Ecological Engineering xxx (2013) xxx–xxx

Contents lists available at ScienceDirect

Ecological Engineering

journal homepage: www.elsevier.com/locate/ecoleng

Escherichia coli removal and internal dynamics in subsurface flow ecotechnologies: Effects of design and plants

Tom Headley[a,*], Jaime Nivala[a], Kinfe Kassa[a], Linda Olsson[b], Scott Wallace[c], Hans Brix[b], Manfred van Afferden[a], Roland Müller[a]

[a] Helmholtz Center for Environmental Research (UFZ), Environmental and Biotechnology Center (UBZ), Permoserstraße 15, 04318 Leipzig, Germany
[b] Department of Bioscience, Plant Biology, Aarhus University, Ole Worms Allé 1, Building 1135, 8000 AarhusC, Denmark
[c] Naturally Wallace Consulting LLC, PO Box 2236, 109 E. Myrtle Street, Stillwater, MN 55082, USA

ARTICLE INFO

Article history:
Received 17 March 2013
Received in revised form 13 July 2013
Accepted 13 July 2013
Available online xxx

Keywords:
Aeration
ANCOVA
Constructed treatment wetland
Depth
Horizontal flow
Pathogen
Phragmites australis
Reciprocating fill and drain
Vertical flow

ABSTRACT

Subsurface flow ecotechnologies encompass a range of different designs, varying in terms of flow configuration, media type, energy requirements and use of wetland plants. This study compared the removal rates and internal dynamics of *Escherichia coli* in a range of commonly used and emerging subsurface flow systems designed for secondary treatment of domestic sewage. Fifteen pilot-scale units were loaded with primary treated sewage in Langenreichenbach, Germany and monitored at the inlet, outlet and a several internal sample points between August 2010 and December 2011. The compared systems spanned a range of energetic intensification levels, including passive horizontal flow (HF) beds (25 cm versus 50 cm deep), moderately-intensified unsaturated pulse-loaded (12 versus 24 times per day) vertical flow (VF) beds (gravel versus sand media), and highly-intensified beds with aeration (HF versus VF) or reciprocating fill and drain hydraulics. Planted (*Phragmites australis*) and unplanted forms were compared for all designs except for the reciprocating system (unplanted only). In general, there was no significant effect of vegetation on *E. coli* removal. Despite receiving the highest loading rates (131–146 L/m^2 d), the aerated HF systems and the reciprocating system achieved the highest log concentration reductions (2.8–4.0 log$_{10}$) and the lowest effluent *E. coli* concentrations (geometric mean less than 1×10^4 MPN/100 mL). The gravel-based VF beds had the lowest log concentration reduction (0.8 log$_{10}$) and highest effluent concentrations ($6.4–8.9 \times 10^5$ MPN/100 mL) at a hydraulic loading rate of 96 L/m^2 d. The design type had an extremely significant effect on areal mass removal rates, with the passive HF beds having the lowest removal rates (50 cm depth significantly better than 25 cm), followed by the unsaturated VF systems (which were not significantly different from one another), while the aerated and reciprocating systems had the highest removal rates. Within the unsaturated VF beds, the use of sand versus gravel substrate, or hourly versus bi-hourly loading regime in the sand-based systems, had no effect on areal load removal. The internal concentration profiles were not significantly different between the unsaturated VF designs, with the exception of the hourly-loaded, planted bed with sand media which had a more rapid rate of concentration reduction with depth. In the HF beds, the internal *E. coli* concentration reduction was significantly faster in the aerated beds than in the non-aerated beds. Depth and plants had no significant effect on the internal concentration profiles within the non-aerated HF beds. Within the aerated systems, horizontal-flow achieved better *E. coli* removal than vertical-flow. Subsurface flow ecotechnologies offer great potential as robust and low-maintenance solutions for reducing the pathogen risk associated with domestic wastewater. The intensified systems produced effluent potentially suitable for restricted surface irrigation, at the cost of higher energy consumption, while the effluent from the other design types would require subsurface irrigation or further disinfection prior to reuse.

1. Introduction

The contamination of drinking and irrigation water with inadequately treated domestic wastewater represents a serious public health concern due to the potential transmission of infectious disease via waterborne pathogenic microorganisms (Asano et al., 2007). Many human settlements throughout the world source their

* Corresponding author at: Constructed Wetland Competence Centre, BAUER Nimr, PO Box 1186, Al Mina, Muscat, Sultanate of Oman,
Tel.: +968 24 699 505/+968 96352920; fax: +968 24699636.
E-mail addresses: tom.headley@bauerenvironment.com, tom.headley@bauer.de, headley_tom@yahoo.com (T. Headley).

0925-8574/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ecoleng.2013.07.062

drinking water from rivers or water bodies that receive upstream inputs of human waste and effluents. Poorly treated wastewater from on-site and decentralised septic tanks and small treatment plants often infiltrates into groundwater, either intentionally or inadvertently. In poor countries, especially in arid regions, wastewater is commonly used as a source of irrigation water, often with little or no prior treatment (Asano et al., 2007). In poor communities and decentralised situations, the capital cost and technical expertise required for on-going operation and servicing typically precludes the successful use of conventional tertiary treatment and disinfection technologies. In 2002, 20% of the population (60 million people) in the USA lived in unsewered residences and relied on decentralised wastewater management systems (Bisesi and Koren, 2003), while 48% of people (2.6 billion) in developing countries worldwide lack access to improved sanitation (WHO and UNICEF, 2010). Hence, there is a widespread need for appropriate technologies that can reduce pathogen risks while being simple and affordable to build, maintain and operate. Natural systems and ecotechnologies, such as treatment wetlands, sand filters and ponds are often hailed as being an appropriate solution for such situations due to their robust operation and low maintenance requirements (Ansola et al., 2003; Mara, 2003). In particular, systems that minimise the risk of human contact with the wastewater, such as subsurface flow wetlands and sand filters, are particularly suitable for decentralised applications where control of public access can be difficult.

There is a diverse range of microorganisms that are potentially present in untreated wastewater and which are associated with water borne diseases, including various species of enteric bacteria, protozoa, cyanobacteria, helminthes, and viruses (Asano et al., 2007). Because of the costs and analytical difficulties associated with identification and enumeration of these organisms in water samples, it is common practice to test for surrogate microorganisms which indicate the presence of faecal contamination. One of the most commonly used indicator organisms is *Escherichia coli*, which is a member of the faecal coliform group of bacteria. *E. coli* is commonly found in the intestinal tracts of humans and other warm-blooded animals and, although most strains are harmless, its presence in water indicates faecal contamination (Mara, 2003). The concentration of *E. coli* in untreated municipal wastewater typically ranges from 10^5–10^8 MPN/100 mL, while the median infectious dose (e.g., the typical dose needed to cause disease in humans) is in the range of 10^6–10^{10} (Asano et al., 2007).

There are several different types of subsurface flow ecotechnologies commonly used for decentralised wastewater treatment, which vary depending on flow configuration, media characteristics, use of plants and electricity requirements (Fonder and Headley, 2010). Such systems are typically designed for removal of particulates and oxygen demanding pollutants rather than for disinfection. However, some level of incidental pathogen reduction does generally occur through the treatment process which can influence downstream reuse options or disinfection requirements. Historically, relatively simple and passive horizontal subsurface flow (HF) wetlands have been most commonly used in many parts of the world and can typically achieve 2–3 $\log_{10}$ reduction in pathogen indicator organisms (Davison et al., 2005; Tanner et al., 2012). However, these systems have limited oxygen transfer rates and therefore require relatively large land areas (Nivala et al., 2013b). While HF wetlands have traditionally been built with wetted depths around 50 cm, there is some evidence that restricting the depth to the upper 25 cm of the bed (where the majority of plant roots occur) can enhance aerobic processes (Garcia et al., 2004; Headley et al., 2005). However, there is little information available about the effect of depth on pathogen removal.

Intermittently pulse-loaded vertical flow (VF) wetlands and sand filters have higher oxygen transfer rates and tend to be more compact than HF wetlands, but they have more specific media requirements and often require energy for pumping the influent. The conventional design for VF systems requires the use of well-graded washed sand with specific particle size characteristics for the main filter media (see for example Brix and Arias, 2005). Experience has shown that if the sand is too fine, not uniform enough or poorly washed, long-term operational problems such as clogging often emerge. In many parts of the world it is difficult or prohibitively expensive to obtain sand media of suitable quality, motivating practitioners to use fine gravel, which is less likely to clog. However, Tanner et al. (2012) reported a substantially poorer *E. coli* removal performance in VF wetlands with a fine gravel substrate compared to coarse sand (1.9 versus 3.2 $\log_{10}$ reduction). A confounding factor when examining the *E. coli* removal performance of vertical flow systems is the wide range of loading regimes used in practice, with the daily hydraulic load being divided up into anything from 4 doses/d (six hourly) (Tietz et al., 2007), 24 doses/d (hourly) (Tanner et al., 2012), to 32 doses/d (every 45 min) (Torrens et al., 2009). There is some suggestion in the literature that dividing the daily hydraulic load into more frequent, smaller pulses should achieve better treatment performance compared to less frequent, larger pulses (Crites and Tchobanoglous, 1998). However, it is difficult to draw conclusions regarding the effect of loading regime on *E. coli* removal.

In an attempt to further reduce the land area requirement of ecotechnology systems, a new generation of intensified wetland technologies has emerged, such as aerated and reciprocating systems, which utilise higher energy inputs to optimise oxygen transfer (Kadlec and Wallace, 2009). Limited information on the pathogen removal performance of these emerging technologies has been published to date.

