Electronic Supplementary Material (ESI) for Environmental Science: Water Research & Technology.


# Supporting Information

## Quaternary treatment with algae-assisted oxidation for antibiotics removal and refractory organics degradation in livestock wastewater effluent

Table S1. LC-MS/MS analysis of selected antibiotics.

| Analyte | Precursor ion (m/z) | Product ion (m/z) | Fragmentor (V) | Collision energy (eV) | Polarity | Retention time (min) |
|---|---|---|---|---|---|---|
| Amoxicillin | 397.8 | 380.9 | 100 | 6 | Positive | 6.36 |
| | | 348.9 | | 15 | | |
| Ampicillin | 381.8 | 332.9 | 100 | 15 | Positive | 7.53 |
| | | 106 | | 20 | | |
| Ceftiofur | 523.7 | 240.9 | 150 | 15 | Positive | 9.64 |
| | | 209.7 | | 20 | | |
| Chlortetracycline | 478.8 | 443.8 | 100 | 20 | Positive | 8.85 |
| | | 154 | | 30 | | |
| Clopidol | 191.9 | 101 | 150 | 30 | Positive | 4.31 |
| | | 87 | | 35 | | |
| Enrofloxacin | 359.9 | 341.9 | 150 | 20 | Positive | 7.66 |
| | | 316 | | 15 | | |
| Erythromycin | 734.2 | 574.8 | 150 | 10 | Positive | 10.34 |
| | | 157.5 | | 30 | | |
| Florfenicol | 355.7 | 335.8 | 100 | 4 | Negative | 9.47 |
| | | 184.9 | | 15 | | |
| Lincomycin | 406.9 | 358.9 | 150 | 15 | Positive | 5.10 |
| | | 126 | | 30 | | |
| Oxytetracycline | 460.9 | 442.9 | 90 | 10 | Positive | 6.93 |
| | | 425.8 | | 15 | | |
| Penicillin-G | 334.9 | 175.9 | 200 | 10 | Positive | 10.81 |
| | | 160 | | 15 | | |
| Penicillin-V | 351 | 192 | 100 | 5 | Positive | 11.56 |
| | | 159.9 | | 5 | | |
| Sulfadiazine | 250.8 | 155.9 | 100 | 10 | Positive | 5.01 |
| | | 108 | | 20 | | |
| Sulfamethazine | 278.9 | 185.9 | 100 | 15 | Positive | 7.55 |
| | | 155.9 | | 15 | | |
| Sulfamethoxazole | 253.8 | 155.9 | 100 | 10 | Positive | 9.41 |
| | | 92 | | 25 | | |
| Sulfathiazole | 255.8 | 155.9 | 100 | 10 | Positive | 5.91 |
| | | 108 | | 20 | | |
| Tetracycline | 444.8 | 409.8 | 150 | 20 | Positive | 7.43 |
| | | 153.8 | | 25 | | |
| Tiamulin | 494.1 | 192 | 150 | 20 | Positive | 11.49 |
| | | 118.9 | | 40 | | |
| Trimethoprim | 290.9 | 229.9 | 150 | 20 | Positive | 6.53 |
| | | 123 | | 25 | | |
| Tylosin | 916.1 | 771.9 | 200 | 25 | Positive | 10.72 |
| | | 174 | | 37 | | |
| Amoxicillin-d4 | 401.8 | 384.8 | 100 | 5 | Positive | 6.36 |
| | | 352.8 | | 15 | | |
| Florfenicol-d3 | 358.7 | 338.6 | 100 | 5 | Negative | 9.47 |
| | | 187.8 | | 15 | | |
| Sulfamethoxazole-d4 | 257.4 | 159.4 | 100 | 10 | Positive | 9.41 |
| | | 95.8 | | 25 | | |

Table S2. Limit of detection (LOD), limit of quantification (LOQ), relative recoveries, relative standard deviation (RSD) of recoveries, and correlation coefficients ($R^2$) of calibration curves for antibiotics examined in this study.

