ANTIBIOTIC RESISTANCE IN EXPERIMENTAL CONSTRUCTED WETLANDS

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Introduction

The emerging problems for public health due to the increase in the resistance of bacteria to antibiotics, make necessary to know how these bacteria are being eliminated in the treatment of the wastewater. The bacterial removal processes and the rates of variation of those bacteria which have some kind of antibiotic resistance, are not well studied in Constructed Wetlands. 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 for the experiment. All of them 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 Figure 1 (Hijosa-Valsero, 2010), CW 4 and 7 are unplanted controls of CW 3 and 6 respectively.

Influent and effluent wastewater was membrane filtered and cultivated in Chromocult (Total Coliforms & *E. Coli*), and SB Agar (*Enterococcus*) (APHA, 1999). Four antibiotics were tested; Amoxicillin (A), Azitromycin (AZ), Amoxicillin + Clavulanic acid (4:1) (AC), and Doxycycline (D). 1000 µg/ml 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 µg/ml (D and A, AZ, AC for *Enterococcus*). Culture medium without antibiotics was used as control (Schwartz, 2003).

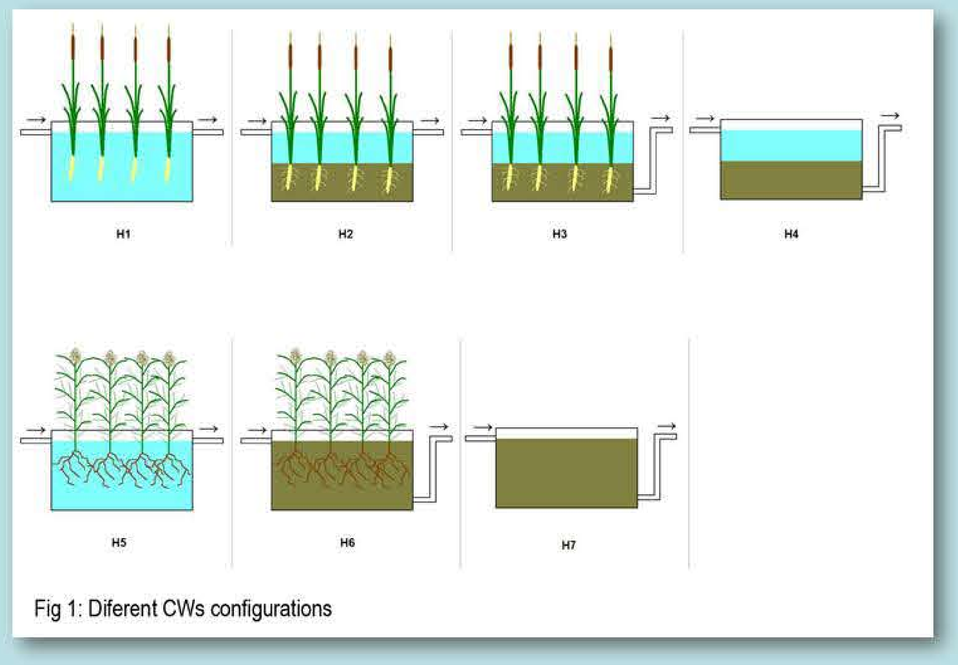

Fig 1: Diferent CWs configurations

Results and Discussion

No significant differences were found between the percentages of antibiotic-resistant bacteria in the effluent of the different CW configurations, as well as between STP and CWs. (Data not shown). With regards the removal of bacteria, all CW configurations worked better than the STP. Sub-surface flow CW, and particularly CW6 (*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 CW6 the differences increase up to 2,6 log units in *E. coli*, 2,9 in CT and 2,3 log units 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. 2). Removal efficiency is lower in unplanted CW 4 and 7 than in their planted replicates, which means some plant effect (higher in case of *Phragmites*) in mortality of bacteria according to García et al. (2008).

