ANTIBIOTIC RESISTANCE IN EXPERIMENTAL CONSTRUCTED WETLANDS

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Abstract

Antibiotic resistance to four antibiotics were tested in the influent and effluents of seven pilot scale constructed wetlands and in a conventional activated sludge treatment plant (STP). Three different flows (sub-surface flow, free-water surface flow, and free-water sub-surface flow), two plant species (cattails and reeds), floating-macrophytes wetlands and non planted wetlands were compared with regards their ability to facilitate antibiotic resistance gene exchange in faecal-indicator bacteria. Three groups of bacteria were studied with regards their ability to resist antibiotics, total Coliforms, *E. coli*, and *Enterococcus.* No significant differences were found in the proportion of antibiotic resistance bacteria present in the effluents of the different CW configurations, nor between CW and the STP. CWs were more efficient than conventional STP in the elimination of bacteria, this means that the loading of antibiotic-resistant bacteria to the environment is much lower when using constructed wetlands.

Introduction

The increase and generalization in the use of antibiotics, and the emerging problems for public health due to the increase in the resistance of bacteria to those antibiotics, make necessary to know how these bacteria are being eliminated in the treatment of the wastewater. Constructed Wetlands are quite well developed as decontamination systems for waste water, but the bacterial removal processes and the rates of variation of those bacteria which have some kind of antibiotic resistance, after crossing the treatment system are not well studied. In this study different CWs configurations (plant species, substrate and flow type, effluent disposition...) were tested to check how the CW design influences the rates of antibiotic resistance bacteria variations in the final effluent. All the CWs designs were compared with a conventional activated sludge treatment plant (STP).

Methods

Seven pilot-scale CWs were set up in the open air inside the facilities of the León conventional activated sludge Sewage Treatment Plant (STP). All constructed wetlands consisted of a fibreglass tank (80 cm width, 130 cm length, 50 cm height) with a surface area of 1 m^2. The CWs differ from each other in their design parameters, which are summarised in table 1 (Hijosa-Valsero, 2010).

Table 1. Pilot plant configuration in León

Systems	Characteristics
1, 5	Hydroponic constructed wetlands (one planted with *Typha* and other with *Phragmites*)
2, 3, 4	Surface flow constructed wetlands (25 cm. average water depth). Systems 2 & 3 planted with *Typha* and system 4 without plants. Systems 3 & 4 part of flow is passing through granular medium. System 2 is surface flow
7, 8	Subsurface flow constructed wetlands (45 cm. average water depth). System 7 planted with *Phragmites* and system 8 without plants.

Influent and effluent wastewater was membrane filtered and cultivated in Chromocult (Merk) (Total Coliforms & *E. Coli*), and SB Agar (Membrane-filter *Enterococcus* selective agar acc. to Slanetz and Barley, Merk) (*Enterococcus*). Four antibiotics were tested for each bacteria group; Amoxicillin (A), Azitromycin (AZ), Amoxicillin + Clavulanic acid (4:1) (AC), and Doxycycline (D). 1000 mg/L solutions of each antibiotic were diluted directly in the culture media to get 5, 50 (A, AZ, AC for E. coli and TC), 1 and 10 mg/L (D and A, AZ, AC for *Enterococcus*). Culture medium without antibiotics was used as control (Schwartz, 2003).

Results and discussion

The results showed no significant differences between the percentages of antibiotic-resistant bacteria found in the effluent of the different CW configurations. No significant differences were either found between STP and CW. (Data not shown). With regards the removal of bacteria, all CW configurations worked better than in the STP Sub-surface flow CW, and particularly CW7 (*Phragmites*, SSF, Gravel substrate), were significantly better than other configurations with regards bacterial groups removal. All these differences mean an average elimination in the CWs of 1,9 logarithm units (2,2 log units in TC, 1,9 in *E. coli*, and 1,6 in *Enterococcus*) (in case of CW7 the differences increase up to 2,9 log units in CT, 2,6 log units in *E. coli*, and 2,3 in *Enterococcus*) and 0,95 logarithm units in STP (1,2 log units in TC, 0,9 in E. Coli, and 0,76 in Enterococcus) (See Fig. 1). This higher removal efficiency of bacteria in the CWs means that a much less number of resistant bacteria are leaving the system, therefore a lower loading of antibiotic resistant-bacteria is produced when using CW in comparison to STP.

