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# Chapter 1
# Removal Optimization and Material Balance of Antibiotics from Freshwater and Seawater Aquaculture Tailwater in Constructed Wetlands

Dan A

## 1. Introduction

I would like to introduce my study on using CWs for antibiotic removal. As you may know, aquaculture is very important in the global food source. In 2020, aquatic production was 123 million tons in the world, of which China accounted for 67 million tons. China was also the largest aquaculture country in the world from 1991 to 2020. With the rapid development of aquaculture, veterinary antibiotics are frequently employed throughout the culturing process and are released directly into the nearby estuaries and sea. Figure 1 illustrates the concentration of antibiotics detected in water and sediment samples in the major mariculture sites in China, where sulfanilamide antibiotics (SAs) are frequently detected in seawater aquaculture with a detection rate of 100% and a concentration of 2.3–291 ng/L in Southeast China (Chen et al. 2017). In Guangdong Province, the exact location of our study, the detection rate is 85–100% with a detection concentration of 0.08–2.09 ng/L (Xu et al. 2019). In addition, sulfanilamide is also detected in freshwater aquaculture ponds and nearby rivers with a maximum concentration of 2.39 mg/L (Le and Munekage 2004).

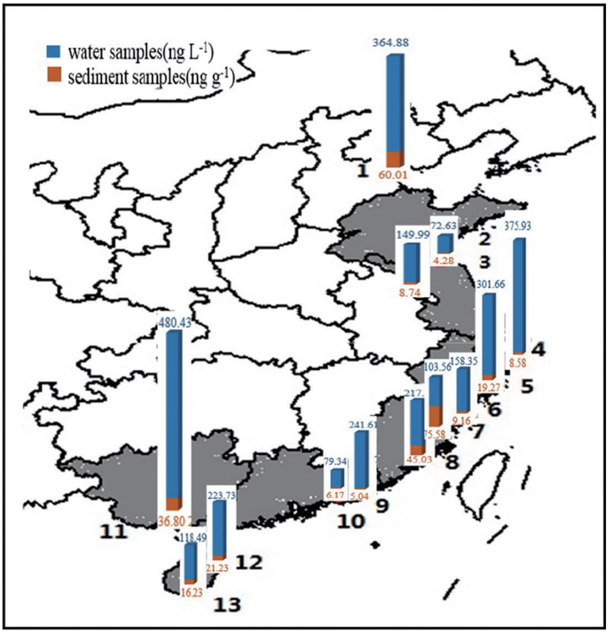



Figure 1: Antibiotics detected in water and sediment samples from key mariculture locations in China.
Source: Chen et al. 2017

The addition of feeds, feces, and veterinary waste led to an excessive content of organic matter, nitrogen, phosphorus, and antibiotics in aquaculture tailwater. Moreover, seawater aquaculture tailwater with different salinity levels was produced. Among them, salt ions cause danger to biological cells and bacteria, resulting in the limitation of the application of biotechnology (Cao et al. 2022; Tang et al. 2019).

CWs are proposed as a cost-effective technology for treating various kinds of wastewaters, including aquaculture tailwater. CWs have also been demonstrated to be effective in the removal of nutrients and antibiotics. Reportedly, when various wetland plants were used for treating aquaculture tailwater in CWs, it was found that the root exudates of common reed and iris had different effects on antibiotic biodegradation (Huang et al. 2019). Furthermore, different types of substrates can also impact the removal efficiency of antibiotics (Liu et al. 2021). This means that the configuration of CWs plays an important role in antibiotic biodegradation.

Figure 2 shows the results of searching for relevant papers by using Web of Science. We found only 23 papers that were related to CWs' aquaculture and antibiotics. Hence, it is necessary to more deeply investigate the removal behavior and mechanism of antibiotics from aquaculture tailwater in CWs.