| Analyte | LOD ($\mu g\ L^{-1}$) | LOQ ($\mu g\ L^{-1}$) | Recovery (%) | RSD (%) | $R^2$ |
|---|---|---|---|---|---|
| Amoxicillin | 2.4478 | 7.7954 | 86.48 | 2.0853 | 0.999 |
| Ampicillin | 1.8591 | 5.9207 | 78.60 | 0.6864 | 0.996 |
| Ceftiofur | 1.9056 | 6.0687 | 100.89 | 5.5949 | 0.999 |
| Chlortetracycline | 1.0123 | 3.2239 | 96.81 | 3.2409 | 0.994 |
| Clopidol | 0.1537 | 0.4895 | 97.38 | 1.8539 | 0.998 |
| Enrofloxacin | 0.2490 | 0.7930 | 107.60 | 6.8056 | 0.998 |
| Erythromycin | 0.2042 | 0.6503 | 149.90 | 6.3993 | 0.999 |
| Florfenicol | 0.2900 | 0.9235 | 99.75 | 1.7638 | 0.999 |
| Lincomycin | 0.0565 | 0.1798 | 92.52 | 0.6704 | 0.999 |
| Oxytetracycline | 1.1001 | 3.5035 | 127.59 | 1.3126 | 0.990 |
| Penicillin-G | 1.6050 | 5.1114 | 98.84 | 4.7310 | 0.997 |
| Penicillin-V | 2.1200 | 6.751 | 97.76 | 7.1800 | 0.996 |
| Sulfadiazine | 1.3924 | 4.4343 | 108.21 | 7.9925 | 0.990 |
| Sulfamethazine | 0.4494 | 1.4311 | 85.98 | 1.9118 | 0.993 |
| Sulfamethoxazole | 0.3971 | 1.2646 | 95.62 | 2.9220 | 0.995 |
| Sulfathiazole | 1.8378 | 5.8530 | 101.89 | 2.6857 | 0.996 |
| Tetracycline | 1.0240 | 3.2600 | 106.66 | 2.0200 | 0.995 |
| Tiamulin | 0.3743 | 1.1919 | 83.62 | 8.3130 | 0.999 |
| Trimethoprim | 0.9678 | 3.0821 | 100.99 | 0.4675 | 0.999 |
| Tylosin | 0.0304 | 0.0968 | 80.97 | 5.5333 | 0.999 |

