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Methanogenic activity in the biomass from horizontal subsurface flow constructed wetlands treating domestic wastewater

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ABSTRACT

The aim of this study was to evaluate the methanogenic activity of horizontal subsurface flow (HSSF) constructed wetlands treating domestic wastewater. The analysis was carried out in four 4.5 m^2 pilot-scale HSSF systems, two planted with *Phragmites australis* and two planted with *Schoenoplectus californicus*. A specific methanogenic activity (SMA) assay was carried out with the microbial biomass attached to the gravel of the HSSF systems to account for the different seasons. Accumulated solids throughout the entire operational time were also assessed. Results showed that biochemical oxygen demand (BOD_5) removal efficiencies averaged 67.6 ± 9.9% with organic loading rates (OLR) of4.4–5.8 g BOD_5 m^{-2} d^{-1}. Total suspended solids (TSS) removals were 92.9 ± 3.4%. Solids accumulation rates ranged between0.7 ± 0.3 and 1.5 ± 0.6 kg TSS m^{-2} $year^{-1}$, respectively. Microbial biomass extracted from the HSSF presented a SMA regarding volatile suspended solids (VSS) of 0.018–1.220 g COD_{CH4} g^{-1} VSS d^{-1}, corresponding to methane productions between 176 and 15227 mg CH_4 m^{-2} d^{-1}. Methanogenic activity after 550d were significantly lower (73.6%) than after 1200d of operation. The influent inlet zone of the HSSF systems showed 14–39% higher methanogenic activity than the middle and exit zones in the first 550d of operation. However, after 1100d of operation, the middle and exit zones presented 18–55% higher methanogenic activity than the inlet zone. The plant species did not affected the methanogenic activity of the biofilm from the HSSF system. The results of the present study showed that microbial biomass development through operation time, seasonality and the applied OLR influence methane production in HSSF systems.

1. Introduction

Horizontal subsurface flow (HSSF) constructed wetlands have been a widely used technology for the removal of organic matter and suspended solids from domestic wastewater (Puigagut et al., 2007; Vera et al., 2011; López et al., 2015). Organic matter removal efficiencies measured as biochemical oxygen demand (BOD_5) and chemical oxygen demand (COD) in HSSF systems have been described in the ranges73–97 and 54–91%, respectively, accounting for average organic removal rates of 14.9 g COD m^{-2} d^{-1} (Vymazal and Kröpfelová, 2009). Suspended solids removal efficiencies have been documented in the ranges of 85–92% in terms of total suspended solids (TSS) (Caselles-Osorio et al., 2007). In this manner, HSSF effluent organic matter and suspended solids concentrations are usually in the ranges of 9–55 and 10–70 mg L^{-1} of BOD_5 and TSS, respectively (Vera et al., 2011).

Specifically, particulate organic matter and suspended solids are mainly removed by such physical mechanisms as filtration and sedimentation (Kadlec and Wallace, 2009). It has been shown that almost 90% of the particles in an HSSF system are removed in the first quarter of the length of the system, reducing the influent organic matter by 50% (García et al., 2004). On the other hand, the dissolved organic matter is removed by biochemical reactions performed by the microbial biofilm attached to the gravel and macrophytes root system (García et al., 2010). This biofilm is mostly composed of anaerobic bacteria and archaea since HSSF deep systems (>0.4m) usually operate at oxidation-reduction potentials (ORP) between −200 and +100 mV and dissolved oxygen (DO) levels < 1 mg L^{-1} (Baptista et al., 2003; García et al., 2003). As a consequence, methane is generated as the final product of the anaerobic digestion of organic matter within an HSSF system.

Methane emissions have been assessed in several HSSF systems showing broad ranges, with values of 19.2–2208 mg CH_4 m^{-2} d^{-1}