Figure 2: The searched results in relevant papers using Web of Science.
Source: Web of Science <https://www.webofscience.com/>

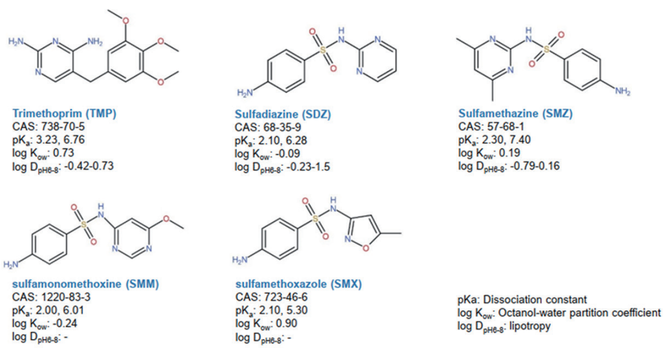
In this research, we prepared three topics, including "The optimization and microbial response mechanism of antibiotic removal from freshwater aquaculture tailwater by CWs," "The optimization and microbial response mechanism of antibiotic removal from seawater aquaculture tailwater by CWs," and "The removal pathways and material balance of antibiotics from freshwater and seawater aquaculture tailwater in CWs," with the three objectives of "The configuration of CWs that affect antibiotic removal by changing environmental factors optimized by orthogonal experiments," "The biodegradation and microbial response mechanism of antibiotics were analyzed by high-throughput sequencing," and "The material balance of antibiotics from aquaculture wastewater in CWs was described by mass balance analysis," respectively.

## 2. Experimental Setup and Investigation

**Topic 1: Optimization and Microbial Response Mechanism of Antibiotic Removal from Freshwater Aquaculture Tailwater by CWs.**

To the best of our knowledge, this marks the inaugural in-depth exploration of the correlation between antibiotic removal and microbial reaction within CWs employed for the treatment of aquaculture wastewater. Trimethoprim (TMP) and sulfonamides [sulfamethoxazole (SMX), sulfadiazine (SDZ), sulfamethazine (SMZ), and sulfamonomethoxine (SMM)] were selected as the targeted antibiotics in this study. Their physicochemical properties are shown in Figure 3a. For synthetic aquaculture wastewater, its composition was prepared according to the actual aquaculture tailwater in Guangdong province, China, by using tap water, fish feed, and veterinary drugs. The fish feed and chemicals are shown in Figure 3b.

(a)



(b)


Figure 3: (a) Physical and chemical properties of trimethoprim and sulfonamide antibiotics, (b) Fish feed, sea salt, and veterinary drugs used in this study.
Source: Author

The average inlet concentrations of TMP, SDZ, SMZ, SMM, and SMX were 6.4 ± 0.7, 15.5 ± 0.3, 3.3 ± 0.3, 5.7 ± 0.2, and 6.6 ± 0.5 mg/L, respectively. While the pH, water temperature (WT), dissolved oxygen (DO), oxygen reduction potential (ORP), chemical oxygen demand (COD), total nitrogen (TN), and total phosphorus (TP), were kept around 7.9 ± 0.1, 19.8 ± 1.9, 9.3 ± 0.3, 90.0 ± 55.0, 326.0 ± 22.0, 10.0 ± 1.1, 1.2 ± 0.1, respectively.

The experimental setup is presented in Figure 4. Nine different CWs were prepared according to the orthogonal test design with three different factors: Factor 1 was the substrate (coral sand, gravel, zeolite), Factor 2 was the plant (reed, canna, yellow flag), and Factor 3 was hydraulic retention time (HRT_1, 2, and 3 days). This experiment was conducted in batch mode. The inlet and outlet samples were collected to determine antibiotic concentrations and measure other water parameters. Additionally, the microbial community from the water samples was analyzed.