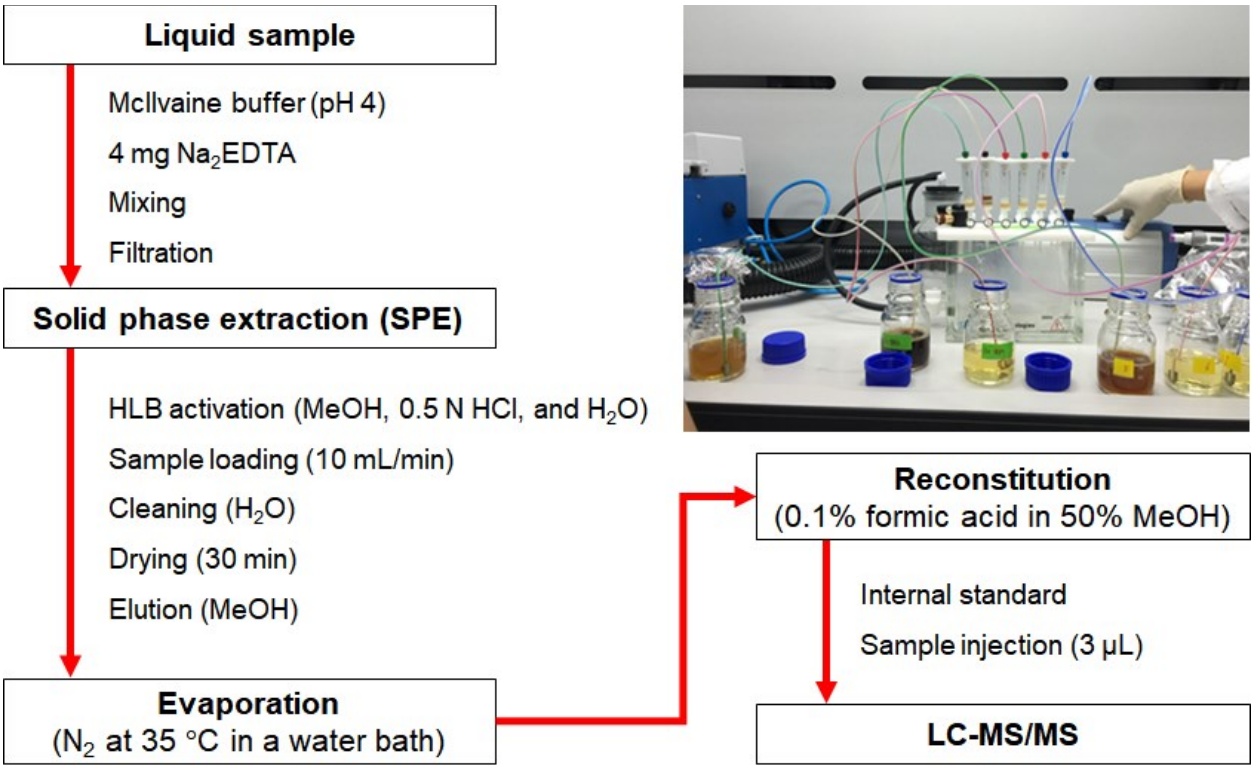


Fig. S1. Sample preparation (extraction and clean-up) procedure for LC-MS/MS analysis of selected antibiotics.

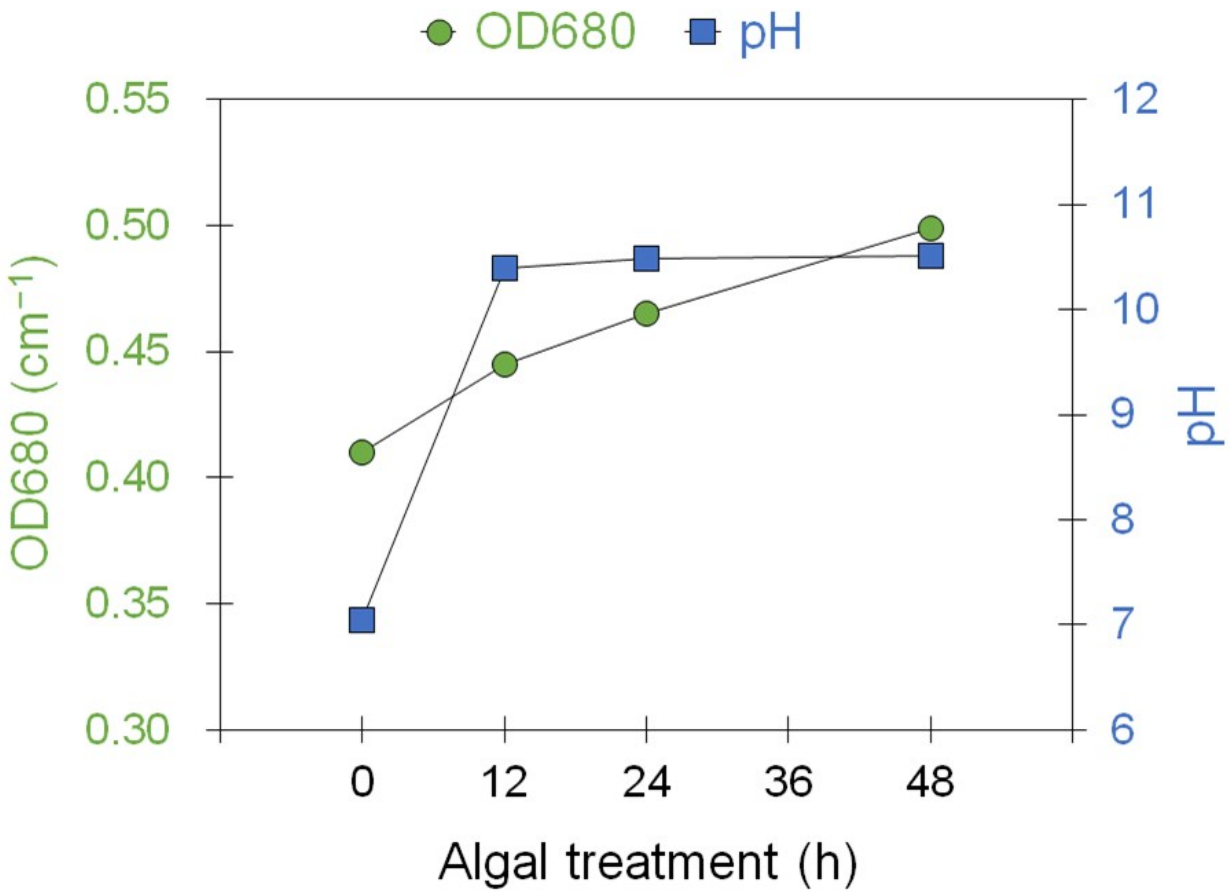


Fig. S2. The growth of algal biomass and the associated pH increase in livestock wastewater effluent. A microalga *Scenedesmus quadricauda* was incubated in the light for 48 h without supplemented $CO_2$. The cell growth was measured by monitoring the optical density at 680 nm (OD680).