The environmental conditions and treatment processes that exist within these different ecotechnologies are quite varied and are likely to result in different rates of pathogen inactivation. The processes by which *E. coli* and pathogens can be removed in subsurface flow wetlands include filtration through the substrate and attached biofilms, sedimentation, aggregation, oxidation, exposure to biocides, antibiosis, predation, attack by lytic bacteria and viruses, natural die-off and competition for limiting nutrients or trace elements (Decamp and Warren, 1998; Gersberg et al., 1989; Green et al., 1997). Vacca et al. (2005) compared the enteric bacterial communities within the media of vertical and horizontal flow systems with sand or expanded clay substrates receiving the same influent and observed differences in the community structure depending on media type, flow direction and the proximity to plant roots. There have been a number of studies that have reported on the removal of pathogens or indicator organisms in one or two of these technologies individually. For example, Decamp and Warren (2000) reported a 2–3 $\log_{10}$ reduction in *E. coli* concentrations in pilot-scale horizontal subsurface flow wetlands. Ausland et al. (2002) reported faecal coliform removal rates of 2.9–6.3 $\log_{10}$ for pulse-loaded vertical flow filters treating septic tank effluent, depending on loading rate and media type. Tietz et al. (2007) observed a 3.5 $\log_{10}$ reduction in *E. coli* concentration in wastewater after passage through pilot-scale vertical flow wetlands with a sand substrate. Baeder-Bederski et al. (2005) reported an *E. coli* reduction of 5 $\log_{10}$ from municipal sewage with a combination of horizontal subsurface flow followed by vertical flow wetlands. However, there is a need for an improved understanding of the relative efficacy of subsurface flow ecotechnologies in removing or inactivating pathogens in wastewater (Werker et al., 2002). Until the recent establishment of an ecotechnology research facility by the Helmholtz Environmental Research Centre in Germany

Please cite this article in press as: Headley, T., et al., *Escherichia coli* removal and internal dynamics in subsurface flow ecotechnologies: Effects of design and plants. Ecol. Eng. (2013), http://dx.doi.org/10.1016/j.ecoleng.2013.07.062

to compare a range of systems designed for secondary treatment of domestic wastewater (Nivala et al., 2013a), there have been no studies enabling direct comparison of *E. coli* attenuation efficiencies of the various subsurface flow designs under the same climatic and wastewater conditions. Furthermore, there is currently very limited published information about the efficiency of intensified systems at removing pathogen indicator organisms.

A commonly debated topic amongst ecotechnologists is the role and necessity of plants in these systems. Plants offer numerous potential advantages in constructed wetland systems, such as passive oxygen transfer to the substrate, uptake of elements, release of root exudates and provision of diverse complex substrates for attached growth microorganisms (Brix, 1997). However, much of the scientific attention to date has focused on the role of wetland plants in removal of organic matter and nutrients from wastewater, with limited published evidence about the importance of vegetation for pathogen removal, especially spanning the range of subsurface flow systems currently in use. Decamp and Warren (2000) reported slightly higher *E. coli* removal rates in planted HF wetlands compared to unplanted beds, although no statistics were conducted to show if the observed differences were significant. Tanner et al. (1994) reported little difference in removal of faecal coliforms between planted and unplanted horizontal subsurface flow systems treating high strength dairy farm wastewaters. Gagnon et al. (2007) showed that higher microbial density and activity occurred in the presence of plants in microcosms studies, but they were not specifically looking at pathogens or indicator organisms. Tietz et al. (2007) found no significant difference in *E. coli* removal between planted and unplanted vertical flow wetlands, although their investigations were conducted on indoor systems where plant growth was likely limited, as indicated by relatively low stem densities when compared to wetland plants growing under outdoor conditions. Under field conditions, Torrens et al. (2009) observed no significant difference in pathogen indicator removal between planted and unplanted vertical flow sand filters which were loaded at relatively high rates with waste stabilisation pond effluent. Kadlec and Wallace (2009) reviewed a large amount of published data for faecal coliform removal in side-by-side planted and unplanted HF and VF systems and concluded that there is often an improvement with the presence of plants, although this was not a universal trend. Thus, there is a need for more conclusive investigations into the effect of wetland plants on the removal of *E. coli* from domestic sewage in the various subsurface flow designs under side-by-side field conditions.

With the above points in mind, it was the main aim of this study to compare *E. coli* removal in a range of commonly used subsurface flow ecotechnologies designed for secondary treatment of primary-settled domestic sewage under the same climatic conditions. Within this aim, several specific objectives were investigated, which were:

- to investigate the internal dynamics of waterborne *E. coli* within various types of subsurface flow ecotechnology systems,
- to determine if the presence of *Phragmites australis* plants have an effect on *E. coli* dynamics and removal in the various design types compared,
- to determine if there is a difference in the *E. coli* removal rates in 25 cm and 50 cm deep horizontal flow systems,
- to determine if there is a difference in *E. coli* removal rates in vertical flow systems with coarse sand versus fine gravel substrate,
- to examine the effect of hourly versus bi-hourly loading regime on *E. coli* removal in vertical flow ecotechnologies with a sand substrate, and
- to determine if flow direction (vertical versus horizontal) has an effect on *E. coli* removal in aerated subsurface flow systems.

2. Materials and methods

2.1. Location and study description

The research was carried out at the Langenreichenbach Ecotechnology Research Facility located near Leipzig in the state of Saxony, Germany (51.5°N, 12.9°E) from August 2010 to December 2011. The location is characterised by a temperate continental climate. The site consists of 15 pilot scale subsurface flow ecotechnologies, representing eight different designs and operational variants (collectively termed system types), including planted (*P. australis*) and unplanted versions of seven of these system types (Table 1). The system types included horizontal flow beds with 25 cm and 50 cm wetted depth, unsaturated vertical flow systems with fine gravel or coarse sand media operated with different loading regimes, aerated beds with vertical or horizontal flow and a reciprocating fill and drain system. It is important to note that the systems were not specifically designed or operated for the purpose of pathogen removal, but rather for removal of particulates, organic matter and, in some cases, nitrogen. Raw sewage from an adjacent wastewater treatment plant received primary treatment in a sedimentation tank from where it was pumped to the individual treatment units using a programmable control system. A full description of the study site and the technical details of the individual treatment units are presented in Nivala et al. (2013a).

2.2. Water sampling and E. coli analysis

Each week, a representative influent sample was collected by sampling the water as it was being pumped from the primary sedimentation tank into one of the treatment units. Due to the vast number of sampling points at the site, a cyclical sampling regime was implemented. The effluent from each bed was generally sampled at least every two to three weeks during the period from August 2010 until the end of December 2011. Internal samples at different fractional distances from inlet to outlet were also collected from each of the horizontal and vertical flow systems on a two to three weekly basis. There were no internal sampling points for the reciprocating beds. For the horizontal flow systems, the internal samples were collected from sampling ports located at mid-depth at distances of 12.5%, 25%, 50% and 75% along the flow path from inlet to outlet. For the unsaturated vertical flow beds, interception pan lysimeters installed at depths of 10 cm, 20 cm and 40 cm from the filter media surface enabled water samples to be collected from the various depths by intercepting the water as it percolated downwards through the filter media. In the aerated vertical flow beds, internal sample ports were located at the midpoints of the upper, middle and lower thirds of the depth profile. For further details about the design and internal sampling equipment for each system, refer to Nivala et al. (2013a).

All samples were collected in sterilised glass bottles and transported in an ice box to the laboratory for analysis within four hours of collection. Water samples were analysed for *E. coli* in the Environmental and Biotechnology laboratory at the Helmholtz Environmental Research Centre in Leipzig, Germany using the Colilert-18 Quanti-Tray™ method (IDEXX, USA) according to the manufacturer's instructions. This is a semi-automatic, enzyme-based method based on the most probable number (MPN) technique. Following appropriate dilution, the multi-trays were incubated for 18 h at 36 °C and the number of fluorescing wells counted under a UV light (366 nm). The MPN/100 mL was then

Please cite this article in press as: Headley, T., et al., *Escherichia coli* removal and internal dynamics in subsurface flow ecotechnologies: Effects of design and plants. Ecol. Eng. (2013), http://dx.doi.org/10.1016/j.ecoleng.2013.07.062

Table 1
Details of the 15 subsurface flow ecotechnology system types compared.

System type abbreviation[a]	Flow configuration[b]	Wetted media depth (m)	Saturation status	Main media type	Loading interval (h)	Surface area (m^2)
Horizontal flow						
H25, H25p	HF	0.25	Saturated	8–16 mm gravel	0.5	5.64
H50, H50p	HF	0.50	Saturated	8–16 mm gravel	0.5	5.64
Vertical flow						
VS1, VS1p	VF	0.85	Unsaturated	0–3 mm sand	1.0	6.20
VS2, VS2p	VF	0.85	Unsaturated	0–3 mm sand	2.0	6.20
VG, VGp	VF	0.85	Unsaturated	4–8 mm gravel	1.0	6.20
Intensified						
VA, VAp	VF + Aeration	0.85	Saturated	8–16 mm gravel	1.0	6.20
HA, HAp	HF + Aeration	1.00	Saturated	8–16 mm gravel	0.5	5.64
R	Reciprocating	0.85	Alternating	8–16 mm gravel	1.0	13.2

[a] Systems planted with *Phragmites australis* are denoted with *p* in the system abbreviation.
[b] HF = horizontal flow; VF = vertical flow.

determined from a manufacturer-supplied table defining the correlation between the number of fluorescing wells and the MPN of *E. coli* in the sample.

