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Comparison of removal efficiency of pathogenic microbes in four types of wastewater treatment systems in Denmark

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ABSTRACT

The aim of the present work was to evaluate and compare the performance in the removal of pathogenic microbes in four different types of decentralized wastewater treatment systems, namely: horizontal flow constructed wetlands (HFCW), vertical flow constructed wetlands (VFCW), biological sand filters (BSF) and biofilters (BF). All the systems analyzed are located in Jutland, Denmark. Water sampling took place during a three months period that covered from winter to spring. Conventional microbial indicators such as *Escherichia coli*, total coliforms (TC), intestinal enterococci and sulphite-reducing clostridia were quantified using traditional microbiological culture methods, whereas *Bacteroides* spp. determination was performed by quantitative PCR (qPCR). Other water quality parameters such as dissolved oxygen, biological oxygen demand (BOD_5), total suspended solids (TSS), pH, temperature, ammonium concentration and conductivity of influent and effluent water samples were also analyzed. The results showed that bacterial indicators significantly reduced in all the systems analyzed. In general, BF showed the best performance in the removal of microbes for all bacteria studied, while BSF demonstrated an improved capacity to eliminate *E. coli* and TC. Contrarily, VFCW seems to be more effective reducing the amount of intestinal enterococci, sulphite-reducing clostridia, and *Bacteroides* spp. In the present study, HFCW were the less efficient wastewater treatment system for the elimination of the evaluated pathogens. However, the performance in the removal of microbes was still significant considering that such systems were the oldest under operation (with over 20 years of continuous task).

1. Introduction

During the last decades, many researchers have focused their attention on the use of natural systems to remove pharmaceuticals, microorganisms, organic matter, and personal care products from urban wastewater. Constructed wetlands (CW), biological sand filters (BSF) and biofilters (BF) have been proven to be an effective technology able to reduce pollution generated from wastewaters, runoff, and other types of pollutants in waters, being specially designed to solve wastewater treatment needs where the centralized systems are not economically or technically viable (Hedmark and Scholz, 2008; Vymazal and Kröpfelová, 2009; Vymazal, 2011; Kurzbaum et al., 2012). In particular, these water treatment technologies have been used in Denmark for > 20 years, and are still being established with very good results to comply with the stringent Danish discharge demands. Horizontal flow constructed wetlands (HFCW) have been used since the early 1980 to treat domestic wastewater generated in urban areas from around 200 Danish municipalities (Brix et al., 2007). The selection of this technology was influenced by the apparent low building costs and minimum operation and maintenance needs, as well as its expected effective performance to treat waters from different origins (Uhl and Dittmer, 2005; Healy et al., 2007; Babatunde et al., 2008; Vymazal and Kröpfelová, 2009). Unfortunately, after some years of implementation most of such systems presented operational problems (clogging), and the pollutants removal expectations were not totally fulfilled. Furthermore, in 1997, Denmark emitted new and more stringent requirements for wastewater treatment that made HFCW obsolete. Following local research and foreign experiences new constructed wetland

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Table 1
Specific details of household wastewater treatment systems analyzed at the present study. VFCW and BSF are unsaturated systems; therefore, residence time is about some hours.

Location	System	Planted*	Area (m^2)	P.E.** served	Recirculation	Phosphorous removal	TRH*** (days)	Years of operation	Organic loading (g/m^2 d)
Bjødstrup	HFCW1	Yes	470	80	No	No	6.12	> 20	8.2
Gronfeld	HFCW2	Yes	1800	220	No	No	42.6	> 20	12.3
Friland	VFCW1	Yes	90	30	Yes	No	< 1	2	20
Tisset	VFCW2	Yes	16	2	No	Chemical	< 1	4	4.7
Astrup	VFCW3	Yes	16	4	Yes	Chemical	< 1	5	15
Logenskovvej	BSF1	No	26	5	Yes	Yes	< 1	5	12
Bojenskovvej	BSF2	No	26	6	No	Chemical	< 1	2	9.8
Friland	BF1	No	50	4	No	Filtralite® P	31	6	4.8
Hanne's	BF2	No	50	6	Yes	Filtralite® P	20.6	6	7.2

* Planted systems with *Phragmites australis*.
** P.E.: person equivalent.
*** TRH: hydraulic residence time.

developments were investigated and implemented; and finally, in 2004, the Danish Environmental Protection Agency (EPA) published a series of guidelines for the design and construction of vertical flow constructed wetlands (VFCW) (Brix and Arias, 2005a,b). Since then, around 1000 VFCW have been built across the country.

