

Antibiotic Resistance in Sewage Treated by Constructed Wetlands

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The emergence of antibiotic-resistant bacteria (ARB) and their dissemination into the environment through antibiotic-resistant genes (ARGs) have been recognized as one of the main concerns of the 21st century. Constructed wetlands (CWs) are nonconventional treatment technologies that mimic the removal processes of natural wetlands, optimizing operational and design parameters to enhance the removal of contaminants. Understanding the behavior of ARGs and ARB under different conditions will allow CWs to be optimized, avoiding an increase in ARG abundances in the final effluents.

Keywords: antibiotic-resistant genes ; sewage ; constructed wetlands

1. Antibiotic-Resistant Genes

Antibiotic-resistant genes (ARGs) are units of nucleic acid information that encode proteins involved in different resistance mechanisms, such as antibiotic inactivation, target site modifications, and reduced antibiotic penetration. In the case of ARGs that are related to the tetracycline family resistance (*tetO*, *tetB*, and *tetM*), these elements encode proteins, which prevent the antibiotic from binding to the ribosome, inhibiting the antibiotic action ^{[1][2]}. This AR is a natural phenomenon used by bacteria that gives them adaptive advantages for obtaining resources in the environment compared to other competing species ^{[3][4]}. The presence of antibiotics in the environment generates a selective pressure that inhibits the growth of susceptible bacteria, favoring intrinsically resistant bacteria or those that have acquired this resistance over time ^[1].

Resistance acquired by bacteria can occur through the transfer of genetic material from other bacteria (of the same or a different species) or point mutations ^[5]. **Figure 1** shows the different processes through which bacteria acquire ARGs. In this case, horizontal gene transfer (HGT) occurs through three genetic mechanisms: 1. Transformation, wherein an extracellular naked ARG is taken up by bacteria that have developed genetic competence; 2. Conjugation, wherein the genetic transfer from antibiotic-resistant bacteria (ARB) to recipient cell occurs through cell-to-cell contact; and 3. Transduction, the mechanism through which an ARG is introduced into a cell by a virus or bacteriophage ^{[5][6]}. Vertical gene transfer is another process of AR acquisition that consists of the transfer of genetic material from parents to offspring by ARB after acquiring an ARG through one of the mechanisms mentioned above. This process allows the resistant bacteria rate in the environment to increase ^[5].

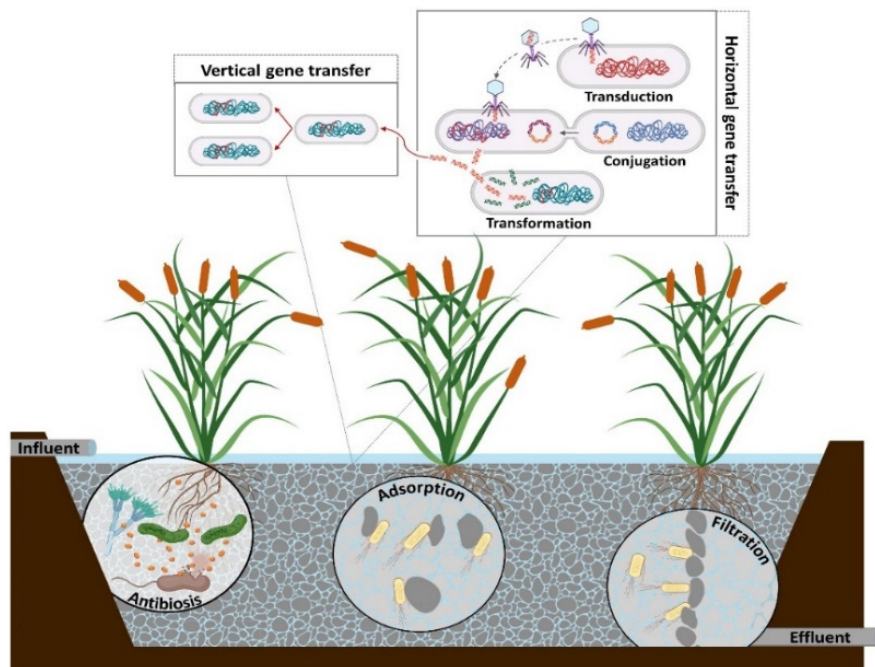


Figure 1. Processes of antibiotic-resistant gene transfer and mechanisms of antibiotic-resistant gene reduction in constructed wetlands. Note: Created with [BioRender.com](https://www.biorender.com) (accessed on 1 April 2022).