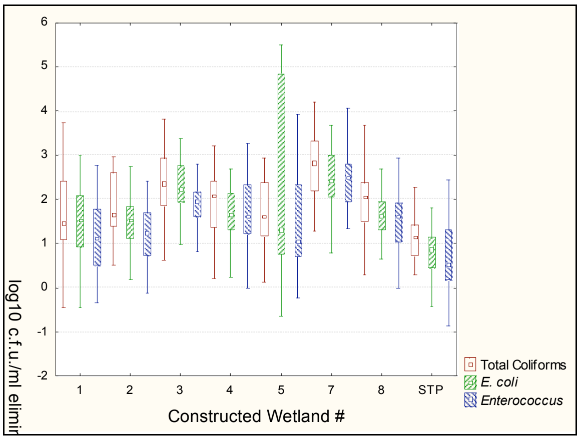

Figure 1. Number of logarithms unit eliminated in CWs

The response of bacteria to antibiotic resistance was strongly dependent on the type of antibiotic used. Doxycicline was the most effective, with no resistances among the bacterial groups tested. Azytromicin also had high rates of effectiveness being the percentage of resistance bacteria between zero and thirty percent. With regards Amoxicillin, almost all total Coliforms were resistant to this antibiotic, even after adding Clavulanic Acid (which is supposed to inhibit beta-lactamase). *E. coli* and *Enterococcus* were nevertheless less resistant to both, amoxicillin and their combination with clavulanic acid. (See Fig. 2)

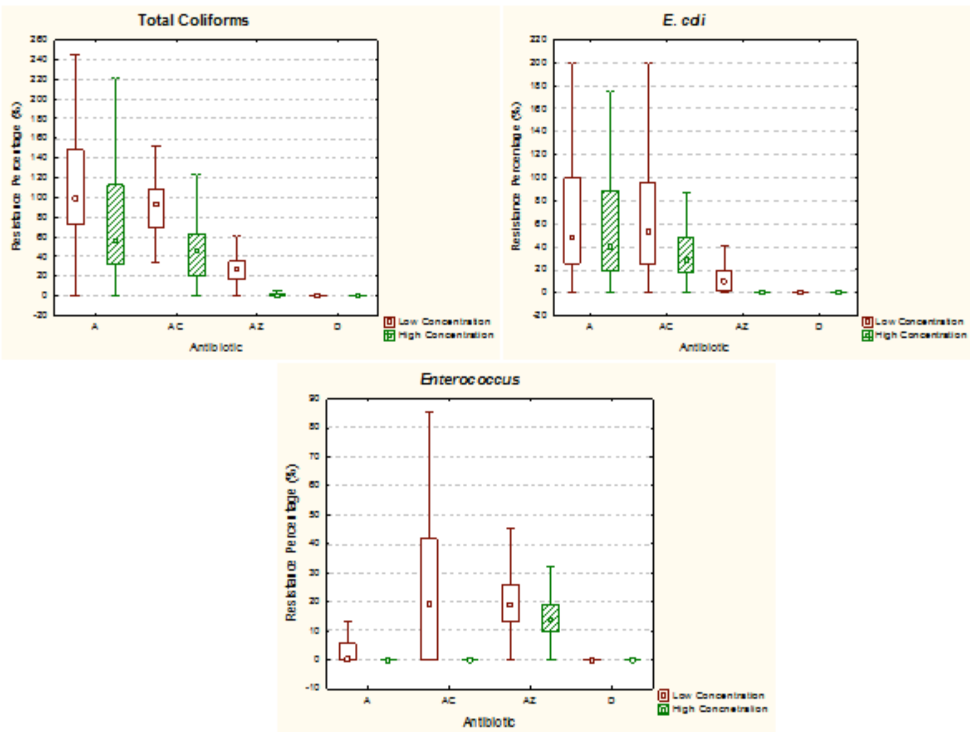

Figure 2. Resistance of bacteria groups to each antibiotic. (A: Amoxicillin, AC: Amoxicillin +Clavulanic Acid, AZ: Azytromicin, D: Doxicicline).

Conclusions

The CW studied did not present differences with regard the proportion of antibiotic-resistant bacteria present in their effluents. No differences between CW and STP were also detected. Neverthless, CW were much more effective in bacteria removal than STP, which means that the release of resistant bacteria to the receiving environment is lower when using CWs.

References

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