For each sample point, the geometric mean of the different sample dates was calculated. Where appropriate, the geometric means were $\log_{10}$ transformed for ease of interpretation. Evapotranspiration in the planted beds generally caused a higher rate of water loss compared to the unplanted beds. Such variations in evaporative water loss between the systems would potentially confound comparisons of *E. coli* concentration removal rates. Thus, the *E. coli* areal load removal rate was calculated for each bed using Eq. (1):

$$E.\,coli \text{ areal load removal rate } (\mathrm{MPN/m^2\ d}) = \frac{(Q_i C_i - Q_o C_o)}{A} \times 10,000 \quad (1)$$

where Q_i is the inflow rate (m^3/d), Q_o is the outflow rate (m^3/d), C_i and C_o are the *E. coli* concentrations in the influent and effluent respectively (MPN/100 mL) and A is the area of the bed (m^2).

At the time of sampling, a separate sample was collected and the water temperature measured immediately using a thermometer. Rainfall and air temperature data for the site were collected using an automatic weather station.

2.3. Statistical analyses

In order to examine the effect of system type (design/operational variant) and plant presence on the $\log_{10}$ *E. coli* Areal Load Removal rates over the study period, a linear mixed model (LMM) with repeated measures approach was used. SPSS® Statistics version 20 (IBM Corporation, NY, USA) was used for these LMM analyses. A LMM was used because it can better handle missing data than analysis of variance (ANOVA), allows for more flexible correlation structures and enables the effect of random covariates such as temperature, influent concentration, system age or date to be included. Furthermore, our data is comprised of repeated observations on the same subjects (beds) over time, which cannot be considered as independent replicates, therefore violating one of the fundamental assumptions of traditional ANOVA. In comparison, the LMM can accommodate correlated data. Following extensive preliminary model parameter exploration taking a penalised likelihood approach by comparing the Bayesian information criterion (BIC) for various model structures, an auto-regressive structure was found to best describe the covariance of the data. System type and plant presence were fixed effects, while sample date and mean monthly air temperature were treated as covariates in the LMM. Variance parameters in the LMM were estimated using the restricted maximum likelihood approach in order to identify significant differences ($p = 0.05$) between the fixed effects (system type, plant presence, date and system type × plant presence interaction). If significant differences were identified, post-hoc Bonferroni comparisons of estimated marginal means were done for the main fixed effects (system type and plant presence) in order to identify between which treatments the differences exist ($p < 0.05$).

In order to determine if the rate of *E. coli* concentration reduction across the internal sampling points varied between the different beds, the analysis of covariance (ANCOVA) procedure described in Zar (2010) was used in a similar way to that of Headley et al. (2005) who applied it to internal data from a horizontal flow wetland. The procedure was used to compare linear regression equations in a two-step process. Firstly, the slopes of the regression equations were compared to determine if they were significantly different ($p = 0.05$). If two or more lines had different slopes, then it was concluded that the regression equations describing the internal rate of concentration reduction are different. If the slopes were found to be the same, then the elevation of the regression lines (y-intercepts) were compared ($p = 0.05$) to determine if the regression equations were the same (slopes and y-intercepts not different), or parallel (share the same slopes, but different y-intercepts). Since all beds in the current study received the same influent, the y-intercepts (influent concentration) should in theory be the same for all of the regression equations. Thus, beds with the same regression slopes will share the same regression equation and can therefore be assumed to have the same internal rate of *E. coli* concentration reduction. The horizontal and vertical flow systems were analyzed separately. In the vertical flow beds, the internal profiles represented the *E. coli* concentration at different depths from 0 cm (influent) to 85 cm (effluent). For the horizontal flow systems, the nominal hydraulic residence time (nHRT) at each internal sample point was inferred using Eq. (2):

$$n\mathrm{HRT} = \left(\frac{V}{Q}\right) y \quad (2)$$

where V is the water volume of the bed (m^3) calculated by multiplying the wetted media volume by the media porosity, Q is the average flow rate through the bed for the study period (m^3/d) based on the average of the mean daily inflow and the mean daily outflow rates, and y is the longitudinal fractional distance of the sample point between the inlet and the outlet (dimensionless). In this way, the horizontal-flow internal profiles represent the *E. coli* concentration reduction over nHRT, enabling beds with different loading rates to be compared directly. The media porosity was measured by filling a 200 L container with dry media and measuring the volume of water required to fill the container to the media surface.

The internal profiles generally displayed a strong exponential decay trend. Thus, the dependent variable (*E. coli* concentration)

Please cite this article in press as: Headley, T., et al., *Escherichia coli* removal and internal dynamics in subsurface flow ecotechnologies: Effects of design and plants. Ecol. Eng. (2013), http://dx.doi.org/10.1016/j.ecoleng.2013.07.062

Table 2
Mean hydraulic loading and *E. coli* concentration and removal rates (±one standard deviation) for the different wetland beds over the study period. Hydraulic parameters are arithmetic means, while *E. coli* data are geometric means. Sample numbers (*n*) are shown in parentheses.

Bed	Hydraulic load		*E. coli*			
	HLR[a] (L/m^2 d)	*n*HRT[b] (days)	Influent concentration (MPN/100 mL)	Effluent concentration (MPN/100 mL)	Log_{10} reduction (MPN/100 mL)	Areal load removal rate (MPN/m^2 d)
H25	18	5.2	$7.4 \times 10^6 \pm 4.1 \times 10^6$ (42)	$2.7 \times 10^5 \pm 2.1 \times 10^5$ (42)	1.4 ± 0.41	$1.3 \times 10^9 \pm 7.5 \times 10^8$
H25p	18	5.6	$7.4 \times 10^6 \pm 4.1 \times 10^6$ (42)	$1.8 \times 10^5 \pm 1.9 \times 10^5$ (42)	1.5 ± 0.41	$1.3 \times 10^9 \pm 7.6 \times 10^8$
H50	36	5.2	$7.4 \times 10^6 \pm 4.1 \times 10^6$ (42)	$3.7 \times 10^5 \pm 2.5 \times 10^5$ (42)	1.3 ± 0.37	$2.5 \times 10^9 \pm 1.5 \times 10^9$
H50p	36	5.4	$7.4 \times 10^6 \pm 4.1 \times 10^6$ (42)	$3.0 \times 10^5 \pm 1.9 \times 10^5$ (42)	1.3 ± 0.37	$2.5 \times 10^9 \pm 1.5 \times 10^9$
HA	131	2.9	$7.6 \times 10^6 \pm 3.2 \times 10^6$ (26)	$6.8 \times 10^2 \pm 3.3 \times 10^3$ (26)	4.0 ± 0.65	$1.0 \times 10^{10} \pm 4.5 \times 10^9$
HAp	131	2.9	$7.6 \times 10^6 \pm 3.2 \times 10^6$ (26)	$2.9 \times 10^3 \pm 7.9 \times 10^3$ (26)	3.3 ± 0.72	$1.0 \times 10^{10} \pm 4.5 \times 10^9$
VA	97	na	$7.7 \times 10^6 \pm 3.2 \times 10^6$ (27)	$5.2 \times 10^4 \pm 1.2 \times 10^5$ (27)	2.1 ± 0.48	$7.3 \times 10^9 \pm 3.1 \times 10^9$
VAp	97	na	$7.6 \times 10^6 \pm 2.9 \times 10^6$ (25)	$5.2 \times 10^4 \pm 1.0 \times 10^5$ (25)	2.1 ± 0.48	$7.2 \times 10^9 \pm 2.9 \times 10^9$
VS1	95	na	$7.2 \times 10^6 \pm 3.2 \times 10^6$ (42)	$1.2 \times 10^5 \pm 6.0 \times 10^5$ (42)	1.6 ± 0.75	$6.4 \times 10^9 \pm 3.2 \times 10^9$
VS1p	95	na	$7.2 \times 10^6 \pm 3.2 \times 10^6$ (42)	$4.4 \times 10^4 \pm 2.4 \times 10^5$ (42)	2.1 ± 0.68	$6.6 \times 10^9 \pm 3.2 \times 10^9$
VS2	95	na	$7.2 \times 10^6 \pm 3.2 \times 10^6$ (42)	$1.7 \times 10^5 \pm 5.8 \times 10^5$ (42)	1.5 ± 0.60	$6.4 \times 10^9 \pm 3.3 \times 10^9$
VS2p	95	na	$7.2 \times 10^6 \pm 3.2 \times 10^6$ (42)	$1.0 \times 10^5 \pm 2.0 \times 10^5$ (42)	1.8 ± 0.56	$6.6 \times 10^9 \pm 3.2 \times 10^9$
VG	96	na	$7.2 \times 10^6 \pm 3.2 \times 10^6$ (42)	$6.4 \times 10^5 \pm 1.4 \times 10^6$ (42)	0.9 ± 0.63	$5.5 \times 10^9 \pm 3.0 \times 10^9$
VGp	96	na	$7.2 \times 10^6 \pm 3.2 \times 10^6$ (42)	$8.9 \times 10^5 \pm 1.7 \times 10^6$ (42)	0.8 ± 0.50	$5.3 \times 10^9 \pm 2.7 \times 10^9$
R	146	2.5	$7.3 \times 10^6 \pm 4.1 \times 10^6$ (36)	$9.3 \times 10^3 \pm 1.3 \times 10^5$ (36)	2.8 ± 0.73	$1.1 \times 10^{10} \pm 6.8 \times 10^9$

na = not applicable.
[a] HLR = hydraulic loading rate (based in inflow rate).
[b] *n*HRT = Nominal hydraulic residence time (based on average of inflow and outflow).

was subjected to a log_{10} transformation in order to generate a straight line relationship. The data were tested to ensure that the assumptions of linearity, normality and homogeneity of variance were not violated. The significance ($p = 0.05$) of the regression equations were tested using the ANOVA procedure. Non-significant regressions were not included in the subsequent ANCOVA. Overall, the VA and VAp beds were the only systems excluded from the ANCOVA, because the assumptions of linearity and homogeneity of variance were violated. If the ANCOVA determined that the slopes or *y*-intercepts were significantly different, then the Tukey multiple comparison procedure was used to identify between which beds the differences existed. SPSS® Statistics version 20 was used for normality, homogeneity and ANOVA testing, while the ANCOVA and Tukey multiple comparison testing were conducted manually by entering the calculation procedures outlined in Zar (2010) into an Excel® 2010 spreadsheet (Microsoft Corporation, WA, USA).