As seen in **Figure 1**, the gene transfer mechanisms mentioned above occur simultaneously within a constructed wetlands (CW) system [7], showing that operational and design conditions are important for reducing ARGs. Therefore, understanding the behavior of ARGs and ARB under different conditions will allow CWs to be optimized, avoiding an increase in ARG abundances in the final effluents.

2. Mechanisms of Antibiotic-Resistant Gene Reduction in Constructed Wetlands

As mentioned above, the effectiveness of ARG reduction in CWs depends on the involved conditions and mechanisms. Different studies have shown a positive correlation between the absolute abundance of ARGs and the rRNA 16S gene, a microbial marker [7][8]. This information could indicate that ARGs are transported by fecal microorganisms in sewage and that the reduction of these microorganisms is related to ARG reductions in the effluents [7][9][9]. The principal mechanisms of ARG reduction in CWs are shown in **Figure 1**. The antibiosis mechanism is related to the production of low molecular weight antibiotics by a group of bacteria or fungi. They can inhibit the growth of ARB in CWs and therefore decrease ARG abundance [10]. Similarly, it has been reported that plant roots are capable of releasing antibiotic compounds. Shirdashtzadeh et al. [11] and Chandrasena et al. [12] found an efficient inhibition of *E. coli* growth due to antibiotic release by the plant *Malaleuca ericifolia*. Likewise, Li et al. [13] reported that extracts from macrophytes, such as *Phragmites communis*, *Typha latifolia*, *Arundo donax*, *Polygonum hydropiper*, and *Polygonum orientale*, achieved reductions close to 100% for coliphages T4 and f2.

Other mechanisms of ARB and ARG reduction in CWs are associated with physical processes, such as filtration and adsorption. In both mechanisms, the support medium and rooting capacity of macrophytes play a fundamental role [7][14]. Liu et al. [15] reported reductions of 50% for the *tet* gene, while Dires et al. [16] achieved an ARB reduction of 77.5%. Both studies suggested that the principal mechanism responsible for achieving these reductions was filtration by zeolite and gravel, respectively. In the case of adsorption, this mechanism is related to the interactions between the contaminants and the support medium or plant roots due to the sorption properties and ionic composition of the medium [17]. Du et al. [8] studied ARG reduction in CWs that used zeolite as a support medium. They determined values of 95.3% for the *sul* and *tet* genes. These results can be explained by the porous morphology and larger surface area of zeolite.

3. Antibiotic-Resistant Gene Reductions in Constructed Wetlands

Different types of ARGs can be detected in sewage [9][18][19]. **Table 1** shows the absolute abundances and reductions of different ARGs in CWs treating sewage. For rural and urban areas, these values in the influents fluctuated between 1.43×10^6 – 1.25×10^8 copies/mL and 1.05×10^4 – 1.58×10^8 copies/mL, respectively. In both types of sewage, the average abundances of ARGs are in the order of 10^7 copies/mL. However, rural areas are characterized by low populations and

scattered households, such that the discharge of untreated sewage into the environment is common, especially in underdeveloped countries [20]. This situation poses a significant risk for antibiotic dissemination into the environment.

Table 1. Absolute abundances and reductions of different antibiotic-resistant genes in constructed wetlands treating sewage.

