CrossMark

Key design factors affecting microbial community composition and pathogenic organism removal in horizontal subsurface flow constructed wetlands

Jordi Morató [a,*], Francesc Codony [a], Olga Sánchez [b], Leonardo Martín Pérez [a,d], Joan García [c], Jordi Mas [b]

[a] *Laboratory of Health and Environmental Microbiology, Department of Optics and Optometry, AquaSost-UNESCO Chair on Sustainability, Technical University of Catalonia, c/Violinista Vellsolà, 37, 08222 Terrassa, Barcelona, Spain*
[b] *Department of Genetics and Microbiology, Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain*
[c] *GEMMA-Group of Environmental Engineering and Microbiology, Department of Hydraulic, Maritime and Environmental Engineering, Universitat Politècnica de Catalunya-BarcelonaTech, c/Jordi Girona, 1–3, Building D1, E-08034 Barcelona, Spain*
[d] *Rosario Chemical Institute (IQUIR-CONICET, UNR), Faculty of Biochemical and Pharmacological Sciences, National University of Rosario, Suipacha 531, 2000 Rosario, Santa Fe, Argentine*

HIGHLIGHTS

- Key design factors affecting HSSF constructed wetlands performance were evaluated.
- Water depth of 0.27 m was more effective than 0.50 m for microbial removal.
- Fine granulometry was more effective than coarse gravel for microbial removal.
- Microbial removal in all HSSF wetlands analysed occurs mainly near the inlet.
- Microbial communities from constructed wetlands were affected by water depth.

ARTICLE INFO

ABSTRACT

Constructed wetlands constitute an interesting option for wastewater reuse since high concentrations of contaminants and pathogenic microorganisms can be removed with these natural treatment systems. In this work, the role of key design factors which could affect microbial removal and wetland performance, such as granular media, water depth and season effect was evaluated in a pilot system consisting of eight parallel horizontal subsurface flow (HSSF) constructed wetlands treating urban wastewater from Les Franqueses del Vallès (Barcelona, Spain). Gravel biofilm as well as influent and effluent water samples of these systems were taken in order to detect the presence of bacterial indicators such as total coliforms (TC), *Escherichia coli*, fecal enterococci (FE), *Clostridium perfringens*, and other microbial groups such as *Pseudomonas* and *Aeromonas*. The overall microbial inactivation ratio ranged between 1.4 and 2.9 log-units for heterotrophic plate counts (HPC), from 1.2 to 2.2 log units for total coliforms (TC) and from 1.4 to 2.3 log units for *E. coli*. The presence of fine granulometry strongly influenced the removal of all the bacterial groups analyzed. This effect was significant for TC ($p = 0.009$), *E. coli* ($p = 0.004$), and FE ($p = 0.012$). Shallow HSSF constructed wetlands were more effective for removing *Clostridium* spores ($p = 0.039$), and were also more efficient for removing TC ($p = 0.011$) and *E. coli* ($p = 0.013$) when fine granulometry was used. On the other hand, changes in the total bacterial community from gravel biofilm were examined by using denaturing gradient gel electrophoresis (DGGE) and sequencing of polymerase chain reaction (PCR)-amplified fragments of the 16S rRNA gene recovered from DGGE bands. Cluster analysis of the DGGE banding pattern from the different wetlands showed that microbial assemblages separated according to water depth, and sequences of different phylogenetic groups, such as Alpha, Beta and Delta-Proteobacteria, Nitrospirae, Bacteroidetes, Acidobacteria, Firmicutes, Synergistetes and Deferribacteres could be retrieved from DGGE bands.

© 2014 Elsevier B.V. All rights reserved.

1. Introduction

Water shortages in arid and semi-arid areas such as the Mediterranean have prompted a need for wastewater treatment and subsequent reuse. Reclamation can be achieved through conventional intensive

* Corresponding author. Tel.: +34 93 7398660; fax: +34 93 7398301.
E-mail address: jordi.morato@upc.edu (J. Morató).

systems or natural, ecologically engineered treatments such as horizontal subsurface flow (HSSF) constructed wetlands. Depending on wastewater type, some pathogenic microorganisms may be present and, therefore, wastewater reclamation processes with disinfection could be required (Asano and Levine, 1998). Thus, research into sewage treatment is needed in order to reduce risks associated with improper sanitation, particularly in terms of wastewater reuse for crop irrigation.

Regardless of their location (water column, biofilms on surface material or sediment pore-water), pathogens must compete with the consortium of organisms surrounding them. As intestinal organisms, most may not survive and may also be destroyed by predation. Water temperature, organic matter concentration and hydraulic conditions such as flow, aspect ratio, and granular media type are some of the most important factors governing occurrence and growth of viable microbes in biofilms developed elsewhere (LeChevallier et al., 1988, 1996; Reasoner et al., 1989; Block, 1992; Block et al., 1993; Van der Kooij et al., 1995).

Molecular fingerprinting techniques (Friedrich et al., 2003; Ibekwe et al., 2003; Vacca et al., 2005) have been used to study the dynamics and structure of microbial communities in constructed wetlands but few studies have been performed on the effect of wetlands on the removal of specific pathogens. Although the work with powerful new techniques, such as quantitative real time polymerase chain reaction (qPCR), will undoubtedly change the scene of health and environmental microbiology during the following years, until now routine examination for pathogenic microorganisms is not recommended because of the high cost of the analysis and the generally low number of a specific pathogen that is present in an environmental sample. Therefore, indicator organisms are routinely used to study microbial removal in constructed wetlands.

