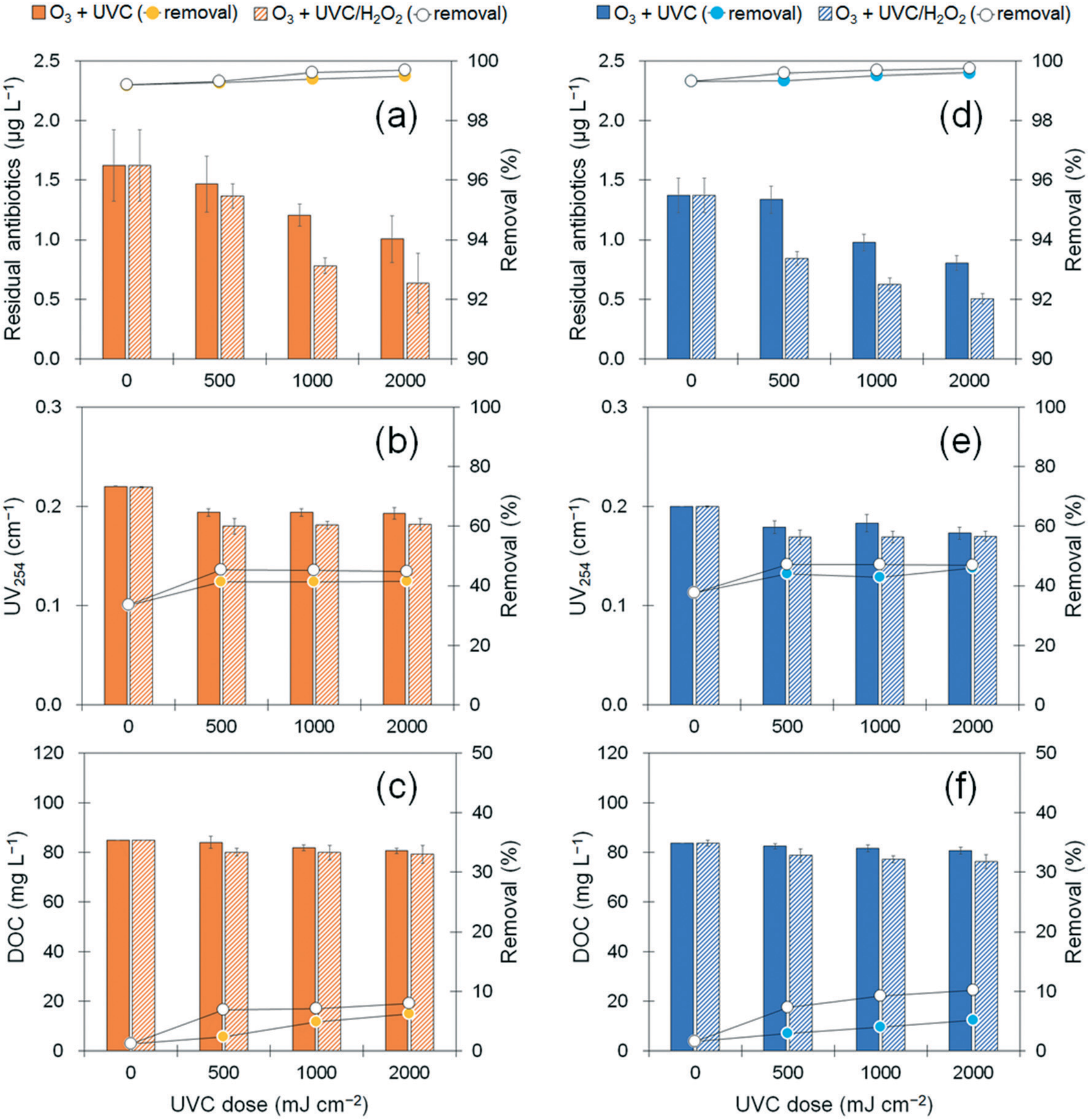

Fig. 6 Comparison of two different treatment methods (O_3 + UVC *vs.* O_3 + UVC/H_2O_2) in the removal of antibiotics, DOC, and UV_{254} from raw neutral (a–c) and algal-treated alkaline (d–f) livestock wastewater. Ozonation (1.5 mg O_3 per mg C, 10 min) was followed by UVC irradiation (0, 500, 1000, and 2000 mJ cm^{-2}). For O_3 + UVC/H_2O_2, H_2O_2 was added to raw and algal-treated wastewater in a final concentration of 0.5 mM just before UVC irradiation.