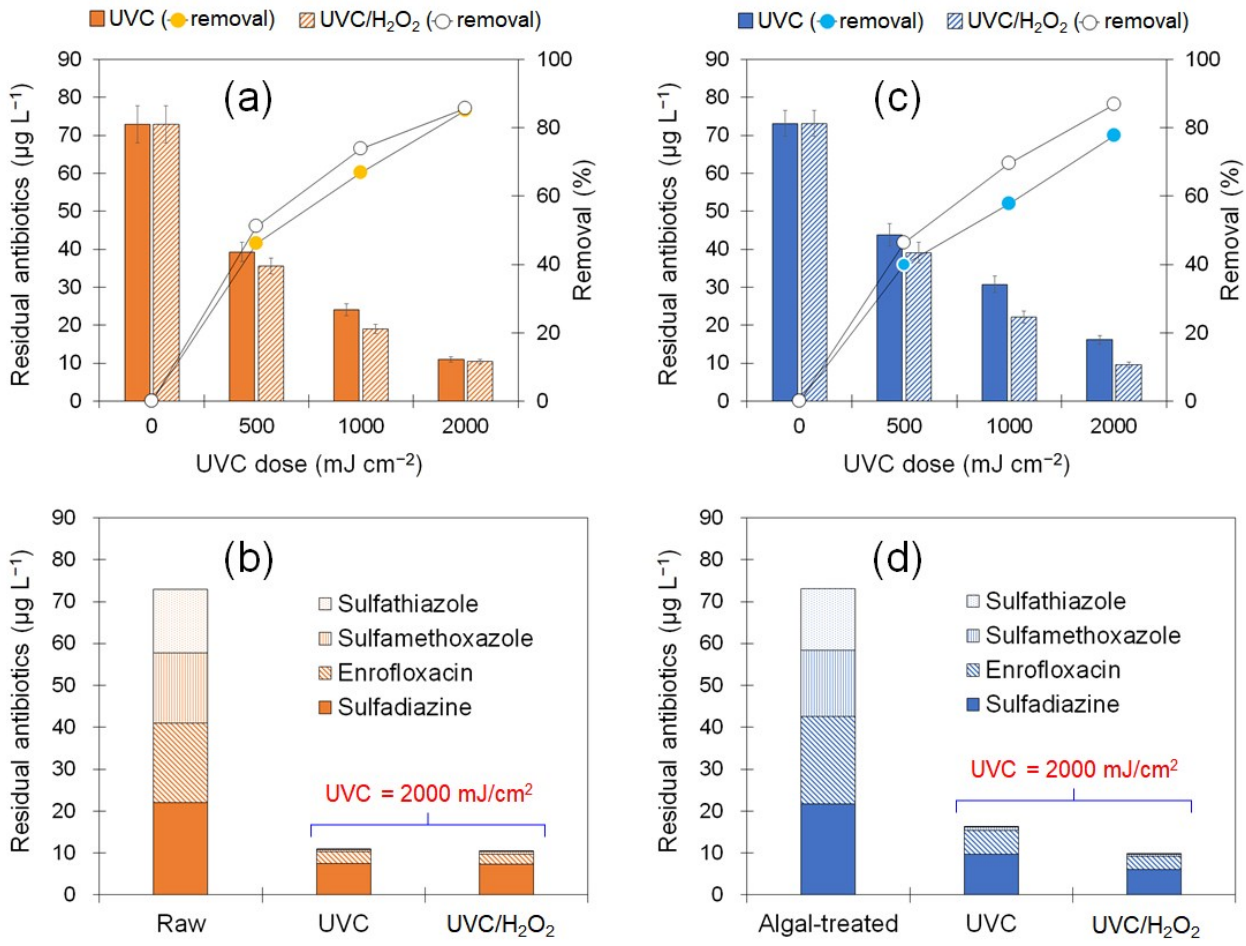


Fig. S3. Treatment of raw neutral (a–b) and algal-treated alkaline (c–d) livestock wastewater using two different methods (UVC Vs. UVC/$H_2O_2$). For UVC/$H_2O_2$, $H_2O_2$ was added to raw and algal-treated wastewater in a final concentration of 0.5 mM just before UVC irradiation (0, 500, 1000, and 2000 mJ $cm^{-2}$). Fig. 3 in the main text was reconstructed here in terms of enrofloxacin, sulfadiazine, sulfamethoxazole, and sulfathiazole. The concentrations of enrofloxacin, sulfadiazine, sulfamethoxazole, and sulfathiazole decreased with increasing UVC dose, and the removal tendency of those antibiotics was relatively independent of the initial pH of the wastewater sample. The percent removals of selected antibiotics in panel (c) include the removal (nearly zero) achieved by algal-treatment.

