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Dynamics of antibiotic resistance genes and their relationships with system treatment efficiency in a horizontal subsurface flow constructed wetland

Hiie Nõlvak *, Marika Truu, Kertu Tiirik, Kristjan Oopkaup, Teele Sildvee, Ants Kaasik, Ülo Mander, Jaak Truu

Institute of Ecology and Earth Sciences, Faculty of Science and Technology, University of Tartu, 46 Vanemuise St., 51014 Tartu, Estonia

HIGHLIGHTS

- ARG were detected in influent, effluent, and media biofilm of a constructed wetland.
- System operation time and temperature affect amounts of ARGs in the effluent.
- ARG abundance in media biofilm and effluent is related to system's treatment efficiency.
- Constructed wetlands are alternatives to conventional treatment for ARG removal.

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ABSTRACT

Municipal wastewater treatment is one of the pathways by which antibiotic resistance genes from anthropogenic sources are introduced into natural ecosystems. This study examined the abundance and proportion dynamics of seven antibiotic resistance genes in the wetland media biofilm and in the influent and effluent of parallel horizontal subsurface flow mesocosm cells of a newly established hybrid constructed wetland treating municipal wastewater. The targeted genes (*tetA*, *tetB*, *tetM*, *ermB*, *sul1*, *ampC*, and *qnrS*) encode resistance to major antibiotic classes such as tetracyclines, macrolides, sulfonamides, penicillins, and fluoroquinolones, respectively. All targeted antibiotic resistance genes were detectable in the tested mesocosm environments, with the *tetA*, *sul1*, and *qnrS* genes being the most abundant in the mesocosm effluents. After initial fluctuation in the microbial community, target gene abundances and proportions stabilized in the wetland media biofilm. The abundance of 16S rRNA and antibiotic resistance genes, and the proportion of antibiotic resistance genes in the microbial community, were reduced during the wastewater treatment by the constructed wetland. The concentration of antibiotic resistance genes in the system effluent was similar to conventional wastewater treatment facilities; however, the mesocosms reduced sulfonamide resistance encoding *sul1* concentrations more effectively than some traditional wastewater treatment options. The concentrations of antibiotic resistance genes in the wetland media biofilm and in effluent were affected by system operation parameters, especially time and temperature. The results also revealed a relationship between antibiotic resistance genes abundance and the removal efficiencies of NO_2–N, NH_4–N, and organic matter. Correlation analysis between the abundance of individual antibiotic resistance genes in the mesocosms influent, effluent and wetland media biofilm indicated that depending on antibiotic resistance gene type the microbes carrying these genes interact differently with microbial communities already present on the wetland media.

1. Introduction

The occurrence and spread of antibiotic resistant bacteria in the environment is a well-recognized concern to the extent where, besides antibiotic residues, the antibiotic resistance genes (ARGs) are being considered as pollutants themselves (Martínez, 2009). Anthropogenic residues contain ARGs that survive and contaminate natural environments, altering natural microbial communities and giving rise to the emergence of antibiotic resistance in the clinical/human setting (Schmieder and Edwards, 2012). However, the transport

* Corresponding author at: Institute of Ecology and Earth Sciences, University of Tartu, 46 Vanemuise St, 51014, Tartu, Estonia. Tel.: +372 737 6843.
E-mail addresses: hiie.nolvak@ut.ee (H. Nõlvak), marika.truu@ut.ee (M. Truu), kertu.tiirik@ut.ee (K. Tiirik), kristjan.oopkaup@ut.ee (K. Oopkaup), teele.sildvee@ut.ee (T. Sildvee), ants.kaasik@ut.ee (A. Kaasik), ulo.mander@ut.ee (Ü. Mander), jaak.truu@ut.ee (J. Truu).

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pathways, development, and environmental reservoirs of resistance determinants are not well understood and need further research (Martínez, 2008; Allen et al., 2010).

Municipal wastewater treatment is one major route by which ARGs from human settings are introduced into natural ecosystems (Servais and Passerat, 2009; Novo and Manaia, 2010). Numerous ARGs encoding resistance for major antibiotic groups, such as tetracycline, aminoglycosides, macrolides, sulfonamides, and β-lactams, have been found both in activated sludge and effluents from wastewater treatment plants (X.X. Zhang et al., 2009). Traditional wastewater treatment facilities (i.e. using activated sludge processes) have also been recognized as possible concentration points and reservoirs for antibiotic resistant bacteria owing to their high concentrations of microbial biomass and the abundance of nutrients (Guardabassi et al., 2002). In wastewater treatment facilities the mainly mobile genetic element (i.e. plasmid) borne ARGs are selected, enriched, and transferred to other bacterial species (Guardabassi et al., 2002; Szczepanowski et al., 2009). While antibiotic resistance in conventional wastewater treatment plants has received attention, antibiotic resistance in alternative wastewater treatment systems, such as constructed wetlands, has been mostly overlooked.

Constructed wetlands (CW) are engineered systems designed and constructed to harness the processes that occur in natural wetlands for the treatment of wastewater. With relatively low setup and maintenance costs and relatively good wastewater purification efficiencies, CWs have proven to be effective alternatives or useful complements to traditional wastewater treatment systems (Scholz and Lee, 2005).