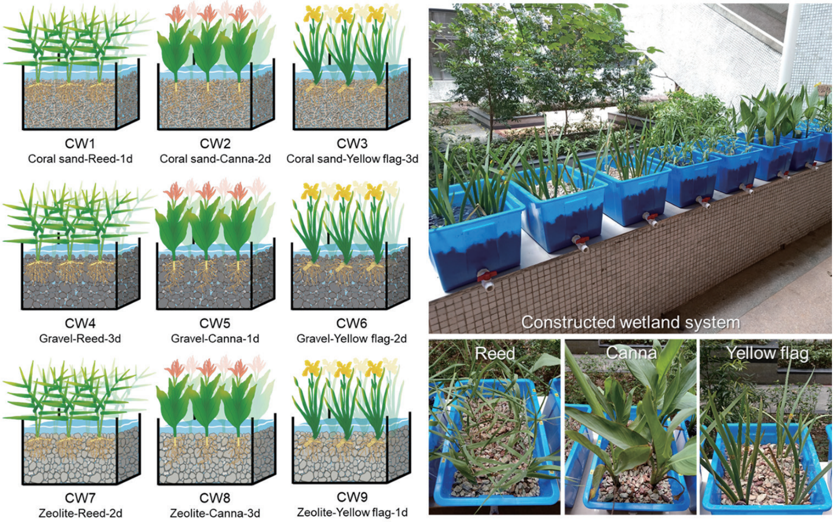



Figure 4: CWs system for antibiotic removal from freshwater.
Source: Author

During the experimental period, the effluent pH and WT reached 7.2–7.8 and 16.8–17.0 °C, respectively. The DO concentration after the CW treatment was reduced to 4.7–5.7 mg/L, implying the high oxygen demand for microbial activities and organic decomposition. The ORP values ranged from -107 to -7 mV. The COD and TP were removed effectively by all CWs, representing 69–76% and 52–85%, respectively. However, TN removal efficiency (4–40%) was quite low. The low temperature probably adversely affected the TN elimination. According to Becerra Jurado et al. (2010) and Du et al. (2016), the nitrification and denitrification rates dropped significantly at 20.0°C and 15.0°C, respectively.

For antibiotic removal, throughout the experiment, we found that the removal efficiency of TMP, SMX, SMM, SDZ, and SMZ were, respectively, 26–96%, -23–87%, -46–86%, -55–80%, and -62–84%

in all CWs. In general, all CWs showed higher TMP removal than SAs. Negative SAs removal was recorded in all CWs. This is probably because SAs can be easily returned to their original forms through the biochemical processes. This was confirmed by Li et al. (2021). The elimination efficiency of SMX was found to be the greatest among SAs. The highest treatment performance was recorded at CW4, which was employed with gravel, common reed, and HRT of 3 days. Its treatment performance was 89%, 61%, 20%, 20%, and 12%, for TMP, SMX, SMM, SMZ, and SDZ, respectively. The substrate was the most important factor for removing antibiotics in this study, followed by the plant and HRT. In addition, aerobic bacteria such as *Hydrogenophaga* and *Pseudomonas* were found to negatively affect TMP and SAs removal. In contrast, the anaerobic bacteria such as *Lacihabitans* and *Ilumatobacter* promoted these antibiotics elimination. The mechanisms of antibiotic removal from freshwater in CWs were summarized and displayed in Figure 5.

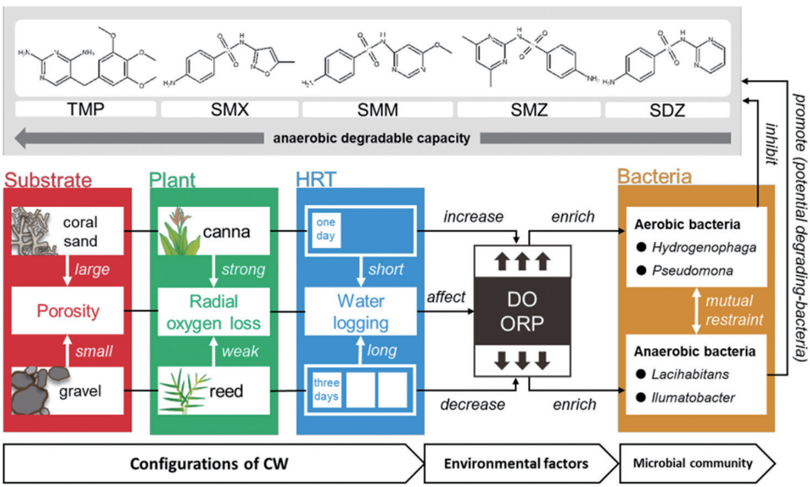



Figure 5: Antibiotic removal mechanisms from freshwater in CWs.
Source: Author

Overall, anaerobic degradation was regarded as the major removal pathway of antibiotics. The specific configuration of CW changed dominant and functional microbes in CWs. The CW configurations using small-porosity gravel, common reed with weak radial oxygen loss, and the longest HRT (3 days) resulted in a drop in the oxygen level. This enriched the anaerobic bacteria, which promoted antibiotic degradation. Conversely, the ones employed with large porosity coral sand, canna, having strong radial oxygen loss, and the short HRT (1 day) led to an increase in the oxygen level, which enhanced aerobic bacteria activities, inhibiting antibiotic degradation.