Separate ozonation test was conducted and the result of antibiotics removal is illustrated in Fig. 8, in which changes in water quality parameters are also shown as a function of ozonation continued for up to 10 min (equivalent to ~1.8 mg O_3 per mg C). A significant removal ($p < 0.05$) of selected antibiotics was achieved by algal treatment, as discussed in section 3.1 (see Fig. 2). Regardless of pH values, >90% removal of total antibiotics was achieved by ozonation for 5 min or longer (see Fig. 8a). Florfenicol accounted for the majority of antibiotic residues in the ozonated wastewater (see ESI† Fig. S6), indicating the recalcitrant characteristics of florfenicol toward ozonation. The removal of florfenicol ranged from only 16 to 34% as a function of O_3 dose (see Fig. 8b). A similar limitation in the degradation of florfenicol with ozonation has been previously reported.[57] Fig. 8b also shows a remarkable increase in the degradability of florfenicol when ozonating algal-treated wastewater, achieving 30–51% removal. Although the fate of florfenicol during O_3-based oxidation is sparse in the literature, we speculate that the oxidation of florfenicol was improved by the faster generation of OH radicals at elevated pH levels, since pH varied with ozonation. The alkaline ozonation decreased the pH of wastewater to a much greater extent than did that observed for neutral ozonation, indicating a higher consumption of OH^- catalyzing the decomposition of O_3 under alkaline conditions. In general, the reactions of organic compounds with OH radicals, close to the diffusion-controlled limit (~10^{10} M^{-1} s^{-1}), are much faster than are

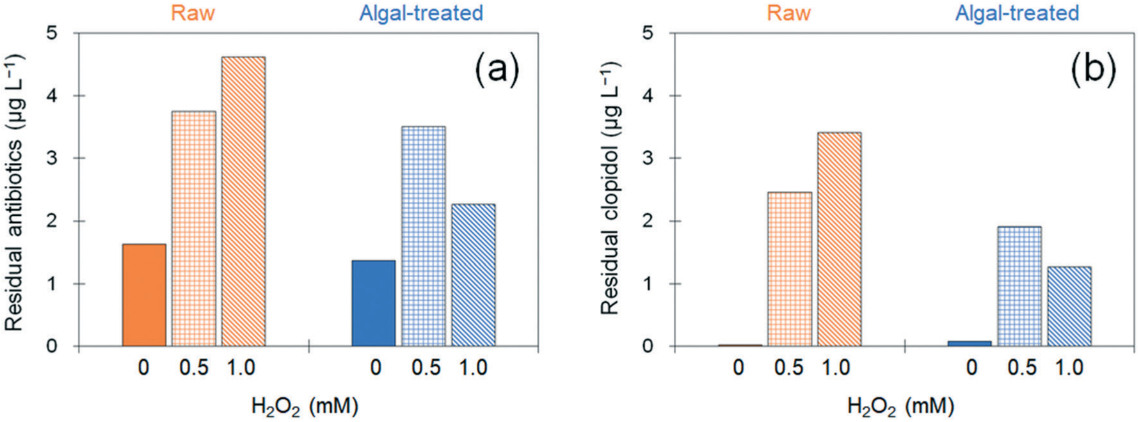

Fig. 7 The effects of H_2O_2 concentrations (0, 0.5, and 1 mM) on the removal of total antibiotics (a) and clopidol (b) upon the ozonation (1.5 mg O_3 per mg C, 10 min) of raw neutral and algal-treated alkaline livestock wastewater.

those with O_3 molecules.[58] Decrement in the pH of wastewater during alkaline ozonation could thus be greater with accumulated carboxylic acids as oxidation byproducts.[31] Considering the concentration of effluent organic matter (5.8–7.2 mM as C) in the wastewater effluent and its reaction with OH radicals (1.4–4.5 × 10^8 M C^{-1}),[59] the radical scavenging of alkalinity (0.5–0.6 mM as HCO_3^-) could be negligible in this study. Higher degradation of antibiotics during ozonation at alkaline pH has been previously reported.[28,32,33]

3.4. The effects of algal treatment on the fate of effluent organic matter by ozonation

The ozonation of livestock wastewater effluent with two different pH values (raw nominally-neutral and algal-treated alkaline, see Fig. 8c) was conducted for up to 10 min (equivalent to ~1.8 mg O_3 per mg C). A slight removal of dissolved organic components was found after the algal treatment alone under autotrophic conditions (see Fig. 6 and 8), which could be associated with a wide variety of non-metabolic processes (physicochemical sorption, ion exchange, complexation, and so forth) on the surface of the algal cells.[60,61] Decreases in the SUVA, color, UV_{254}, COD, and DOC were found upon ozonation, and the removal of those parameters was always higher in alkaline ozonation than in neutral ozonation (see Fig. 8d–h). The chromaticity decreased steeply, but was not completely removed, for the given time of ozonation. The UV_{254} decreased stepwise as the O_3 dose was increased, whereas DOC change was negligible under the given ozonation conditions. Effluent organic matter was also determined as COD, and the removal was relatively higher than was the DOC removal, probably because of the oxidation of reduced inorganic species, which could also be oxidized quantitatively under the test conditions for COD measurement.