Various types of CWs are often combined in sequence as hybrid systems to enhance wastewater treatment efficiencies; the treatment performance of CWs is mainly based on the combined action of microbes and filter material which may be complemented by plants (Truu et al., 2009). Although the removal of both medical and veterinary antibiotics in CWs have been targeted in recent years (Conkle et al., 2008; Hijosa-Valsero et al., 2011; Hussain et al., 2012), the presence and removal of ARG-carrying microbes within such systems have received less attention. Currently, antibiotic resistance studies in CWs have mostly been limited to testing the susceptibility of fecal indicator bacteria (*Escherichia coli*, *Enterococcus*) isolates to antibiotics (Helt et al., 2012; Sidrach-Cardona and Bécares, 2012) which covers only a very small fraction of bacterial community. However, little research has been done to estimate the extent and types of antibiotic resistance genes in the CWs whole microbial communities.

This research had three goals. First, to study the abundance and dynamics of seven ARGs (*tetA*, *tetB*, *tetM*, *ermB*, *sul1*, *ampC*, and *qnrS*) and their proportion in microbial communities in the influent, effluent, and wetland media biofilm of horizontal subsurface flow filter mesocosms (HSSF MCs) of a newly established pilot scale hybrid CW treating municipal wastewater. Second, to evaluate correlations between environmental factors and ARG abundance as well as ARG proportions in community in both the effluent water and wetland media biofilm. Finally, to assess the relationships between ARG removal and wastewater purification efficiency in the HSSF MCs.

2. Materials and methods

2.1. Site description and sampling

150-day municipal wastewater treatment experiment was conducted from June to November 2009 in Nõo village, Estonia. Nõo village is the center of a parish that has a permanent population of about 1500 people. Small meat processing and dairy industries are situated near the village. The village's activated sludge wastewater treatment facility treats domestic municipal wastewater combined with the effluents from the dairy and meat industries. This study was conducted in an unplanted hybrid CW system fed with raw wastewater pumped from the inlet of the activated sludge treatment plant. The pilot system consisted of a septic tank (2 m^3), followed by six parallel vertical subsurface flow mesocosms (total area 6 m^2), a collection well, and 21 parallel HSSF MCs (each MC cell: length — 1.5 m, width — 0.2 m, depth — 0.6 m). A detailed description of the system is given by Nurk et al. (2009). The three HSSF MCs used in this study were filled with light expanded clay aggregates (LECA) with 2–4 mm particle size forming the wetland media and providing high surface area environment for microbial biofilm attachment. The hydraulic loading rate was ≤20 mm d^{-1} and the wastewater retention time in the HSSF MCs was 1.2 days. The HSSF MCs influent was pretreated wastewater which had passed through the septic tank and the vertical subsurface flow mesocosms. The characteristics of the influent to the HSSF MCs and the difference in respective parameter values (removal efficiencies) between influent and effluent of the studied mesocosms are shown in Table 1.

Sampling began after 26 days of regular operation of the CW system and samples were collected five times during the five-month trial period. Between the second and the third samplings partial clogging with biomass occurred in pipes connecting the collection well and HSSF MCs causing uneven distribution of wastewater to the parallel MC cells. The clogging was removed by dismantling, wash-out and reattachment of the pipes, as soon as it was detected. The clogging had a short term effect on the system reflected in higher deviation between parallel MCs wastewater treatment efficiency on third sampling (Table 1); by the fourth sampling this effect was not perceivable any more. Grab samples of wastewater were taken from the collection well located before the inlet to the HSSF MCs and from the outlets of each HSSF MC. During each sampling event also five subsamples from the wetland media (25–35 cm depth) of each HSSF MC were collected at even spacing along the longitudinal axis; all mesocosm media subsamples were mixed to form a composite sample. Water temperature and pH inside the mesocosms were measured during each sampling. The water temperature in mesocosms

Table 1
Wastewater quality parameters of HSSF MCs influent with means and standard deviations (given in parentheses, n = 3) of treatment efficiencies (TE) and pH differences (ΔpH) between influent and effluent of HSSF MCs at each sampling.

	Days of HSSF MCs operation									
	Day 26		Day 45		Day 64		Day 94		Day 150	
	Conc.[a]	TE[b]	Conc.	TE	Conc.	TE	Conc.	TE	Conc.	TE
NO_3–N	10.30	0.03 (±11.08)	10.80	46.11 (±10.99)	11.00	45.09 (±37.00)	13.03	45.81 (±2.31)	14.10	26.24 (±3.25)
NO_2–N	0.51	−0.78 (±29.72)	0.49	54.49 (±13.58)	1.58	81.48 (±12.49)	1.19	56.97 (±6.33)	0.47	12.27 (±7.80)
NH_4–N	6.41	2.55 (±16.72)	8.00	17.54 (±7.21)	15.50	8.60 (±7.64)	13.9	23.26 (±1.50)	3.34	27.05 (±1.90)
N_{total}	19.40	−1.37 (±9.24)	21.60	37.35 (±3.08)	29.10	23.02 (±23.56)	31.60	33.97 (±1.28)	20.90	25.04 (±2.92)
BOD_7	27.0	70.37 (±19.51)	23.75	62.66 (±4.22)	19.0	40.35 (±3.04)	30.0	90.11 (±1.02)	19.0	92.11 (±1.39)
TOC	16.00	−25.83 (±15.93)	16.22	53.27 (±10.30)	24.20	23.94 (±17.86)	26.00	23.41 (±6.96)	11.60	52.99 (±20.31)
ΔpH	1.05 (±0.07)		0.59 (±0.09)		0.81 (±0.27)		0.62 (±0.04)		0.53 (±0.02)	

[a] Conc.— water quality parameter concentrations in mg l^{-1}, except pH.
[b] Treatment efficiency in %.