Microbial Monitoring of a Subsurface Constructed Wetland Treating Domestic Wastewater

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INTRODUCTION

Microbial monitoring of constructed wetlands (CWs) treating domestic wastewater is generally insufficient (Vacca *et al*., 2005), despite the need of more steady knowledge about its biocenosis. Since one of the goals of domestic wastewater treatment is to improve the quality of receiving water bodies, mainly of watersheds with bathing areas, removal of microbial groups of sanitation importance is indisputable. Beyond the information about the sanitation quality of the treated wastewater, knowledge on the dynamics of microbial communities established in such systems is also important. Structure of bacterial communities has been estimated by polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) analyses on different kinds of environmental samples, including CWs (Calheiros *et al*., 2008).

This study describes the removal of coliform bacteria and the density of heterotrophic bacteria within a horizontal subsurface flow CW operating with domestic wastewater (mean flow 49 m^3 day^{-1}), coming from a population of about 300 inhabitants. In addition, the dynamics of microbial communities established in the system was assessed by molecular analysis.

METHODS

Between November 2007 and September 2008, monthly sampling campaigns were carried out at a CW in northwest Portugal. Domestic wastewater and substratum samples were taken from the inlet (IN) and outlet (OUT) of a horizontal subsurface flow CW planted with *Phragmites australis*. Determinations of Biochemical Oxygen Demand (BOD_5), Chemical Oxygen Demand (COD) and Total Suspended Solids (TSS) were carried out using standard procedures, respectively: mercury-free pressure measurements using the OxiTop® system, dichromate digestion method and filtration under vacuum through a 47mm diameter 934-AHWatman filter. Bacterial enumeration of some microbial groups - Recovered Heterotrophics (RH), Total Coliforms (TC) and Fecal Coliforms (FC) - was carried out based on selected decimal diluted samples by the recovery of colony forming units (CFU L^{-1}) on solid culture media, using the spread plate method. Dynamics of the bacterial communities within the CWs was analysed by PCR-DGGE based on 16S rRNA bacterial gene.

RESULTS AND DISCUSSION

The CW was able to polish the wastewater to levels of discharge concerning organic matter and suspended solids, however COD and TSS removal efficiencies (Table 1) were lower (44-65%) than that reported for other similar systems (Vymazal, 2009).

Concerning the selected microbial groups (TC and FC), a reduction of one log cycle occurred within the systems (Table 2). Higher removal would be needed for the outlet wastewater to be in compliance with the discharge limits demanded to bathing waters. On substratum samples, FC were

not recovered, however the recovery of background microflora, either on upstream (up) or downstream (dw) substratum samples, reached densities of about 10^3-10^4 CFU g^{-1} (data not shown).

Table 1. Mean values of BOD_5, COD and TSS, determined on samples of wastewater (ww) collected at the inlet (IN) and outlet (OUT) of the CW.

Parameters	Mean (min.-max.)		Removal efficiency (%)
Samples	IN - ww	OUT - ww	
BOD_5[a] (mg L^{-1})	91 (50-160)	35 (16-52)	61
COD[b] (mg L^{-1})	157 (104-229)	88 (33-141)	44
TSS[c] (mg L^{-1})	17 (8-32)	6 (2-12)	65

[a]n=10; [b]n=8; [c]n=5

Table 2. Mean densities of Recovered Heterotrophics (RH), Total (TC) and Fecal Coliforms (FC) estimated from wastewater (ww) inlet (IN) and outlet (OUT) samples (n), and upstream (up) and downstream (dw) samples of substratum.

Microbial group	min.-max. - CFU L^{-1}		Eff. (%)[b]	min.-max. - CFU g^{-1}	
	IN-ww	OUT-ww		up – sub	dw - sub
RH[a]	$31x10^7$– $45x10^8$	$32x10^6$– $48x10^7$		$12x10^4$- $54x10^5$	$15x10^4$-$35x10^5$
TC[a]	$27x10^5$– $25x10^7$	$37x10^4$– $19x10^6$	92	NCD - $68x10^3$	NCD - $8x10^2$
FC[a]	$2x10^5$– $6x10^7$	$2x10^4$– $16x10^5$	97	NCD	NCD

[a]n=10; [b]Eff. (%) = mean removal efficiency (%); NCD – no colonies detected

The molecular analyses of substratum samples expressed on DGGE band profiles of 16S rRNA genes showed between 19 and 31 bands. The *Shannon-Weaver* (H) and the equitability (E) indexes exhibited similar values, both in space and in time, for upstream and downstream substratum samples, suggesting the stability of bacterial communities along the reed beds substratum.

CONCLUSIONS

The need of microbial monitoring of domestic wastewater treated in CW is crucial; although there was a reduction, according to legislation, the outlet densities of the microbial indicators were still high for water discharge in bathing areas. The substrate RH density was found to be lower than that published in other studies (Calheiros *et al*, 2008; Truu *et al*., 2005). The DGGE analysis revealed a high bacterial diversity within the system, with no relevant differences at the inlet and outlet of the bed.

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