The reduction of UV_{254} was associated with the degradation of aromatic compounds and subsequent conversion to low-molecular substances such as ketone, carboxylic acids, and aldehydes.[62,63] The low abatement of DOC could be attributed to accumulated low-molecular compounds, which are generally not susceptible to O_3 and OH radicals and thus are difficult to completely mineralize. Among the integrated oxidation processes examined in this study, O_3 + UVC/H_2O_2 achieved the greatest removal of the DOC from algal-treated wastewater, but it accounted for only 11% (see Fig. 6f). Numerous studies have shown greater removal of UV-absorbing materials than of effluent organic matter quantified as DOC during ozonation, resulting in a decrease in the level of SUVA.[64,65] The observations were also consistent with the results shown for the fluorescence measurements of this study. Fig. 9 shows that the fluorescent moieties of effluent organic matter dramatically decreased with increasing O_3 dose regardless of pH values examined. The fluorescence spectra of organic components in livestock wastewater effluent show a typical feature of humic substances, and a negligible change in the fluorescence intensity was found after the 12 h cultivation of microalgae in the effluent. Moreover, tryptophan-like components that are typically associated with microbial activities were found in neither raw nor algal-treated wastewater. The profile of dissolved organic matter can be changed by extracellular polymeric substances formed during biological metabolism and biomass decay,[36,66] but there were no observations supporting such changes in this study. The fluorophores originating from humic-like substances are generally enriched with aromatic constituents,[67–69] which are preferentially reactive to electrophilic oxidants (*e.g.*, O_3).[58] The effluent treated by alkaline ozonation showed a relatively lower intensity of the fluorophores, which coincided with a greater reduction in UV-absorbing components than when neutral ozonation was done (see Fig. 8f). This indicates either the degradation or mineralization of the corresponding fluorophores or functional groups that are present in the chemical structure of the fluorescent organic matter.[70,71]

For alleviating the burden of receiving water bodies, quaternary-treated wastewater can be further treated with biologically-active processes. Biological treatment following