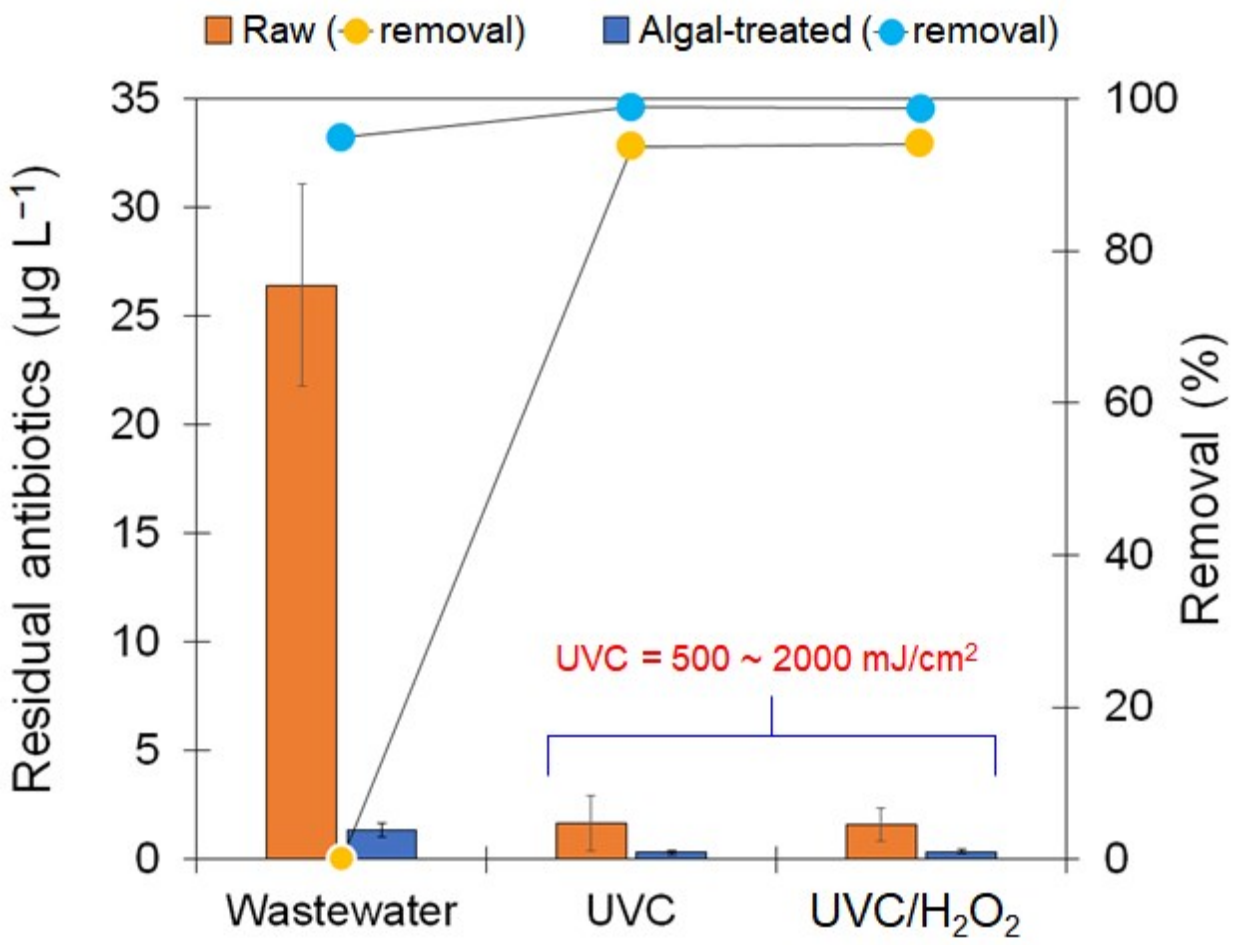


Fig. S4. Treatment of raw and algal-treated livestock wastewater using two different methods (UVC Vs. UVC/$H_2O_2$). For UVC/$H_2O_2$, $H_2O_2$ was added to raw and algal-treated wastewater in a final concentration of 0.5 mM just before UVC irradiation (0, 500, 1000, and 2000 mJ $cm^{-2}$). Fig. 3 in the main text was reconstructed here in terms of ceftiofur, penicillin-V, and tylosin. The total concentration of ceftiofur, penicillin-V, and tylosin in the raw wastewater was 26.4 μg $L^{-1}$, which was effectively removed by all treatment methods proposed in this study. For UVC-based treatment of algal-treated wastewater, the percent removals of the selected antibiotics include the removal (95%) achieved by algal-treatment.