3. Results and discussion

3.1. Inlet and outlet concentrations and log_{10} reductions

The inlet *E. coli* concentrations ranged from a minimum of 2.2×10^6 to a maximum of 2.4×10^7 MPN/100 mL, with geometric mean of 7.2×10^6–7.7×10^6 MPN/100 mL depending on the bed (Table 2). These concentrations are considered typical of primary treated sewage or effluent from a well-functioning septic tank (Crites and Tchobanoglous, 1998). The mean hydraulic loading rates and hydraulic residence times for each system are also shown in Table 2. The effluent water temperature ranged from 0.1 to 21.3 °C and followed a seasonal trend.

In general, the subsurface flow ecotechnologies examined were effective at reducing the concentration of *E. coli* to varying degrees, ranging from 0.8 (VGp) to 4.0 (HA) log_{10} reduction. Despite having the highest loading rates of all the systems, the aerated horizontal flow beds (HA and HAp) and the reciprocating bed (R) had the highest *E.* coli log_{10} concentration reduction rates, with 4.0, 3.3 and 2.8 Logs, respectively. These beds all produced effluents with geometric mean concentrations below 10,000 MPN/100 mL (Table 2 and Fig. 1). There is a scarcity of published *E. coli* reduction data for intensified systems. With mean *E. coli* concentrations below 10^5 MPN/100 mL, the effluents from these systems are considered to be acceptable for restricted irrigation reuse on crops that are not eaten raw, pending demonstration that the concentrations of intestinal nematodes are also less than 1 egg/L (Mara, 2003). The aerated vertical flow beds (VA and VAp) and VS1p also satisfied this *E.* coli requirement, with geometric mean effluent concentrations of 5.2×10^4 and 4.4×10^4 MPN/100 mL, respectively (2.1 log_{10} reduction). However, intestinal nematodes were not measured in this study.

The HA bed performed the best out of all the systems, with a geometric mean *E. coli* concentration of 680 MPN/100 mL which is below the threshold value of less than 1000 MPN/100 mL that is generally considered acceptable for unrestricted irrigation of salad crops and uncooked vegetables (Mara, 2003). However, confirmation that the concentration of intestinal nematodes was less than 1 egg/L would be required before unrestricted irrigation could be permitted. Such high removal of *E. coli* is very impressive given the high loading rate received by this intensified system and demonstrates a clear benefit of the added energy inputs used for aeration.

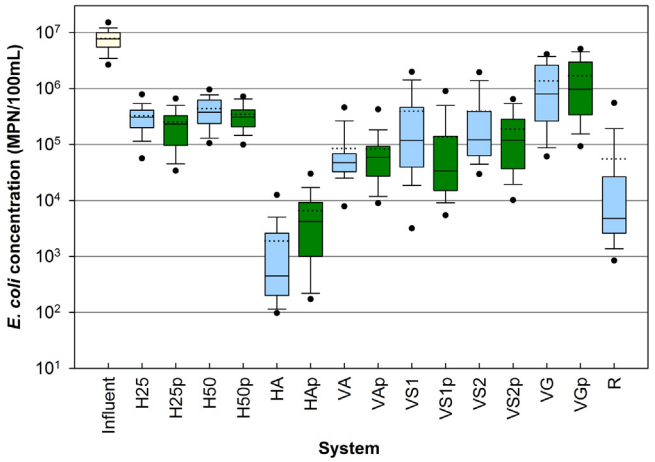

Fig. 1. Box and whisker plot of influent and effluent *E. coli* concentrations from each treatment system for the study period. Lines within the boxes are the medians, dotted lines are the means, boundaries of the boxes are the 25th and 75th percentiles, error bars are the 10th and 90th percentiles, while the dots represent the 5th and 95th percentiles of the data.

Good performance was also maintained by the HA bed through winter, with a mean effluent concentration of 1297 MPN/100 mL and $\log_{10}$ reduction of 4.2 ($n = 11$) for sampling days when the mean air temperature was below 5 °C (average temperature for this subset of data was 2.8 °C). Even on the coldest sampling day, when the mean air temperature was −4.3 °C (December 2010), HA produced an effluent *E. coli* concentration of 6090 MPN/100 mL (2.8 $\log_{10}$ reduction). Maintaining effective treatment performance in cold northern climates was one of the main drivers behind the development of such aerated intensified designs, albeit for pollutants other than pathogens (Wallace and Kadlec, 2005).

The sand-based vertical flow systems displayed intermediate performance, with 1.5–2.1 $\log_{10}$ reductions and mean effluent *E. coli* concentrations of approximately 10^5 MPN/100 mL. Torrens *et al.* (2009) observed similar *E. coli* reduction rates of approximately 1.5 $\log_{10}$ for 65 cm deep vertical flow filters, both with and without plants. Arias et al. (2003) reported a faecal coliform reduction of 1.7 $\log_{10}$ in a VF wetland with 0.8 m depth of filter sand. However, these removal rates are substantially lower than the reductions of over 2.9 $\log_{10}$ reported by Ausland et al. (2002) and Tietz et al. (2007) for faecal coliforms and *E. coli* respectively. The gravel-based VF systems (VG, VGp) performed the worst of all systems, achieving less than 1 $\log_{10}$ reduction in *E. coli* concentrations, followed by the passive horizontal flow systems (H50, H50p, H25, H25p) which achieved 1.3–1.5 $\log_{10}$ reductions on average. Tanner et al. (2012) observed a similar decreased *E. coli* removal efficiency in gravel compared to sand-based VF wetland pilot systems (sand 1.7 times better than gravel), although they experienced generally higher removal rates overall. None of the unsaturated VF systems produced an effluent that is considered suitable for above-ground irrigation of crops and such effluent would need to be applied sub-surface in order to minimise the risk to public health. The performance of the horizontal flow systems is within the range reported for faecal coliforms in Kadlec and Wallace (2009) from a number of full-scale HF wetland studies. However, Tanner et al. (2012) reported a higher mean *E. coli* reduction of 2.8 logs for a HF wetland in New Zealand of similar scale and receiving a similar loading rate to the current study. This may be due to the fact that the HF wetland in New Zealand experienced higher water temperatures year-round of 12–30 °C, compared to 0.1–21 °C in the present study. Kadlec and Wallace (2009) examined several data sets and summarised that the effect of temperature on removal of pathogen indicator organisms is not clear.

3.2. Areal *E. coli* load removal rates

Differences in the log *E. coli* areal load removal rate ($\log_{10}$ [MPN/m^2 d]) between the various system types (varying based on design or loading regime in the case of the VS systems) and between planted and unplanted versions of each system type were compared using a linear mixed model. The load removal was compared because it represents the total quantity of *E. coli* organisms removed, taking into account both the differences in evapotranspirative water loss between planted and unplanted systems, and the fact that not all systems received the same hydraulic load. Such hydraulic differences made it difficult to compare the relative efficiency of these different systems using concentration data alone.

Differences in the log *E. coli* areal load removal rates between the different system types compared were extremely significant ($p < 0.0005$; Table 3). Air temperature also had a significant influence on the *E. coli* load removal rate overall ($p < 0.0005$) with higher load removals at warmer temperatures, largely due to the fact that the influent *E. coli* concentration generally increased with air temperature, leading to higher *E. coli* loads in summer. However, plants had no significant effect on the *E. coli* load removal rates overall ($p = 0.865$). Several studies have indicated no effect of plants on *E. coli* or faecal coliform concentration reduction in both gravel-based HF systems (Tanner et al., 1994; Rivera et al., 1995) and sand-based VF beds (Tietz et al., 2007; Torrens et al., 2009). This is most likely because the major *E. coli* removal pathways in subsurface flow wetlands are physical processes such as sedimentation, straining and entrapment in biofilms attached to the substrate (Stevik et al., 2004; Stott and Tanner, 2005; Weber and Legge, 2008; Williams et al., 1995), coupled with the biological processes of predation and competition by microorganisms such as protozoa (Decamp and Warren, 1998; Decamp et al., 1999; Stott et al., 2001, 2003a,b; Wand et al., 2007), bacteria such as *Bdellovibrio bacteriovorus* (Wand et al., 2007), and natural die-off (Karim et al., 2004; Wand et al., 2007). Based on the evidence available, it seems that the presence of *P. australis* roots and rhizomes has little impact on these pathogen removal processes.

Table 3
Results of the linear mixed model repeated measures analysis comparing the log *E. coli* areal load removal rates for the different beds. System type and plant presence are fixed main factors, while air temperature is a covariate.