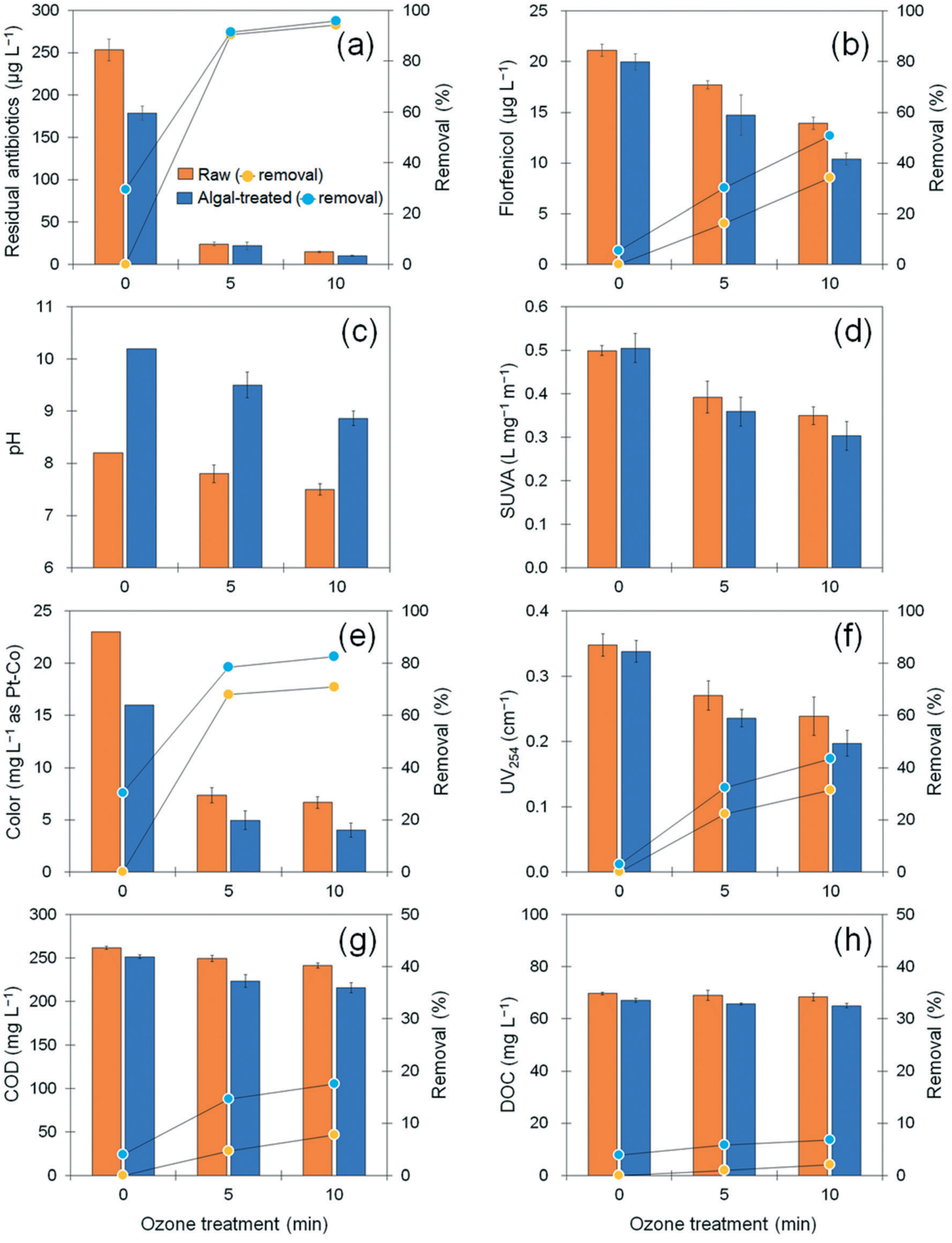

Fig. 8 The changes in total antibiotics (a), florfenicol (b), pH (c), SUVA (d), color (e), UV_{254} (f), COD (g), and DOC (h) upon ozonation. The ozonation of raw and algal-treated livestock wastewater was carried out for up to 10 min (equivalent to ~1.8 mg O_3 per mg C). For ozonation of algal-treated wastewater, the percent removals of antibiotics and organic-related parameters include the removal achieved by algal-treatment.

ozone oxidation is a well-established strategy to effectively remove effluent organic matter by converting refractory organic compounds into biodegradable fractions, rather than by completely oxidizing them. We previously determined that pretreatment with proper levels of O_3 substantially improves the bioavailability of organic matter for mixotrophic microalgae in the bioremediation of piggery wastewater.[64] In the previous study, we also noticed that *S. quadricauda* performed mixotrophic growth depending on culture conditions, in addition to the common photoautotrophic