**Topic 2: Optimization and Microbial Response Mechanism of Antibiotic Removal from Seawater Aquaculture Tailwater by CWs**

In this study, we carried out an experiment in the greenhouse of Zhongkai University of Agriculture and Engineering in Guangzhou. The materials used in this experiment were the same as in Topic 1, except for Factor 3. In this experiment, Factor 3 was salinity, including three salt levels of 4‰ (low), 8‰ (middle), and 12‰ (high). The experimental design is shown in Figure 6.

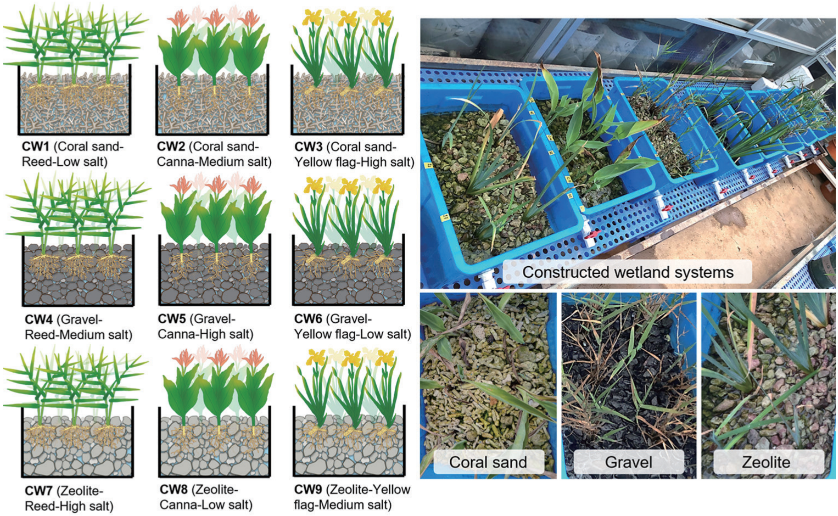



Figure 6: CWs system for antibiotic removal from seawater.
Source: Author

Unlike Topic 1, the HRT in this topic was fixed within 2 days. The tidal flow constructed wetlands (TFCWs) in this experiment were operated in intermittent mode, one day with synthetic wastewater (wet phase), and the other day without wastewater (dry phase), for the purpose of improving aerobic conditions in TFCWs, besides the water parameters mentioned in Topic 1. In this experiment, electrical conductivity (EC) and salinity (SAL) were also measured.

The results showed that during the experiment, the TFCWs demonstrated effectiveness in SAs removal with a removal efficiency of 59–92% for SMX, 38–97% for SMM, 30–94% for SDZ, and 34–92% for SMZ. In contrast, it was ineffective in TMP removal (-88–7%). The best treatment performance was recorded at CW9 (zeolite-yellow flag-12‰) for SAs, while CW4 (gravel-reed-8‰) showed the highest TMP removal. The TMP removal was dependent on anaerobic conditions and substrate adsorption. The zeolite improved pH, salt neutralization, and oxygen enrichment, which had the ability to enrich potential aerobic degrading bacteria (such as *Enterobacter* and *Sulfuritalea*). Among wetland plants, yellow flag performed the best in antibiotic removal in TFCWs. It enhanced bacteria with aerobic function and stress tolerance. Unlike with substrate and plant, salinity had no significant influence on antibiotic removal. The biodegradation mechanism of antibiotics from seawater in CW systems was summarized in Figure 7.