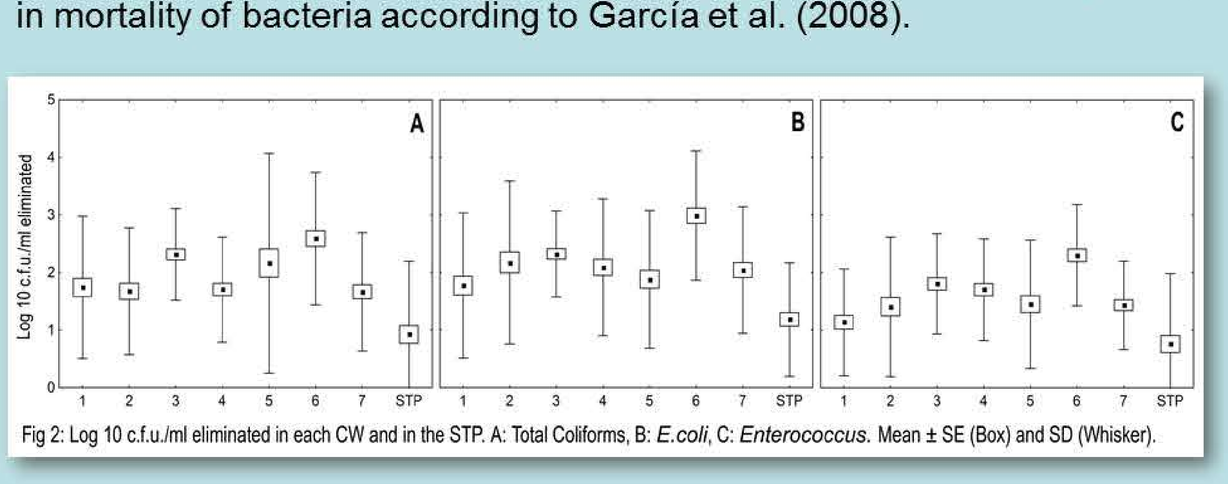

Fig 2: Log 10 c.f.u./ml eliminated in each CW and in the STP. A: Total Coliforms, B: *E.coli*, C: *Enterococcus*. Mean ± SE (Box) and SD (Whisker).

The response of bacteria to antibiotic resistance was strongly dependent on the antibiotic used. Doxycycline presents no resistants. Azytromicin had high rates of effectiveness being the percentage of resistance between 0 and 30%. 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. 3)

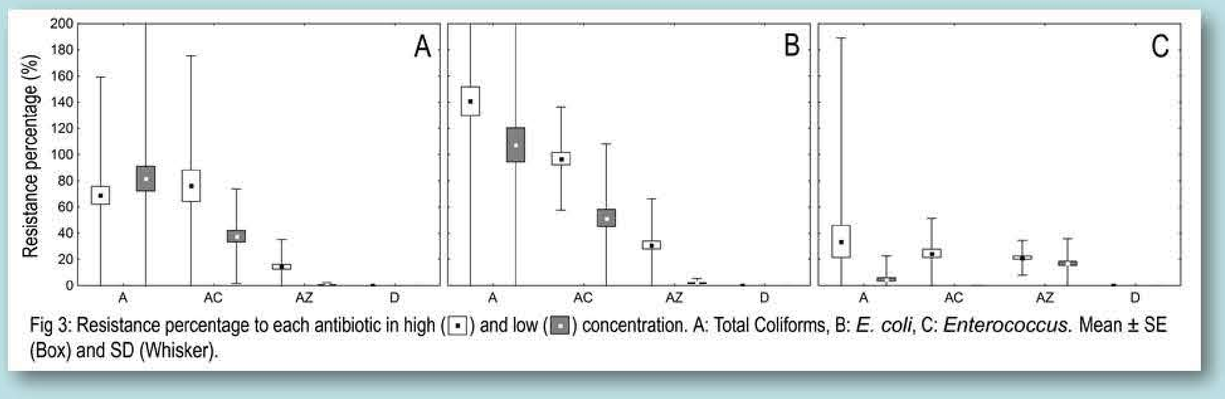

Fig 3: Resistance percentage to each antibiotic in high (▪) and low (▪) concentration. A: Total Coliforms, B: *E. coli*, C: *Enterococcus*. Mean ± SE (Box) and SD (Whisker).

Conclusions

- There are no differences in antibiotic resistance proportion between CWs and STP and between different CWs.
- Better elimination rates was found in CW than in STP.
- Planted CW eliminates more bacteria than its unplanted replicates.
- Sub Superficial Flow (SSF) is the configuration which eliminates more bacteria.
- The release of resistant bacteria to the receiving environment is lower when using CWs due to bacteria elimination.

References

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