Biological sand filters (BSF) are another technological solution for decentralized domestic wastewater treatment frequently used in different countries around the world (Healy et al., 2007; Bali et al., 2011; Stauber et al., 2012). These systems were widely used in Denmark since 1997 to treat domestic wastewater, and currently this technology is nationally accepted (Brix and Arias, 2005a,b). BSF use similar operational principles than VFCW but the construction guidelines suggest the need of larger treatment surfaces and therefore higher construction costs.

Biofilters (BF) are a different technology developed in Norway during the early 90s to meet the needs exerted by the unfavourable climatic conditions for plant development where constructed wetlands could not achieve their full potential. BF pollutant removal mechanisms rely on the combination of oxic-anoxic environments and the use of specific light weight aggregates and specific media (Fitralite-P®) to remove phosphorus (Jenssen et al., 2010). There are only two BF constructed in Denmark that were built in 2003 as a part of an industrial sponsored research initiative looking for a common decentralized wastewater treatment solution at the Nordic countries. The high construction costs of such systems combined with the possibility to use other equally efficient and more economical alternatives to wastewater treatment explains why no more BF have been constructed in Denmark since then. However, BF are still widely used in Norway and Sweden.

Sanitary risk is directly associated with the presence of microbial pathogens in waters, especially those present in untreated wastewater. Pathogenic organisms should be removed before water discharge to the environment in order to ensure population safety (Graczyk and Lucy, 2007). The reuse of treated wastewater is also a major challenge as global warming increases and water scarcity increases, especially in warm latitudes. In general, natural wastewater treatment systems are not designed but for secondary treatment, and not to remove microbial pollution. It is known that these systems could act as excellent bacterial sinks through a combination of complex physical, chemical and biological factors that actively participate in the reduction of the number of bacteria present in water (Vymazal, 2005; Wu et al., 2016). In the last 15 years, significant resources have been invested to improve the understanding of the mechanisms involved in the removal of microbes at decentralized systems (Arias et al., 2003; Hansen et al., 2004; Ibekwe et al., 2003; Karim et al., 2004; Vacca et al., 2005; Winward et al., 2008; Adrados et al., 2014; Morató et al., 2014; Wu et al., 2016; Alexandros and Akratos, 2016; Akunna et al., 2017). However, there is still a lack of information from comparative studies evaluating the removal of microbes between natural wastewater treatment systems actively working during long-term operation periods.

Therefore, the aim of the present work was to evaluate the performance in the removal of conventional indicator organisms and pathogenic microbes (*Escherichia coli*, total coliforms, intestinal enterococci, sulphite-reducing clostridia and *Bacteroides* spp.) for a series of different non-conventional wastewater treatment systems (HFCW, VFCW, BSF and BF) located at Denmark. In addition, systems capability to improve wastewater physicochemical parameters was also considered.

2. Material and methods

2.1. Site description

Samples were taken from real-operating decentralized wastewater treatment systems constructed in the vicinity of Aarhus (Jutland, Denmark). All the selected systems have been effectively functioning for several years and are representative of similar systems used all over the world. The analyzed systems correspond to horizontal flow constructed wetlands (HFCW), vertical flow constructed wetlands (VFCW), biological sand filters (BSF) and biofilters (BF) with expanded clay aggregate as filtering and bed material. The operative and design characteristics are shown in Table 1. A general scheme of each kind of treatment system is presented in Fig. 1.

2.2. Sample collection

Grab samples were collected between March and June (2014) in three sampling campaigns (approximately one per month) over three consecutive days ($n = 9$); except for BF where the first campaign did not take place ($n = 6$). Influent and effluent water samples were collected from each system in 1 L sterile glass bottles and transported under refrigeration (4 °C) to the laboratory within 24 h for the microbiological analysis.

2.3. Physicochemical parameters

Water temperature, dissolved oxygen (O_2), pH and electric conductivity were measured *in-situ* using commercially available calibrated electrodes (Hach Lange BmbH, Barcelona). Samples were immediately transported under refrigeration to the laboratory of the Department of Bioscience (Aarhus University) for further analysis. Additional water quality parameters evaluated included total suspended solids (APHA 2540 D method), ammonia nitrogen (APHA 4500 NH_3 D method) and BOD_5 (APHA 5210B method) (APHA, 2012).

2.4. Microbiological analyses

Total coliforms, *E. coli* and intestinal enterococci were determined by the membrane filtration method (0.45 μm pore size sterile cellulose, Millipore, MA, USA) with subsequent colony counting, and were