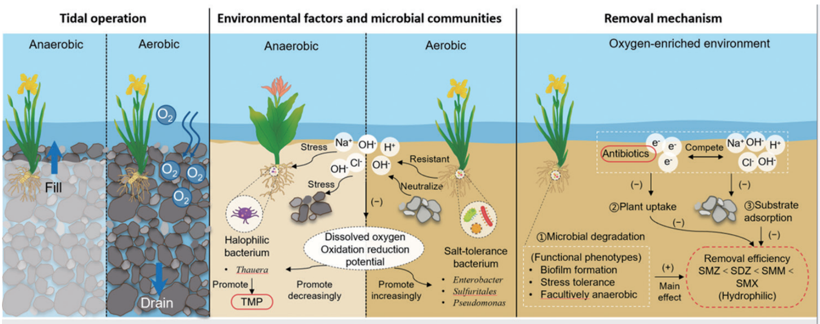



Figure 7. Biodegradation mechanisms of antibiotics in CWs.
Source: Author

In general, microbial degradation was regarded as the primary route for eliminating antibiotics from seawater in TFCWs. The substrate adsorption and plant uptake had minimal direct impacts on antibiotic elimination. Nevertheless, the ion exchange capacity of the substrate, the salinity tolerance of plants, and the salinity level each impacted the pH and EC values of the system, as well as the formation of rhizosphere biofilm, microbial functional characteristics, oxygen levels, and the structure of the microbial community. These factors determined the types and distribution of crucial functional bacteria, thereby indirectly influencing antibiotic biodegradation.

**Topic 3: Removal Pathways and Material Balance of Antibiotics from Freshwater and Seawater Aquaculture Tailwater in CWs.**

The main purpose of this experiment was to evaluate the removal pathways and material balance of antibiotics from freshwater and seawater in CWs. In order to achieve this purpose, we installed CW systems for fresh and seawater treatment. The experimental setup diagram is presented in Figure 8.

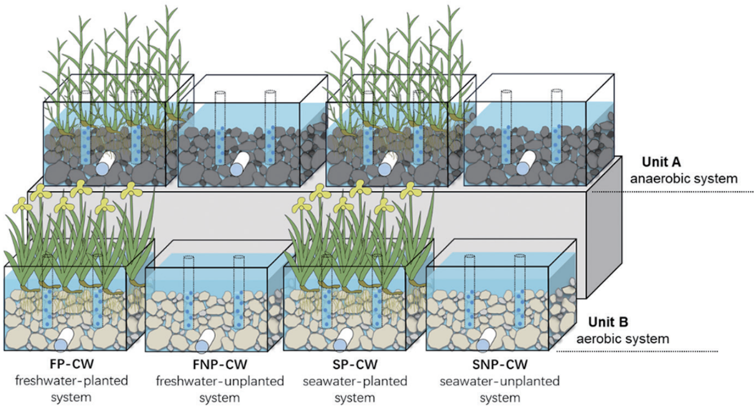



Figure 8: Design of the hybrid CWs.
Source: Author

The tidal flow series CW includes two units A and B. Unit A was designed similarly to the CW with the best treatment performance in Topic 1, employing gravel, common reed, and wetting mode. While Unit B was designed similar to the best performing treatment in Topic 2, with zeolite, yellow flag, and alternate wetting and drying modes. A control subunit without plants was prepared for each unit. This experiment was operated in sequencing batch modes with freshwater or seawater. The planted or unplanted CWs operated with freshwater were referred to as FP-CW and FNP-CW, while those with seawater were termed SP-CW and SNP-CW.

After the CW treatment, we observed that the removal efficiency of SAs from freshwater ranged from 7% to 87%, while it ranged from 7% to 94% for seawater. The planted CWs showed higher SAs removal efficiency (7–94%) than unplanted CWs (5–89%). Additionally, Unit B exhibited better SAs treatment performance. For TPM removal, its elimination efficiency from freshwater (>90%) was higher than that from seawater (-28–80%). Unlike SAs, TPM was removed more effectively in the unplanted CWs (52–98%) than planted CWs (-28–98%). Unit A removed TMP at a rate of 14–93% TMP, which was better than Unit B (-48–91%). Figure 9 shows the substrate adsorption and plant accumulation of antibiotics in CWs of freshwater and seawater.