In general, most studies on fecal microorganism removal in constructed wetlands only describe total and fecal coliform removal (Kadlec and Knight, 1996; Wang et al., 2005; Tanaka et al., 2006; Tunçsiper, 2007). Research using experimental, pilot and full-scale constructed wetlands has shown that fecal coliform bacteria inactivation usually ranges between 1.25 and 2.5 log units (Gersberg et al., 1989 a,b; Hiley, 1990; Rivera et al., 1995; Williams et al., 1995; Decamp et al., 1999; Arias et al., 2003; Vacca et al., 2005; Vymazal, 2005). However, fecal coliform inactivation rates of 3.0 log units and higher have been recorded in tertiary HSSF constructed wetlands treating slaughterhouse wastewater with extremely high influent concentrations of fecal bacteria (from 6.0 to 11.0 log units/100 mL). Furthermore, removals of 2.4 to 5.3 orders of magnitude for cultivable *Salmonella* cells were found (Pundsack et al., 2001). The high degree of inactivation observed in these wetlands was related to the high influent microbial concentration (Rivera et al., 1995).

It must also be taken into account that the concentration of coliform bacteria in wastewaters is subject to significant daily fluctuations, so the highest concentration in the influent of a given constructed wetland system will not necessarily coincide with the highest concentration in the effluent (Cooper et al., 1996).

In any case, subsurface flow constructed wetlands offer a suitable combination of physical, chemical and biological mechanisms required to remove pathogenic organisms. Physical factors include filtration and sedimentation (Gersberg et al., 1989a; Pundsack et al., 2001), while chemical mechanisms combine oxidation and adsorption to organic matter (Gersberg et al., 1989a). The biological removal features include oxygen release and bacterial activity in the rhizosphere, aggregation and retention in biofilms (Hiley, 1995; Brix, 1997), potential production of bactericidal compounds or antimicrobial activity of root exudates (Kickuth and Kaitzis, 1975; Seidel, 1976; Axelrood et al., 1996), as well as predation by nematodes and protists (Decamp and Warren, 1998; Decamp et al., 1999), attack by lytic bacteria and viruses (Axelrood et al., 1996), natural die-off (Gersberg et al., 1989a,b) and competition for limiting nutrients or trace elements (Gersberg et al., 1987a,b).

Fecal bacteria removal in constructed wetlands has been related to environmental factors such as granular medium or type of plant.

Some studies appear to show that granular media and the presence or absence of plants (macrophytes) in constructed wetlands are important for fecal bacteria inactivation, while in others' works there is no evidence of this fact. On the one hand, wetland plants play several roles in the HSSF constructed wetland. Their root systems provide surfaces for the attachment of microorganisms, enhance filtration effects, and stabilize the bed surface. The roots contribute to the development of microorganisms by the release of oxygen and nutrients. Moreover, the plants give the treatment site an attractive appearance. The effect of macrophytes on the system efficiency seems to vary depending on the season, wastewater type and plant species (Stein and Hook, 2003). On the other hand, the use of a small size granular medium instead of a large size one seems to improve the microbial inactivation ratio between 1.0 and 2.0 log units for both fecal coliforms and somatic coliphage removal (Ottová et al., 1997; Garcia et al., 2003). Nevertheless, several researchers have obtained contradictory results, and links between microbial removal and environmental factors have still not been definitively understood.

Another important design parameter for constructed wetlands is water depth. From practical experience, water depth in subsurface flow constructed wetlands has been normally set at 0.60 m because this is the maximum depth at which the roots and rhizomes of the macrophytes grow, and it is therefore the maximum depth at which the macrophytes can have effects on the process (Cooper et al., 1996). The mass transfer theory dictates that water depth influences the oxygen transfer coefficient from the atmosphere to the water. Water depth also determines the fraction of water volume in contact with the underground biomass of the macrophytes. Therefore, despite the fact that only the surface area is in contact with atmosphere, it can be reasonably supposed that water depth influences the efficiency of the subsurface flow constructed wetlands. However, the information available on the effect of water depth is scarce and contradictory (US EPA, 2000; Coleman et al., 2001).

Thus, the removal of pathogens in subsurface flow constructed wetlands used for wastewater treatment seems to be a very complex process and may vary in time and space, depending on many factors. Even though possible mechanisms of bacterial removal have been discussed in many papers (Burger and Weise, 1984; Armstrong et al., 1990; Morales et al., 1996; Decamp and Warren, 2000), no systematic analyses on the removal processes and the fate of potential pathogenic bacteria in constructed wetlands are yet known. In terms of bacterial removal, constructed wetlands are generally considered to feature a combination of chemical and physical factors, including mechanical filtration and sedimentation (Pundsack et al., 2001). Also, the relative importance of the different biochemical reactions that can take place in these natural treatment systems plays a determinant role on the efficiency of the system and the microbial removal.

In view of all the above, the objective of the present work was to evaluate and clarify the role of different design key factors, such as granular media, water depth and season effect, that could affect the removal of microbial indicators in order to improve our understanding of the microbial reduction in constructed wetlands. We have also performed a molecular analysis using denaturing gradient gel electrophoresis (DGGE) with the aim to determine the effect of wetland design on the microbial diversity of gravel biofilms.

2. Materials and methods

2.1. Pilot plant system

The pilot HSSF system used in this study treats part of the urban wastewater generated by the Can Suquet housing development in the municipality of Les Franqueses del Vallès (Barcelona, north-east Spain). A detailed description of the plant can be found in García et al.