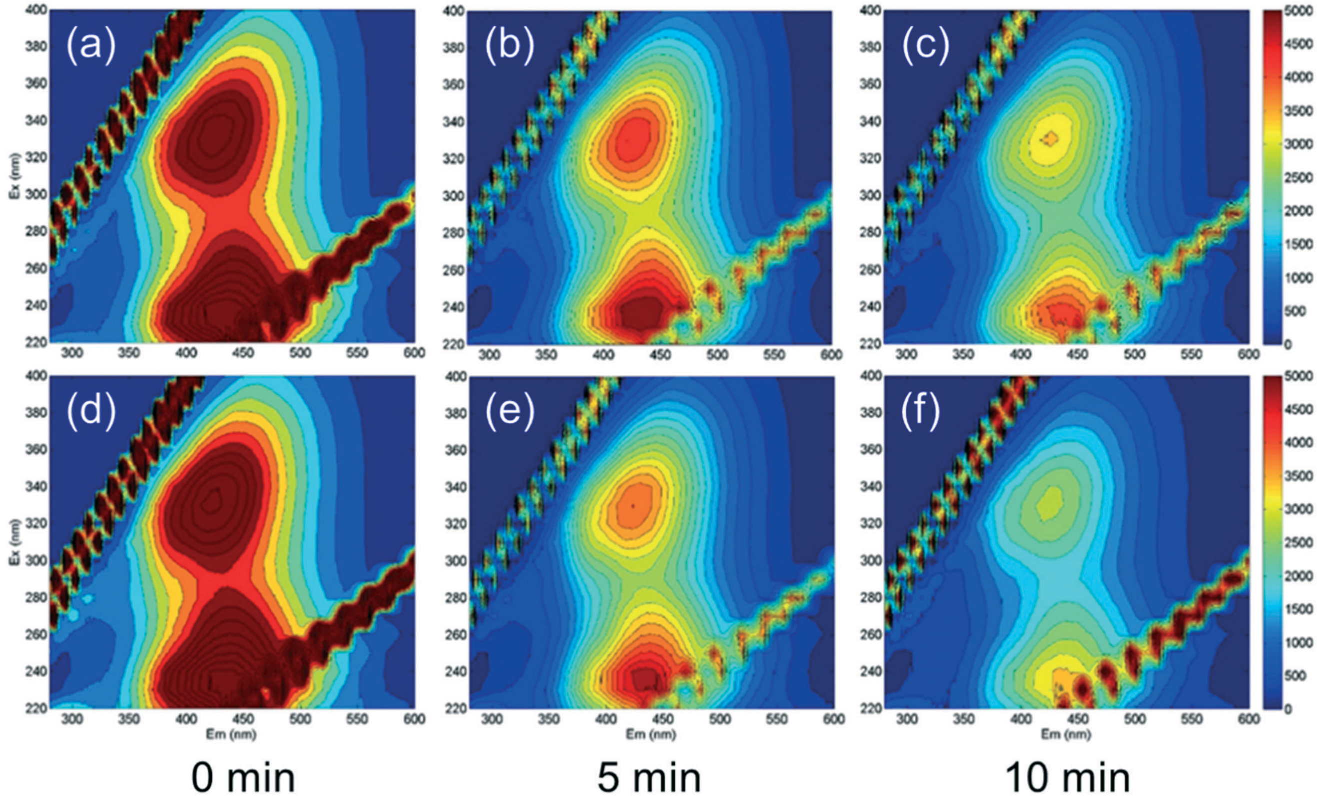

Fig. 9 The fluorescence excitation–emission matrices of effluent organic matter remaining after ozonation of raw neutral (a–c) and algal-treated alkaline (d–f) livestock wastewater effluent at three different doses of 0, 0.9, and 1.8 mg O_3 per mg C (equivalent to 0, 5, and 10 min).

growth when using CO_2 as the sole carbon source. This observation reveals the high adaptability of switchable algal treatment to fluctuating livestock wastewater quality, indicating the operational diversity of algal treatment to remove biodegradable organic components in a single biological reactor.

4. Conclusions

Algal treatment was combined with subsequent ozonation and/or photolytic oxidation to improve the antibiotics removal and refractory organics degradation in livestock wastewater effluent. The algal treatment removed effectively some antibiotics (chlortetracycline, erythromycin, oxytetracycline, penicillin-G, tetracycline, and tiamulin) that were highly resistant to UVC treatment (UVC and UVC/H_2O_2). In addition to reducing antibiotics directly, the algae-induced alkaline conditions ($pH > 10$) were synergistic with H_2O_2 (0.5 mM) in the removal of sulfamethazine, lincomycin and trimethoprim during UVC irradiation (up to 2000 mJ cm^{-2}). Similarly, the removal of clopidol by UVC treatment was always higher in algal-treated alkaline wastewater than in raw neutral wastewater, and the degradation of O_3-resistant florfenicol was improved upon ozonation (~1.8 mg O_3 per mg C) in the alkaline wastewater.

This study also corroborated the effectiveness of integrating ozonation and UVC treatment in removing antibiotics from livestock waster effluent. The ozonation dominated the degradation of selected antibiotics, but required further removal of accumulated antibiotics resistant to ozone oxidation. Considering the total mass of antibiotics released to water bodies, subsequent UVC treatment is still considered valid as a promising complementary strategy for robust removal of accumulated antibiotics resistant to ozone oxidation. O_3/H_2O_2 could be applied for process simplicity; however, the removal of antibiotics by ozonation was considerably inhibited by addition of H_2O_2, whereas UVC performance was generally improved by adding H_2O_2.

Our results indicate that the oxidation of algal-treated alkaline wastewater could be a better option to effectively remove antibiotics remaining in secondary- or tertiary-treated effluent, depending on the physicochemical characteristics of antibiotics. In contrast to increasing pH of wastewater with caustic chemicals, the alternative strategy proposed in this study could make alkaline oxidation realistic and economically more feasible. Moreover, this algae-induced alkalization is readily convertible to mixotrophic biotreatment for removal of carbonaceous organic residues in oxidized wastewater effluent. Despite quaternary treatment using oxidation-based processes, effluent organic matter (determined as DOC) mostly remained. In addition to recarbonation of the treated wastewater, a biologically active treatment process should be considered for further removal of the biodegradable organic fraction that was identified by characterizing effluent organic matter.

Conflicts of interest

There are no conflicts of interest to declare.

Acknowledgements

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