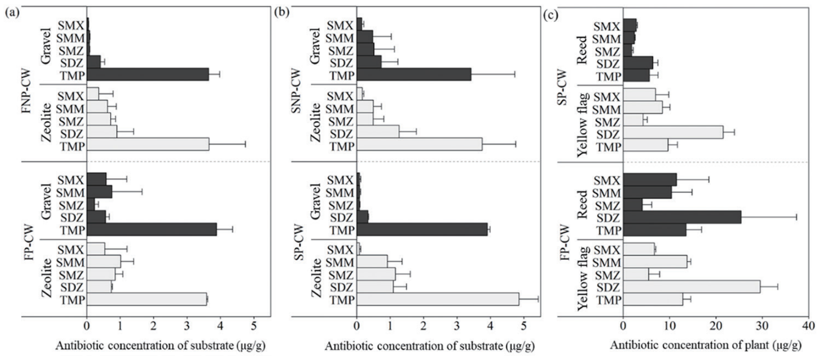



Figure 9: Distribution of antibiotics in substrates from the (a) freshwater and (b) seawater systems, and (c) in plants.
Source: Author

In general, zeolite demonstrated better antibiotic removal than gravel, as evidenced by the higher concentration of antibiotics absorbed in zeolite. The highest concentration of antibiotics found in the substrate was recorded for TMP in both systems. The TMP level in substrates was approximately 7 times higher than that of SAs. Also, we found that there were no significant differences between the treatment performances in freshwater and seawater systems, indicating that substrate adsorption of antibiotics is not impacted by salinity stress. For plant accumulation, the yellow flag showed a higher accumulation of antibiotics than the common reed. The antibiotic concentrations in plants from the freshwater system were around two times higher than those from the seawater, suggesting that plant uptake was inhibited by salinity stress. The antibiotic removal pathways were depicted in Figure 10.

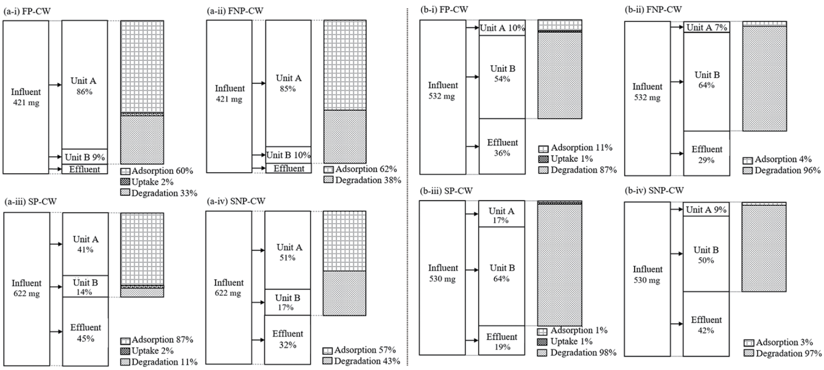



Figure 10: Mass balance model of (a) TMP and (b) SMX in the (i) FP-CWs, (ii) FNP-CWs, (iii) SP-CWs and (iv) SNP-CWs.
Source: Author

During the experiment, substrate adsorption was exhibited as the main pathway for TMP removal, representing 62–87%, followed by degradation (11–48%) and plant uptake (approximately 2%). Whereas degradation was the principal mechanism for removing SAs, accounting for 72–98%, followed by adsorption (1–24%), and plant uptake (only 1–6%).

## 3. Conclusion

In this research, we conducted three experiments to evaluate how different configurations of CWs influence the removal of antibiotics, elucidate the mechanisms of biodegradation and microbial response to antibiotics, and conduct a mass balance analysis. Our findings demonstrated that CWs were effective in removing antibiotics, with SAs and TMP showing higher removal efficiency in seawater and freshwater CWs, respectively. The specific configuration of CWs influenced the composition of dominant and functional microbes within them. Microbial degradation emerged as the primary pathway for SAs removal, accounting for 72% to 98% of removal, while substrate adsorption was the primary mechanism for TMP removal, accounting for 62% to 87% of removal. These results provided valuable insights for the utilization of CWs in the advanced purification of aquaculture wastewater containing veterinary drugs.

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Chapter 1. Removal Optimization and Material Balance of Antibiotics from Freshwater and Seawater Aquaculture Tailwater in Constructed Wetlands

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