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Methanogenic activity of accumulated solids and gas emissions from planted and unplanted shallow horizontal subsurface flow constructed wetlands

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ABSTRACT

Anaerobic processes play an important role in horizontal subsurface flow (HSSF) constructed wetlands (CW) and methane generation contributes to overall greenhouse gas emissions. Plants and bed depth are two main factors that can influence anaerobic processes but the effect of plants' presence and species on methane emissions on shallower HSSF (0.3 m bed depth) has not been studied. We measured CH_4 and CO_2 emissions from a pilot plant constituted of five HSSF units in parallel with different plant species (CW1-UN: unplanted, CW2-*JE*: *Juncus effusus*, CW3-*IP*: *Iris pseudacorus*, CW4-*TL*: *Typha latifolia* L. and CW5-*PA*: *Phragmites australis*). Shallow HSSF beds showed high methane emissions (averaging 440 $mgCH_4/m^2{*}d$) even at low loading rates (<5.0 $gBOD_5/m^2{*}d$). Differences in mean emissions between units were not significant. However, the unplanted unit showed lower methane emissions during cold periods and higher emissions during warmer periods than planted units. Temperature was the main variable determining CO_2 and CH_4 emission for all units, except for CO_2 emissions in CW2-*JE* unit. Temperature models explained 63–98% of seasonal variability and predicted zero emissions of CH_4 and CO_2 at temperatures of $9 \pm 1\,^{\circ}C$ and $7.6 \pm 1.3\,^{\circ}C$, respectively. Organic matter accumulation (volatile solids, VS), specific methanogenic activity (SMA) and methane potential (MP) of accumulated solids were also determined. None of these parameters showed significant differences among CW units, except the higher values of MP in CW2-*JE* unit. While VS content and minimum SMA clearly increased with operation time, maximum SMA and MP remained stable. Potential methane emissions can be estimated from the product of surface density of VS and SMA, the measured emissions fitting well in the estimated range from batch assays.

1. Introduction

Constructed wetlands (CWs) are engineered treatment systems for wastewater effluents in which macrophytes play several roles helping to stabilise the surface of the beds, promote microbial activity and processes, provide good conditions for physical filtration and insulate the surface against coldness (Vymazal, 2011; Button et al., 2015). Organic matter production and plant uptake of nutrients as well as root-zone oxygen and organic carbon release were identified as key factors influencing nutrient transformation and removal. Studies showed that the above-ground and below-ground parts of the macrophytes increase microorganism diversity and provide large surface areas for the development of biofilm which is responsible for most of the microbial processes occurring in CWs (Button et al., 2015; Chen et al., 2014).

Most studies have shown that planted horizontal subsurface flow (HSSF) CWs achieve higher treatment efficiency than unplanted systems, at least for the removal of some pollutants such as nitrogen (Tanner, 2001; Chen et al., 2014). Even recent studies also show a higher performance of planted systems in biological oxygen demand (BOD_5) and chemical oxygen demand (COD) removal (Leto et al., 2013; Button et al., 2015; Toscano et al., 2015). Removal efficiency is usually less affected by the plant species than by the presence or absence of plants but some studies reported higher removal for selected plant species. While some studies found a higher efficiency in nutrient removal in CWs planted with *Typha sp* in comparison with other species (Maltais-Landry et al., 2009; Leto et al., 2013), other studies reported better results for *Scirpus validus* (Fraser et al., 2004), *Iris pseudacorus* (Villaseñor et al., 2007),

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Juncus effusus (Carballeira et al., 2016) or *Phragmites australis* (Toscano et al., 2015).

Besides plants, substrate depth in HSSF CWs appeared as a main variable in determining the system performance with the effective substrate depth usually ranging from 0.5 to 0.6 m. Shallower HSSF systems (0.2–0.3 m medium depth) force all of the wastewater through the rooting zone of the plants, which increased the treatment performance as has been shown by García et al. (2005). In contrast, Nivala et al. (2013) reported that deeper HSSF systems (0.5 m) had higher areal oxygen consumption rates (OCRs) than shallow systems (0.25 m). On the other hand, shallow beds showed the greatest effect of vegetation on areal oxygen consumption rates, presumably because the plant rhizosphere was able to occupy a greater portion of the overall bed volume and, on a volumetric basis, the shallow systems perform better than the deeper beds (Nivala et al., 2013).

Some studies on shallower CW systems (0.25–0.40 m effective depth) have been carried out for secondary treatment of municipal or domestic pre-treated wastewater, for the treatment of swine, winery and tannery industry effluents and for nitrogen removal from fish farm effluents (Carballeira et al., 2016). García et al. (2005) reported that the shallow beds removed more COD, BOD_5, ammonia and phosphorus than the deeper bed at a low surface loading rate (SLR). Pedescoll et al. (2011) demonstrated that shallow beds planted with *Phragmites australis* are good systems that can remove COD and ammonium efficiently. The higher efficiency observed in shallower beds was related to their less reducing conditions as indicated by higher redox potential and slightly higher dissolved oxygen concentration (García et al., 2004). The difference in redox status between shallower and deeper beds can lead to differences in the biochemical processes and in particular in methane generation and emission from the wetland. If oxygen reached a higher part of the bed in shallower wetlands, anaerobic methanogenic processes would be less predominant and methane generation and emissions would probably be lower than from deeper wetlands.