Sewage	Flow Configuration	Macrophyte Type	Support Medium	HRT	ARGs	Absolute Abundance (Copies/mL)		Reduction (Ulog)	Range	Ref
						Influent ($\times 10^6$)	Effluent ($\times 10^6$)			
Urban	HSSF, VSSF, SF, VSSF–HSSF	<i>P. australis</i> , <i>T. angustifolia</i> , <i>T. dealbata</i> , <i>C. alterfolius</i> , <i>I. tectorum</i>	tuff, gravel, sand, zeolite	0.18–6	<i>sul1</i>	8.18	7.10	0.33	–0.49–1.01	[18] [21] [22] [23] [24] [25] [26]
					<i>sul2</i>	8.95	7.48	0.35	0.04–0.9	
					<i>sul3</i>	6.92	6.04	0.13	–0.27–0.75	
					<i>ermB</i>	4.25	1.56	0.21	0.08–0.82	
					<i>ermC</i>	4.43	1.62	0.42	0.30–0.67	
					<i>tetM</i>	0.91	0.25	0.36	0.34–0.63	
					<i>tetO</i>	0.01	0.04	0.34	0.32–0.90	
					<i>tetX</i>	0.04	0.005	0.60	0.31–0.69	
					<i>floR</i>	0.00007	0.00004	0.75	–0.02–0.88	
					<i>cmlA</i>	2.64	0.07	0.36	0.29–0.74	
Rural	SF–VSSF, SF	<i>P. australis</i> , <i>T. dealbata</i> , <i>T. orientalis</i> , <i>P. cordata</i> , <i>M. vercillatum</i> , <i>I. tectorum</i>	chaff, soil	0.25–1.5	<i>sul1</i>	1.14	0.05	0.70	0.42–1.55	[27] [28]
					<i>sul2</i>	0.01	0.001	0.65	0.41–1.34	
					<i>sul3</i>	1.47	0.003	0.78	0.22–0.78	
					<i>tetM</i>	1.02	0.01	0.66	0.30–2.69	
					<i>tetO</i>	0.42	0.002	0.73	0.51–1.69	
					<i>ermB</i>	0.83	0.007	2.03	0.12–2.03	
					<i>ermC</i>	0.02	0.01	0.24	0.14–0.27	

Note: HSSF: horizontal subsurface flow; VSSF: vertical subsurface flow; SF: surface flow; VSSF–HSSF: hybrid vertical subsurface flow–horizontal subsurface flow; SF–VSSF: hybrid superficial flow–vertical subsurface flow; *P. australis*: *Phragmites australis*; *T. angustifolia*: *Typha angustifolia*; *T. dealbata*: *Thalia dealbata*; *C. alterfolius*: *Cyperus alterfolius*; *I. tectorum*: *Iris tectorum*; HRT: hydraulic retention time; ARGs: antibiotic-resistant genes.

The occurrence of ARGs in influent with an average abundance of 2.0×10^7 copies/mL shows the risks associated with the occurrence of HTG and ARB proliferation. These processes can trigger the dissemination of AR into the environment [7][21]. Moreover, concentrations of antibiotics, pesticides, disinfectants, and heavy metals in sewage can increase this risk [5][26].

In addition, **Table 1** shows the operating and design parameters of the CWs used by the evaluated studies to reduce ARG abundances in sewage. These parameters include conventional flow configurations, such as SF, HSSF, VSSF, and also hybrid configurations. The type of macrophytes used, support medium, and hydraulic retention time (HRT) can also be visualized.

Regarding the performance of CWs at reducing ARG abundances in sewage, the ARG reductions were above 0.3 ulog except for *ermB* and *tetM*. However, these systems achieved variable reductions that can be observed in the wide ranges reported. These ranges fluctuated from negative values (−0.49 to −0.02 ulog) to values above the average of 0.3 ulog. For urban sewage, negative reductions were reported for *sul1*, *sul2*, and *sul3*, with values of −0.49, −0.27, and −0.02 ulog, respectively. These negative reductions indicate an increase in ARG abundance in the effluent. This behavior can be related to the presence of antibiotic and coselective agents in CWs. In the case of rural sewage, higher reductions were reported for *tetM* and *ermB*, with values of 2.69 and 2.03 ulog, respectively. Regarding the wide ranges of reductions reported (−0.49 to 2.69 ulog), this result indicates that the ARG reductions in CWs depend on operational and design parameters.

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