Factor	$df_{denominator}$	$df_{numerator}$	p
System type	7	98	<0.0005
Plant presence	1	101	0.865
System × plants	6	100	1.000
Air temperature	1	156	<0.0005

The results of the post-hoc comparisons are shown in Fig. 2. The passive horizontal flow systems had significantly lower *E. coli* load removal rates than all the other systems, with H25 and H25p having the lowest load removal rates of all systems (9.10 and 9.11 $\log_{10}$, [MPN/m^2 d], respectively), followed by H50 and H50p (9.40 and 9.41 $\log_{10}$, [MPN/m^2 d] respectively). Thus, the depth of horizontal flow systems had a significant effect on *E. coli* load removal rate, with 50 cm wetted gravel depth being better than 25 cm. However, this can be at least partly explained by the fact that the shallower beds received approximately half the influent areal loading rate of the deeper beds. Experience has shown that, for most pollutants in constructed wetlands, areal removal rates tend to increase as the influent loading rate increases (Tanner et al., 1998).

There was no significant difference in mean load removal rate amongst the vertical flow systems, which ranged from 9.73 (VGp) to 9.86 (VA) $\log_{10}$ [MPN/m^2 d]. The unsaturated gravel-based VF systems (VG and VGp) had higher areal load removal rates than the

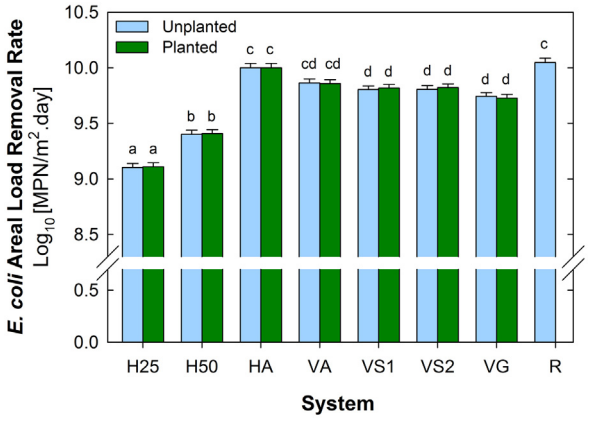

Fig. 2. Mean log *E. coli* areal load removal rates for the 15 beds. Systems with different letters above their columns have significantly different means ($p < 0.05$) according to the Bonferroni post-hoc comparisons following the linear mixed model analyses.

Please cite this article in press as: Headley, T., et al., *Escherichia coli* removal and internal dynamics in subsurface flow ecotechnologies: Effects of design and plants. Ecol. Eng. (2013), http://dx.doi.org/10.1016/j.ecoleng.2013.07.062

passive HF systems, even though the HF beds produced lower effluent *E. coli* concentrations. These higher load removal rates occurred because the VF systems were loaded at a rate three to five times higher than the HF beds (Table 2). Interestingly, the use of sand or fine gravel, or loading hourly or bi-hourly, had no significant effect on the overall *E.* coli load removal rate in the unsaturated vertical flow systems. Thus, the differences in effluent *E. coli* concentrations between the VG and VS systems seen in Fig. 1 and Table 2 cannot be considered significant, as evidenced by the large variance in the data. This is in contrast to the findings of Tanner et al. (2012) who observed lower effluent *E. coli* concentrations and higher $\log_{10}$ reductions in sand based VF wetlands than in those with a fine gravel substrate. However, no statistical analysis was conducted by Tanner et al. (2012) to determine if the observed differences were in fact significant. Ausland et al. (2002) also observed poorer faecal coliform removal in VF filters (unplanted) loaded at 80 mm/d as the media particle size increased from sand to fine gravel (2–4 mm), although they were comparing expanded clay aggregates rather than alluvial gravel and their filters were loaded 12 times per day rather than 24, which may account for the difference in results to the current study. Further detailed investigation is warranted into the effect of media size on pathogen removal in unsaturated VF systems.

The different loading regimes examined here (dividing the daily load into 12 or 24 equal pulses) for the sand-based vertical flow filters had no effect on *E. coli* removal. Both loading regimes can be considered satisfactory from an *E. coli* perspective. However, this is in contrast to the observations of other authors who have reported a significant improvement in *E. coli* concentration reduction when the daily load is divided into smaller, more frequent doses. For example, Torrens et al. (2009) found better performance in sand-based VF beds when the HLR of 800 mm/d was delivered in 32 rather than 16 pulses per day. However, their systems received a HLR eight times higher than in the current study which would have resulted in at least partial saturation of the media during loading, making the hydraulic conditions within the substrate and the general performance somewhat different. Further investigations are necessary to determine if a larger difference between the loading regimes (e.g., 6 pulses/d versus 48 pulses/d) under the conditions of the current study would result in any effect on *E. coli* removal.

Intensification significantly improved *E. coli* removal, with the reciprocating (*R*) and aerated beds (HA, HAp, VA and VAp) having the highest load removal rates overall (10.05, 10.00, 10.00, 9.86 and 9.86 $\log_{10}$, [MPN/m^2 d] respectively), which were not significantly different from each other ($p > 0.05$). Of the intensified systems, VA and VAp had the lowest areal load removal rates and highest effluent concentrations by at least one order of magnitude. Post-hoc testing indicated that VA and VAp were not significantly different from the unsaturated VF systems (Fig. 2) and therefore sat on the verge between the other intensified systems and the unsaturated VF beds with regards to *E. coli* load removal.

In general, there was a clear relationship between *E. coli* areal load removal rate and the degree of system intensification, with passive (no electricity consumption) horizontal flow wetlands performing the worst, while the intensified aerated and reciprocating systems had the highest rates of *E. coli* removal per square meter. In the middle were the unsaturated vertical flow systems, which can be considered to have an intermediate level of intensification ("semi-intensive") due to the use of pumps for pulse loading. This is the first reported case where the effect of intensification on the rate of *E. coli* removal in subsurface flow ecotechnologies has been clearly demonstrated. In each case, the main aim of intensification was to increase the rate of oxygen supply to the subsurface environment to improve the removal efficiency for oxygen demanding substances, such as organic matter and ammoniacal nitrogen (Nivala et al., 2013b). Fortuitously, the evidence from the current study demonstrates that there is an additional benefit from the enhanced oxygen availability provided by such intensification measures, in the form of enhanced *E. coli* removal. While some studies have indicated that pathogen removal may be related to the dissolved oxygen concentration in the water (Williams et al., 1995), there is limited published information to date about the effect of aeration on the removal of *E. coli* and other pathogens. It is conceivable that the aerobic conditions created by aeration of the saturated media lead to a change in the microbial ecology and trophic structure, facilitating the establishment of communities of organisms which grazed or predated on the *E. coli* bacteria. Further research into the microbial ecology of aerated subsurface flow ecotechnologies is warranted. The trend of increasing *E. coli* areal removal rate with increasing intensification may also be partly explained by the fact that the more heavily intensified systems generally received higher influent loading rates, which invariably resulted in increased areal removal.

3.3. Internal concentration profiles

The internal longitudinal $\log_{10}$ *E. coli* concentration profiles for the HF systems, with fractional distance from inlet to outlet represented as nominal hydraulic residence time (*n*HRT), are shown in Fig. 3. The $\log_{10}$ concentrations generally follow a linear reduction over the length of the HF beds, except for HAp, which showed an inflection at the halfway point of the bed, after which the mean $\log_{10}$ concentration displayed little further reduction. In some beds (H50, H50p, H25 and HAp) the outlet samples showed a slight deviation from the overall profile, with a small increase in *E. coli* concentration between the 75% point and the outlet. This is likely related to the fact that the internal samples and the outlet samples were collected using different methodologies. The internal samples were collected in situ directly from a pipe positioned at mid-depth within the gravel substrate. In comparison, the outlet water was collected via a perforated pipe on the bottom of each bed and then passed through an outlet chamber and length of pipe before reaching the outlet sample collection point. Thus, the outlet water was collected from a different depth and may have undergone some additional transformations prior to being sampled, compared to the samples collected in situ. More consistent profiles may have been obtained by collecting the outlet sample in the same way as the internal samples.