In fact, CWs may emit variable amounts of carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O), which are important greenhouse gases and main contributors to the global climate change. Mander et al. (2014) point out that several studies have shown that extensive aquatic macrophyte cover significantly suppresses CH_4 emission in FWS CWs and artificial riverine wetlands. However, the effect could depend on the macrophyte species, as some aerenchymatous macrophytes such as common reed (*Phragmites australis*) as well as willow plants can inhibit CH_4 emission from wetlands while other wetland plants such as *Juncus effusus* and *Typha latifolia* L. among others were considered important emitters of methane (Mander et al., 2014). Bateganya et al. (2015) reported that planted treatments had significantly lower CH_4 emissions compared to the unplanted but significantly higher CO_2 emissions.

On the other hand, a lower water table level caused a significant increase in CO_2 and N_2O emission and a decrease in CH_4 emission in comparison to a higher water table level in both natural marsh (Yang et al., 2013) and HSSF CWs (Mander et al. 2011). In addition, Mander et al. (2015) found that **s**hort-term fluctuations in the water table of HSSF treating wastewater significantly enhanced CO_2 and N_2O emission. However, increasing or lowering the water table level probably causes a different effect on gas emissions than that of designing shallow CWs with a constant water table level. However, studies regarding gas emissions from shallower CW systems (0.25–0.40 m effective depth) are scarce. Maltais-Landry et al. (2009) reported CH_4 emissions of 20–120 $mgCH_4/m^2{*}d$ in 0.3 m deep HSSF systems treating fish farm effluent for nitrogen removal, lower values being obtained from planted and artificially aerated units. Corbella and Puigagut (2015) reported CH_4 emissions ranging from 71 to 391 $mgCH_4/m^2{*}d$ in 0.3 m deep HSSF (*Phragmites australis*) treating municipal wastewater and found that emissions depended on the pre-treatment method.

To our knowledge, there are no studies about the effect of plants presence and species on gas emissions from shallower CWs used for secondary treatment of municipal wastewater. Another point of interest is the impact of microbial communities on gas emissions (Garcia et al., 2007; Mander et al., 2014). Zhu et al. (2007) found that methane flux was directly influenced by the quantitative variation in methanogenic and methanotrophic bacteria in both wetlands. The intensity of methanogenesis can be determined throughout batch assays carried out with the accumulated solids from the wetland media (Garcia et al., 2007). The aim of this work is to study the role of plants and the usefulness of anaerobic assays on the estimation of methane and carbon dioxide emissions from shallow HSSF CWs. The impact of the seasonal development of vegetation and other environmental factors on greenhouse gas emissions is also assessed.

2. Materials and methods

2.1. Plant description

The pilot plant was built in 2009 at the outdoors of the Science Faculty of the University of A Coruña, in A Coruña (Spain) (latitude/longitude: 43.326382, −8.410240) and was in operation from October 2009 to March 2012. The pilot plant was constituted of five HSSF CW units in parallel, including an unplanted control unit while the others were planted with a different plant species each: unplanted (CW1-UN), *Juncus effusus* (CW2-*JE*), *Iris pseudacorus* (CW3-*IP*), *Typha latifolia* L. (CW4-*TL*) and *Phragmites australis* (CW5-*PA*). Each CW unit had an overall surface of 12 m^2 (3 m width × 4 m long), gravel media of 6–12 mm in size and 35 cm depth (30 cm of water depth at the outlet) and 1% slope in the flow direction. The plant location corresponded to a temperate and humid oceanic climate. Average rainfall during the monitoring periods ranged from 0.7 to 8.3 mm/d, whilst the temperature of the influent wastewater showed a variation ranging from 13 to 19 °C. Other details of plant design and operation have been reported elsewhere (Carballeira et al., 2016).

2.2. Gravel sampling procedure and biological anaerobic assays

At each wetland unit, four sampling points were considered, two being placed near the inlet and the other two near the outlet (Fig. 1A). To obtain the samples, a 12.7 cm diameter steel cylinder was inserted in the gravel until the bottom of the bed. Subsequently, the gravel inside the cylinder was collected by using a gardening shovel. At the same time, a sample of the liquid that remained in the cylinder was taken. A composite, representative sample was obtained from both fractions. The two samples obtained at each location (i.e. near the inlet and near the outlet) were combined in order to obtain an integrated sample. The samples were stored in plastic bottles and covered with treated effluent from the corresponding unit in order to avoid biomass aeration. Once transported to the laboratory, the gravel samples were washed by shaking and brushing them in order to withdraw the solids trapped by or attached to the gravel. The cleaned gravel particles were removed by passing the sample throughout a 0.2 mm mesh sieve. The resulting volume containing the solids was concentrated by sedimentation. This procedure has been previously tested and applied in other CW systems (Ruiz et al., 2010).

Organic matter content (volatile solids, VS), specific methanogenic activity (SMA) and methane production potential (MP) were determined for the integrated samples taken from close to the inlet and outlet zones. Anaerobic assays were carried