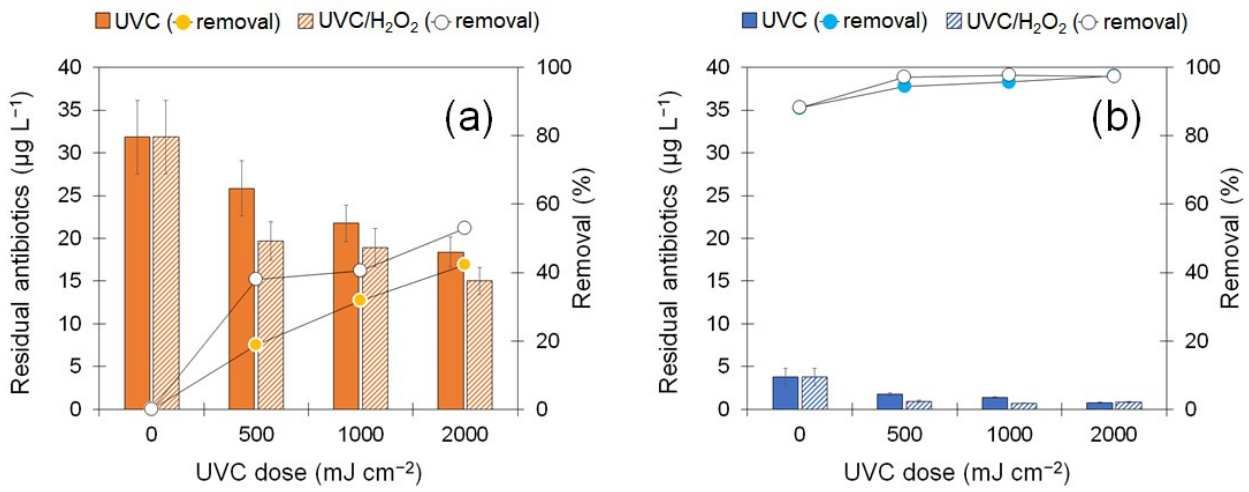


Fig. S5. Treatment of raw neutral (a) and algal-treated alkaline (b) livestock wastewater using two different methods (UVC Vs. UVC/$H_2O_2$). For UVC/$H_2O_2$, $H_2O_2$ was added to raw and algal-treated wastewater in a final concentration of 0.5 mM just before UVC irradiation (0, 500, 1000, and 2000 mJ $cm^{-2}$). Fig. 3 in the main text was reconstructed here in terms of chlortetracycline, erythromycin, oxytetracycline, penicillin-G, tetracycline, and tiamulin. The antibiotics were resistant to both UVC and UVC/$H_2O_2$, but it could be easily removed by algal treatment alone. The percent removals in panel (b) include the removal (88%) achieved by algal-treatment prior to UVC-based treatment of livestock wastewater.