The relationship between $\log_{10}$ *E. coli* concentration and *n*HRT was similar for the 50 cm and 25 cm deep HF systems, both with and without plants (H50, H50p, H25 and H25p). However, the aerated HF systems (HA and HAp) showed a much more rapid rate of *E. coli* concentration reduction, achieving an *E. coli* concentration of 5.4 $\log_{10}$ (≈250,000 MPN/100 mL) after a *n*HRT of only 17.5 h (fractional distance of 25%) compared to the non-aerated HF beds which did not attain this concentration until the outlet (mean *n*HRT of 5.2–5.6 days). This was supported by the ANCOVA comparison of the linear regression equations for the relationship between $\log_{10}$ *E. coli* concentration (MPN/100 mL) and *n*HRT in the HF systems (Table 4). The linear regression equations generally provided a good fit to the data (high r^2) and had slopes extremely significantly different from zero ($p<0.0001$). The ANCOVA showed that the slopes of the HF regression lines were extremely different [$F(5,559) = 177.4$, $p < 0.0001$]. In other words, the internal rate of *E.* coli concentration reduction against *n*HRT was not the same for all systems. Tukey HSD post-hoc comparisons ($p < 0.01$) revealed that the differences in slopes were only between the aerated and non-aerated systems (Table 4). The rate of *E. coli* concentration reduction against *n*HRT was significantly greater in HAp and HA compared to the other HF systems, which all shared the same

Please cite this article in press as: Headley, T., et al., *Escherichia coli* removal and internal dynamics in subsurface flow ecotechnologies: Effects of design and plants. Ecol. Eng. (2013), http://dx.doi.org/10.1016/j.ecoleng.2013.07.062

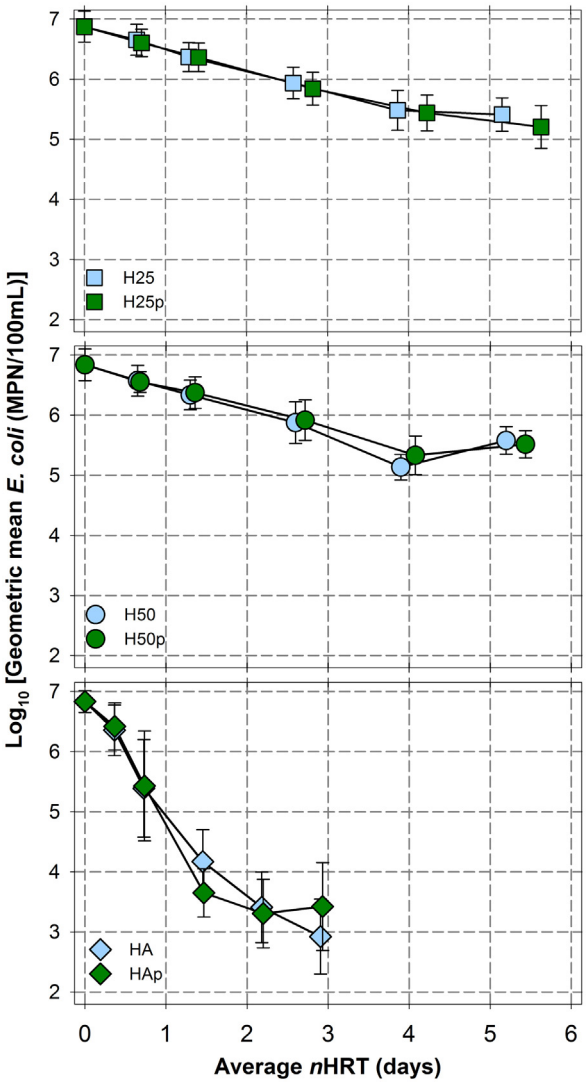

Fig. 3. Internal profiles for the horizontal flow systems. Values shown are geometric means. Error bars = ±standard deviation; $n = 17$ (HA, HAp, H25, H25p) and 14 (H50, H50p).

slope. Thus, there was no significant effect of plants across the HF systems, and the depth of the non-aerated HF systems (25 cm versus 50 cm) had no effect on the rate of *E. coli* concentration reduction against *n*HRT. As previously noted, Tanner et al. (1994) and Rivera et al. (1995) found no clear effect of plants on faecal coliform removal in gravel-based HF systems.

The internal depth profiles of $\log_{10}$ *E. coli* concentration for the VF systems are shown in Fig. 4. The unsaturated VF systems (VS1, VS1p, VS2, VS2p, VG and VGp) displayed a linear rate of $\log_{10}$ concentration reduction with depth, indicating that bed depth is important for *E. coli* removal and that lower outlet concentrations could potentially be achieved with deeper beds. Conversely, in the aerated saturated VF beds (VA and VAp), the $\log_{10}$ *E. coli* concentration decreased sharply from the inlet to the first depth sample point (0.1 m), but then remained stable through subsequent depths. Regressions for VA and VAp therefore failed the tests of linearity and homogeneity of variance and were excluded from the subsequent ANCOVA testing. This lack of a linear concentration-depth profile can be explained by the occurrence of extensive mixing caused by the aeration in the VA and VAp beds, with the air bubbles rising turbulently from the bottom of the bed towards the top in the opposite direction to the water flow which travelled from top to the bottom. Thus, the hydraulics of the aerated VF beds more closely resembles a completely stirred tank reactor (CSTR) than it does plug flow. In a completely mixed reactor, inflowing water mixes almost instantly with the contents of the reactor resulting in a uniform concentration of constituents within the vessel (Fogler, 2005), which also means that there is the potential for continuous contamination of the outflowing water with influent. This partially explains why the aerated VF beds achieved 1.2–1.9 $\log_{10}$ poorer *E. coli* reduction than the aerated HF beds, despite receiving the same areal and volumetric loading rates. In contrast to the aerated VF beds, the mixing created by the aeration in HA and HAp was in a direction perpendicular to the hydraulic flow path which is likely to have broken any short-circuit pathways and created reactor hydraulics somewhere between completely-mixed and plug-flow. Hydraulic tracer studies are needed to confirm the hydraulic characteristics of the aerated VF and HF systems.

The ANCOVA testing showed that extremely significant differences existed amongst the slopes of the unsaturated VF regression lines [$F(5,361) = 4.68$, $p = 0.0004$]. Thus, the rate of *E.* coli concentration reduction against depth was not the same for all systems. There were no obvious differences in the depth profiles for the sand based VF systems, except that the hourly-loaded planted bed (VS1p) displayed a slightly more rapid rate of concentration

Table 4
Comparison of linear regression equations for the relationships between $\log_{10}$ *E. coli* concentration (dependent variable) and fractional distance through the wetland (independent variable). ANCOVA indicated that the differences between slopes are extremely significant for both the horizontal flow ($p < 0.0001$) and vertical flow ($p = 0.0004$) systems. The superscript letters associated with each slope reflect the results of Tukey HSD testing, with slopes that are not significantly different ($p < 0.01$) sharing the same letter.

Flow direction	System	Independent variable (x)	Slope (b)	y-intercept (a)	r^2
Horizontal	H25	*n*HRT (days)	−0.30[a]	6.76	0.718
	H25p	*n*HRT (days)	−0.22[a]	6.65	0.755
	H50	*n*HRT (days)	−0.29[a]	6.71	0.699
	H50p	*n*HRT (days)	−0.24[a]	6.69	0.714
	HA	*n*HRT (days)	−1.47[b]	6.61	0.843
	HAp	*n*HRT (days)	−1.34[b]	6.46	0.717
Vertical	VS1	Depth (m)	−1.95[d]	6.64	0.577
	VS1p	Depth (m)	−2.26[e]	6.41	0.546
	VS2	Depth (m)	−1.75[d]	6.77	0.748
	VS2p	Depth (m)	−1.91[d]	6.72	0.668
	VG	Depth (m)	−1.44[d]	6.85	0.504
	VGp	Depth (m)	−1.08[d]	6.77	0.443
	VA*	Depth (m)	−1.82	5.99	0.413
	VAp*	Depth (m)	−1.76	5.88	0.370

* Regressions for VA and VAp failed the tests of linearity and homogeneity of variance and were therefore excluded from the subsequent ANCOVA testing.

Please cite this article in press as: Headley, T., et al., *Escherichia coli* removal and internal dynamics in subsurface flow ecotechnologies: Effects of design and plants. Ecol. Eng. (2013), http://dx.doi.org/10.1016/j.ecoleng.2013.07.062

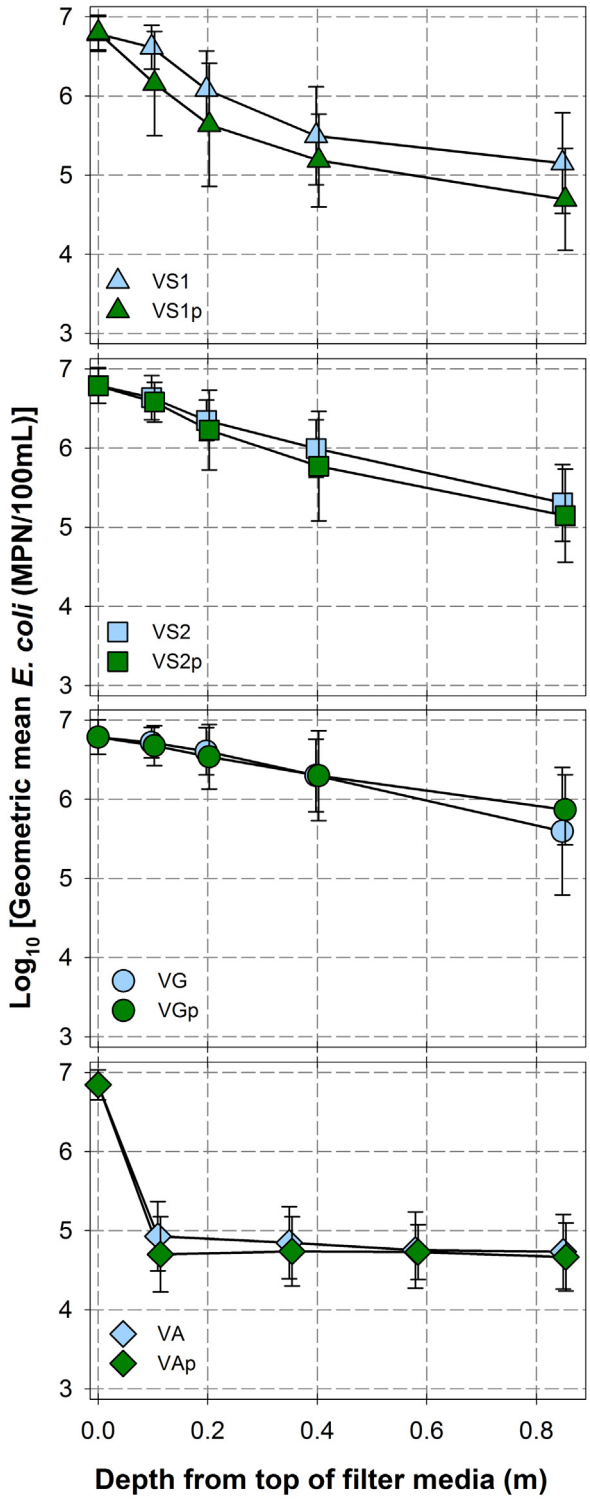

Fig. 4. Internal profiles for the vertical flow systems. Values shown are geometric means. Error bars = ±standard deviation; n = 13 (VS1p, VS2), 14 (VS1, VS2p, VG, VGp) or 17 (VA, VAp).

decrease over the first 10 cm (Fig. 4). This was borne out by the Tukey HSD comparisons, which revealed that VS1p was the only system with a significantly different slope ($p < 0.01$), while the other systems all shared the same slope. In other words, the rate at which the *E. coli* concentration decreased with depth was significantly greater in VS1p than in the other beds. This indicates that, of all the unsaturated VF design factors compared in this study, a planted, hourly-loaded sand-based VF wetland requires less depth to achieve a given level of *E. coli* reduction. However, changing any one of these three design variables (planted to unplanted, sand media to gravel, or hourly to two-hourly loading frequency) caused this advantage to be lost. Planting had no effect on gravel-based beds (VG versus VGp), or sand-based beds that were loaded every 2 h (VS2 versus VS2p). Reducing the loading interval from two hours to one hour in the sand-based beds only improved the rate of *E. coli* reduction when plants were present; without plants, there was no effect of loading frequency. Tietz et al. (2007) found no significant difference between planted and unplanted sand-based VF beds in the amount of microbial biomass at different depths or the overall *E. coli* concentration reduction.

The gravel based VF beds appeared to have a slower rate of *E. coli* concentration reduction with depth compared to the sand-based beds (Fig. 4), indicating that the gravel beds would either need to be deeper or to receive a lighter loading rate to achieve similar effluent concentrations. However, the variation within the data, as indicated by the error bars in Fig. 4, proved to be greater than any differences in the rate of *E. coli* reduction with depth between the gravel and sand-based VF systems. Thus, VG and VGp were shown statistically to have the same rate of *E. coli* reduction with depth (slope) as the sand-based systems (except for VS1p).

4. Conclusions

This study enabled, for the first time, the removal rates and internal dynamics of *E. coli* in several common and emerging subsurface flow ecotechnologies designed for secondary treatment of sewage to be compared side-by-side, both with and without vegetation. The removal of *E. coli* depended largely on the design, especially the degree of energetic intensification. In general, there was no significant effect of vegetation. Despite receiving the highest loading rates, the intensified aerated HF systems (HA and HAp) and reciprocating system (*R*) outperformed the other designs in all of the monitored *E. coli* reduction metrics, producing effluent suitable for restricted irrigation reuse (pending intestinal nematode monitoring and local regulations) in the smallest footprint, albeit at the highest electricity consumption. The HA bed performed the best, with a geometric mean effluent *E. coli* concentration of 680 MPN/100 mL at a mean hydraulic loading rate of 131 L/m^2 d (*n*HRT of 2.9 days). In comparison, the effluent concentrations of the passive horizontal flow beds were almost three orders of magnitude higher, despite receiving substantially lower loading rates (*n*HRT of 5.2–5.6 days). This was also reflected in the internal concentration profiles, which showed a significantly faster rate of reduction in the aerated HF beds, which achieved in the equivalent of less than one day *n*HRT the same concentration (5.4 $\log_{10}$ MPN/100 mL) that the passive HF beds achieved at their outlets after more than five days *n*HRT. Thus, the additional input of electricity and equipment for aeration yielded the benefit of greatly enhanced *E. coli* removal efficiency. Further research is needed to determine if the enhanced *E. coli* removal was due to the direct creation of aerobic conditions in the filter bed and resultant changes in the microbiological ecology and trophic structure (grazing, predation or competition), or secondary effects such as nutrient deficiency, modified mixing or the physical agitation created by the aeration.

Amongst the aerated systems it was found that a horizontal flow path was better than the vertical flow path. This was mainly due to greater mixing within the VA and VAp beds leading to continuous cross-contamination of the effluent with the incoming wastewater

Please cite this article in press as: Headley, T., et al., *Escherichia coli* removal and internal dynamics in subsurface flow ecotechnologies: Effects of design and plants. Ecol. Eng. (2013), http://dx.doi.org/10.1016/j.ecoleng.2013.07.062

which partly counteracted the increased removal efficiency caused by the aeration.

In the passive HF beds (H25, H25p, H50 and H50p), neither depth nor plants had a significant effect on the internal rate of concentration reduction against *n*HRT, indicating that retention time within the bed of gravel is a key factor governing *E. coli* reduction in non-aerated HF systems and that macrophytes are not important for the physical, chemical or biological processes that may be responsible for *E. coli* retention or die-back. The passive HF systems had significantly lower areal load removal rates than all of the other designs, partly because they received the lowest influent areal loading rates. Accordingly, the shallow (25 cm deep) passive HF beds had significantly lower areal removal rates than the 50 cm deep beds. More research is needed to see if there is a difference in areal removal rates when HF systems with different depths are loaded at the same areal loading rate.

The unsaturated sand-based VF systems displayed an intermediate level of performance amongst all of the systems, while the gravel-based vertical flow beds (VG and VGp) performed the worst of all systems in terms of concentration, with less than 1 $\log_{10}$ reduction. However, they achieved higher areal load removal rates than the passive HF systems (H50, H50p, H25 and H25p), due partly to the fact that they received influent loading rates approximately three to five times higher than these HF beds. There was no significant difference in *E. coli* areal load removal amongst the various unsaturated vertical flow systems. Thus, the use of sand or gravel substrate, or hourly versus bi-hourly loading regime in the sand-based systems had no effect on areal *E. coli* load removal. There were no differences between the rates of *E. coli* concentration reduction with depth in the various unsaturated VF systems (planted and unplanted), with the exception of the planted sand based hourly loaded bed (VS1p) which experienced a higher rate of concentration reduction with depth. This indicates that the combination of sand media, hourly loading and vegetation requires less depth to achieve a given effluent *E. coli* concentration than the other design configurations compared. However, further research is needed to validate this claim since no significant differences were found in the overall areal load removal rates of the various unsaturated VF systems.

The areal load removal rate has proved to be a useful performance metric in this study for comparing the relative efficiency of a range of different wetland systems that, by virtue of their various designs, are typically operated at different hydraulic loading rates. However, it is apparent that there are also limitations in using this parameter due to the fact that areal removal efficiencies often increase in response to an increase in areal loading rate, without necessarily delivering lower concentrations at the outlet. It is clear that a range of performance parameters need to be used in order to adequately and comprehensively compare the relative merits of different treatment system designs that are loaded at different areal and volumetric loading rates.

This study has highlighted the potential offered by subsurface flow ecotechnologies designed and operated for secondary treatment of domestic sewage as operationally simple, low-maintenance solutions for reducing the pathogen risk associated with wastewater. *E. coli* reduction is a function of design (especially degree of energy input to enhance oxygen transfer) and loading rate in such systems. At one end of the spectrum, lightly-loaded passive HF wetlands can achieve low to moderate levels of *E. coli* removal without the need for electricity. These systems also typically produce an effluent that is low in organic matter and suspended solids, making them suitable for use in combination with other technologies for mitigating public health risks, such as chlorine or ultra-violet disinfection or application below-ground using subsurface drip-irrigation. At the other end of the spectrum, aerated HF systems were shown to be capable of consistently reducing *E. coli* concentrations to very low levels (less than 1000 MPN/100 mL) at relatively high loading rates through the input of moderate amounts of electricity to drive air pumps. Despite the need for more complex design and infrastructure installation, these systems are still operationally simple and robust when compared to conventional activated sludge technologies. Even lower *E. coli* concentrations should be achievable by reducing the loading rate, potentially negating the need for any additional disinfection processes. The smaller area requirement of the intensified systems lends them to applications where space is limited, electricity is available and/or high levels of treatment performance are needed. The sand-based unsaturated vertical flow systems sit somewhere in the middle, offering a good compromise of moderate levels of *E. coli* removal, potentially without the use of electricity where sufficient site topography allows for gravity-loading. Further research is warranted to see if the same trends hold true for other pathogenic organisms, such as parasites and viruses. Also, the potential merits of combining different systems in series to enhance pathogen removal should be investigated.Acknowledgements

This work was supported by funding from the German Ministry of Education & Research (BMBF) within the context of the SMART Project (Ref. 02WM1080). All authors thank Katy Bernhard (Helmholtz Center for Environmental Research–UFZ, Germany) for her invaluable role in constructing, operating, and monitoring the research facility at Langenreichenbach. We are also especially grateful to Grit Weichert and Petra Hoffmann (UFZ, Germany) for their endless support with sampling and laboratory analysis. Jaime Nivala also acknowledges the Helmholtz Interdisciplinary Graduate School for Environmental Research (HIGRADE) and UFZ for additional funding and support.

References

Ansola, G., Gonzales, J.M., Cortijo, R., de Luis, E., 2003. Experimental and full-scale pilot plant constructed wetlands for municipal wastewaters treatment. Ecol. Eng. 21, 43–52.