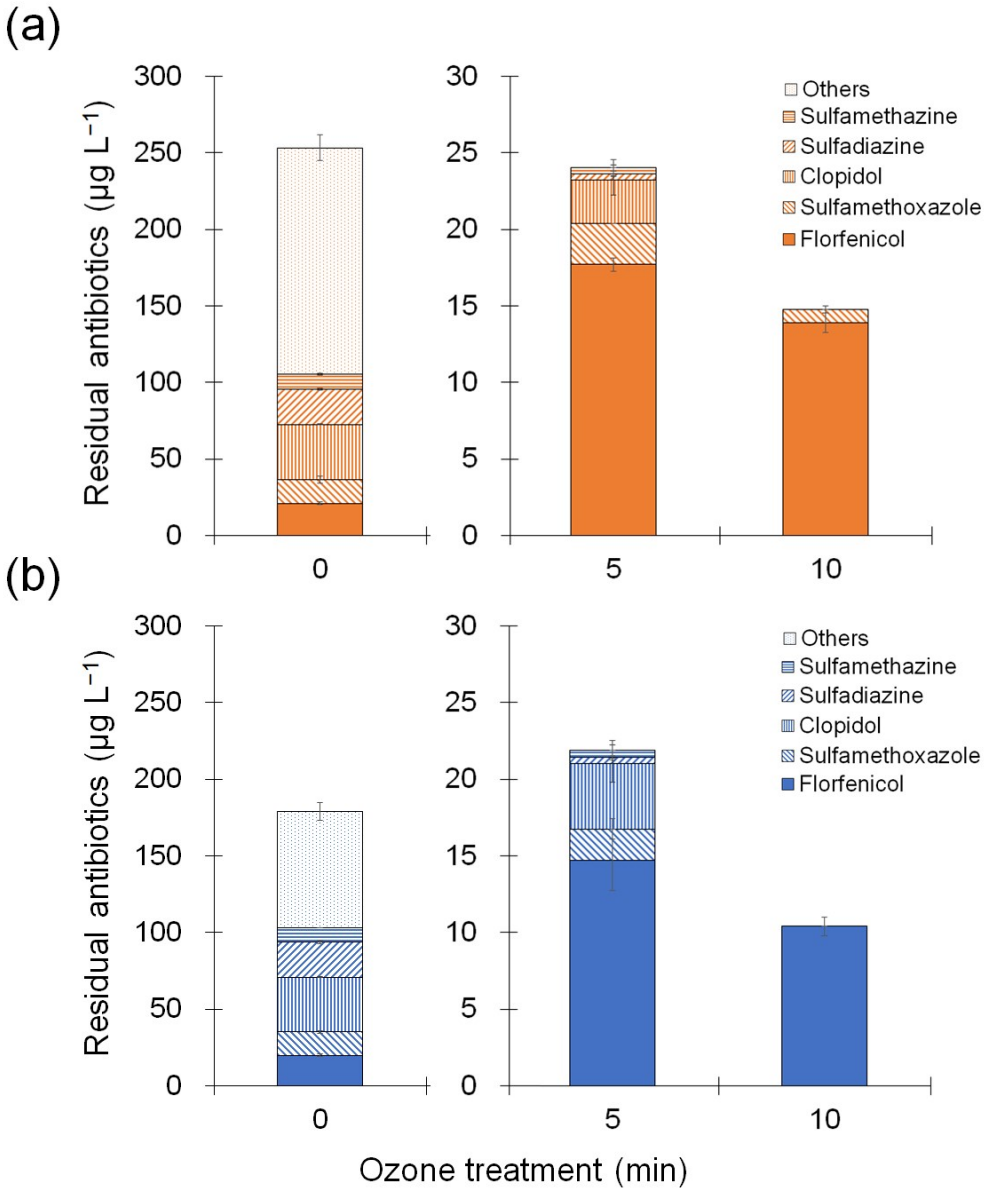


Fig. S6. The effects of ozonation on the removal of antibiotics from raw neutral (a) and algal-treated alkaline (b) livestock wastewater. The ozonation of raw and algal-treated livestock wastewater was carried out for up to 10 min (equivalent to ~1.8 mg-$O_3$/mg-C). Fig. 8a in the main text was reconstructed here in terms of clopidol, florfenicol, sulfadiazine, sulfamethazine, and sulfamethoxazole. The other antibiotics were not detected in the ozonated wastewater.