Arias, C.A., Cabello, A., Brix, H., Johansen, N.-H., 2003. Removal of indicator bacteria from municipal wastewater in an experimental two-stage vertical flow constructed wetland system. Water Sci. Technol. 48 (5), 35–41.

Asano, T., Burtin, F.L., Leverenz, H.L., Tsuchihashi, R., Tchobanoglous, G., 2007. Water Reuse: Issues, Technologies, and Applications. McGraw-Hill, NY, USA.

Ausland, G., Stevik, T.K., Hanssen, J.F., Koehler, J.C., Jenssen, P.D., 2002. Intermittent filtration of wastewater-removal of fecal coliforms and fecal streptococci. Water Res. 36 (14), 3507–3516.

Baeder-Bederski, O., Duerr, M., Borneff-Lipp, M., Kuschk, P., Netter, R., Daeschlein, G., Mosig Mueller, R.A., 2005. Retention of *Escherichia coli* in municipal sewage by means of planted soil filters in two-stage pilot plant systems. Water Sci. Technol. 51 (9), 205–212.

Bisesi, M., Koren, H., 2003. Handbook of Environmental Health, Volume 1: Biological, Chemical, and Physical Agents of Environmentally Related Disease, fourth ed. CRC Press, Boca Raton, FL.

Brix, H., 1997. Do macrophytes play a role in constructed treatment wetlands? Water Sci. Technol. 35 (54), 11–17.

Brix, H., Arias, C., 2005. The use of vertical flow constructed wetlands for on-site treatment of domestic wastewater: new Danish guidelines. Ecol. Eng. 25, 491–500.

Crites, R.W., Tchobanoglous, G., 1998. Small and Decentralised Wastewater Management Systems. WCB/McGraw-Hill, Boston, USA.

Davison, L., Headley, T., Pratt, K., 2005. Aspects of design, structure, performance and operation of reed beds—eight years' experience in northeastern New South Wales, Australia. Water Sci. Technol. 51 (10), 129–138.

Decamp, O., Warren, A., 1998. Bacterivory in ciliates isolated from constructed wetlands (reed beds) used for wastewater treatment. Water Res. 32 (7), 1989–1996.

Decamp, O., Warren, A., 2000. Investigation of *Escherichia coli* removal in various designs of subsurface flow wetlands used for wastewater treatment. Ecol. Eng. 14, 293–299.

Decamp, O., Warren, A., Sanchez, R., 1999. The role of ciliated protozoa in subsurface flow wetlands and their potential as bioindicators. Water Sci. Technol. 39 (7), 65–70.

Fogler, H.S., 2005. Elements in Chemical Reaction Engineering, fourth ed. Prentice Hall, NJ, USA.

Fonder, N., Headley, T., 2010. Systematic classification, nomenclature and reporting for constructed treatment wetlands. In: Vymazal, J. (Ed.), Water and Nutrient

Management in Natural and Constructed Wetlands. Springer Science + Business Media B.V., Dordrecht pp. 191–219.
Gagnon, V., Chazarenc, F., Comeau, Y., Brisson, J., 2007. Influence of macrophyte species on microbial density and activity in constructed wetlands. Water Sci. Technol. 56 (3), 249–254.
Garcia, J., Aguirre, P., Mujeriego, R., Huang, Y., Ortiz, L., Bayona, J.M., 2004. Initial contaminant removal performance factors in horizontal flow reed beds used for treating urban wastewater. Water Res. 38, 1669–1678.
Gersberg, R.M., Gearheart, R.A., Ives, M., 1989. Pathogen removal in constructed wetlands. In: Hammer, D.A. (Ed.), Constructed Wetlands for Wastewater Treatment: municipal, industrial and agricultural. Lewis Publishers Inc., MI, USA, pp: 431–445.
Green, M.B., Griffin, P., Seabridge, J.K., Dhobie, D., 1997. Removal of bacteria in subsurface flow wetlands. Water Sci. Technol. 35 (5), 109–116.
Headley, T.R., Herity, E., Davison, L., 2005. Treatment at different depths and vertical mixing in a 1-m deep horizontal subsurface flow wetland. Ecol. Eng. 25, 567–582.
Kadlec, R.H., Wallace, S.D., 2009. Treatment Wetlands, second ed. CRC Press, Boca Raton, FL.
Karim, M.R., Manshadi, F.D., Karpiscak, M.M., Gerba, C.P., 2004. The persistence and removal of enteric pathogens in constructed wetlands. Water Res. 38 (7), 1831–1837.
Mara, D., 2003. Domestic Wastewater Treatment in Developing Countries. Earthscan, London, UK.
Nivala, J., Headley, T., Wallace, S., Bernhard, K., Brix, H., van Afferden, M., Müller, R., 2013a. Comparative analysis of constructed wetlands: design and construction of the ecotechnology research facility in Langenreichenbach. Germany. Ecol. Eng., http://dx.doi.org/10.1016/j. ecoleng.2013.01.035.
Nivala, J., Wallace, S., Headley, T., Kassa, K., Brix, H., van Afferden, M., Müller, R., 2013b. Oxygen transfer and consumption in subsurface flow treatment wetlands. Ecol. Eng, http://dx.doi.org/10.1016/j.ecoleng.2012.08.028.
Rivera, F., Warren, A., Ramirez, E., Decamp, O., Bonilla, P., Gallegos, E., Calderon, A., Sanchez, J.T., 1995. Removal of pathogens from wastewaters by the root zone method (RZM). Water Sci. Technol. 32 (3), 211–218.
Stevik, T.K., Aa, K., Ausland, G., Hanssen, J.F., 2004. Retention and removal of pathogenic bacteria in wastewater percolating through porous media: a review. Water Res. 38, 1355–1367.
Stott, R., May, E., Matsushita, E., Warren, A., 2001. Protozoan predation as a mechanism for the removal of *Cryptosporidium* oocysts from wastewaters in constructed wetlands. Water Sci. Technol. 44 (11–12), 194–198.
Stott, R., May, E., Mara, D.D., 2003a. Parasite removal by natural wastewater treatment systems: performance of waste stabilisation ponds and constructed wetlands. Water Sci. Technol. 48 (2), 97–104.
Stott, R., May, E., Ramirez, E., Warren, A., 2003b. Predation of *Cryptosporidium* oocysts by protozoa and rotifers: implications for water quality and public health. Water Sci. Technol. 47 (3), 77–83.
Stott, R., Tanner, C.C., 2005. Influence of biofilm on removal of surrogate faecal microbes in a constructed wetland and maturation pond. Water Sci. Technol. 51 (9), 315–322.
Tanner, C.C., Clayton, J.S., Upsdell, M.P., 1994. Effect of loading rate and planting on treatment of dairy farm wastewaters in constructed wetlands. I. Removal of oxygen demand, suspended solids and faecal coliforms. Water Res. 29 (1), 17–26.
Tanner, C.C., Sukias, J.P.S., Upsdell, M.P., 1998. Relationships between loading rates and pollutant removal during maturation of gravel-bed constructed wetlands. J. Environ. Qual. 27, 448–458.
Tanner, C.C., Sukias, J.P.S., Headley, T.R., Yates, C.R., Stott, R., 2012. Constructed wetlands and denitrifying bioreactors for on-site and decentralised wastewater treatment: comparison of five alternative configurations. Ecol. Eng. 42, 112–123.
Tietz, A., Kirschner, A., Langergraber, G., Sleytr, K., Haberl, R., 2007. Characterization of microbial biocenosis in vertical subsurface flow constructed wetlands. Sci. Total Environ. 280, 163–172.
Torrens, A., Molle, P., Boutin, C., Salot, M., 2009. Removal of bacterial and viral indicators in vertical flow constructed wetlands and intermittent sand filters. Desalination 246, 169–178.
Vacca, G., Wand, H., Nikolausz, M., Kuschk, P., Kästner, M., 2005. Effect of plants and filter materials on bacteria removal in pilot-scale constructed wetlands. Water Res. 39, 1361–1373.
Wand, H., Vacca, G., Kuschk, P., Krueger, M., Kaestner, M., 2007. Removal of bacteria by filtration in planted and non-planted sand columns. Water Res. 41 (1), 159–167.
Wallace, S.D., Kadlec, R.H., 2005. BTEX degradation in a cold-climate wetland system. Water Sci. Technol. 51 (9), 165–171.
Weber, K.P., Legge, R.L., 2008. Pathogen removal in constructed wetlands. In: Russo, R.E. (Ed.), Wetlands: Ecology, Conservation, & Restoration. Nova Science Publishers Inc., New York, USA, pp. 177–211.
Werker, A.G., Dougherty, J.M., McHenry, J.L., Van Loon, W.A., 2002. Review: treatment variability for wetland wastewater treatment design in cold climates. Ecol. Eng. 19, 1–11.
Williams, J., Bahgat, M., May, E., Ford, M., Butler, J., 1995. Mineralisation and pathogen removal in gravel bed hydroponic constructed wetlands for wastewater treatment. Water Sci. Technol. 32 (3), 49–58.
World Health Organisation and United Nations International Children's Emergency Fund, 2010. Progress on Sanitation and Drinking-Water: 2010 Update. WHO Press, Geneva, Switzerland.
Zar, J., 2010. Biostatistical Analysis, fifth ed. Pearson Prentice Hall, NJ, USA.

Please cite this article in press as: Headley, T., et al., *Escherichia coli* removal and internal dynamics in subsurface flow ecotechnologies: Effects of design and plants. Ecol. Eng. (2013), http://dx.doi.org/10.1016/j.ecoleng.2013.07.062