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(Tanner et al., 1997; Grünfeld and Brix, 1999; García et al., 2007; Wang et al., 2013; Mander et al., 2014; Corbella and Puigagut, 2015; López et al., 2015). These methane emissions are the result of the net flux of methane production and consumption by methanogenic and methanotrophic activities, respectively (Niu et al., 2015). These microbial activities are influenced by the type of macrophyte used (Niu et al., 2015). Seasonality also affect methanogenic activity in terms of radiation which is higher in spring and summer, increasing sediment temperature and promoting macrophytes photosynthesis generating exudates which are easily biodegradable substrates for methanogenic communities (Johansson et al., 2004; Barbera et al., 2014; Maucieri et al., 2017; Vidal et al., 2007). Macrophytes gas transport system (oxygen to the rizhosphere and methane to the atmosphere) also inhibits methanogenic activity and promotes methanotrophic activity (Grünfeld and Brix, 1999; DeJournett et al., 2007). The HSSF design (*e.g.* depth or the water table position) also influence whether methanogenic activity or other biochemical reactions predominate in the system (Aguirre et al., 2005). Finally, one of the most important factors influencing HSSF methane production are the organic loading rate (OLR) and substrate availability (Corbella and Puigagut, 2015; López et al., 2015). Organic matter availability is an important factor since wastewater is constituted by a complex mixture of organic compounds that include both settleable and dissolved organic matter (with a biodegradable and a non-biodegradable fraction) (Sadecka et al., 2013; Navia et al., 2003). It has been demonstrated that the particle-size distribution is a key factor in removal efficiency for conventional wastewater treatment systems. Elmitwalli et al. (2001) have proved that colloidal organic matter measured as COD (COD_c) has the highest maximum methane production potential (86%), followed by the settleable COD fraction (COD_{set}) (78%) and the soluble COD fraction (COD_s) (62%). However, Caselles-Osorio and García (2006) demonstrated that HSSF microcosms fed with COD_{set} and COD_c (starch) or COD_s (glucose) did not show significant differences in COD_s removal. Nevertheless, these authors did find significantly higher biofilm development in HSSF systems fed with COD_s than those fed with COD_{set} and COD_c (Caselles-Osorio and García, 2006). A similar behavior also has been seen in HSSF systems fed with hydrolyzed effluents (through hydrolytic anaerobic digesters as primary treatment), showing increments of up to 81% in methane emissions (Corbella and Puigagut, 2015). The clogging of filter media is another factor to consider since the highest solids accumulations are reported near the inlet of HSSF system, the organic matter content of which accounts for up to 20% of the total solids (90% of this fraction corresponding to the recalcitrant portion) (Caselles-Osorio et al., 2007). However, HSSF systems usually show the highest methane emissions rates (25–95%) in the same inlet zone (Mander et al., 2015). In terms of vertical distribution Nurk et al. (2005) found that the bottom of an HSSF showed a respiration rate 30% lower than in the surface layers with values of 0.056 ± 0.006 and 0.039 ± 0.014 mg CO_2 dm^{-2} d^{-1}, respectively. Besides that the respiration rates resulted negatively correlated to CH_4 emission ($\rho = -0.93$) (Nurk et al., 2005). Finally, Samsó and García (2013) described an active microbial zone pushed from inlet to outlet as the organic matter availability change in function of operational time.

Despite this existing research, little is known about the effect of OLR and particle-size distribution in the spatiotemporal variations of methane production by microbial biomass from HSSF mesocosmic systems treating domestic wastewater. Therefore, the aim of this study was to evaluate the methanogenic activity of microbial biomass attached to HSSF gravel as a function of organic matter loading rates and taking into account the effects of the species of macrophytes.

Table 1
Operational conditions.

Parameter	Unit		Value
Design parameters			
Surface area	m^2		4.5
Aspect ratio	–		2
Water level	m		0.4
Average high	m		0.57
Theorical volume	m^3		1.28
Operation parameters	Unit	F/W	S/Sm
HLR	mm d^{-1}	30.4 ± 0.5	30.1 ± 0.5
OLR	$gBOD_5$ m^{-2} d^{-1}	5.8 ± 0.4	4.4 ± 0.1
HRT	d	3.7 ± 0.1	3.8 ± 0.1

HLR: hydraulic loading rate; OLR: organic loading rate; HRT: hydraulic retention time; F/W: Fall/Winter season; S/Sm: Spring/Summer season.

2. Material and methods

2.1. Description of the HSSF pilot plant

The HSSF pilot plant start-up was in July of 2011 and was located in Hualqui, in the Biobío Region, Chile (36°59′26.93″ south and 72°56′47.23″ west). Influent was extracted after a pretreatment sand trap and bar screen (40 mm) of a wastewater treatment plant of a rural community of 20,000 inhabitants (López et al., 2015). Fig. 1 shows the pilot plant diagram. Influent was pumped into a primary treatment process consisting of a settling tank (630 L) with a subsequent septic tank (1200 L). After primary treatment, wastewater was conducted to four parallel HSSF constructed wetlands systems. Two HSSF systems were planted with *Phragmites australis* (HSSF-Phr1 and HSSF-Phr2) and the other two with *Schoenoplectus californicus* (HSSF-Sch1 and HSSF-Sch2). For data analysis, the HSSF-Phr1 and HSSF-Phr2 averaged results will be referred to as HSSF-Phr. Similarly, HSSF-Sch1 and HSSF-Sch2 will be refereed as HSSF-Sch.

Table 1 shows the design and operational parameters for evapotranspiration and rainfall data. Three sampling tubes were installed for gravel sampling and *in situ* parameter monitoring. Each tube was located at a different distance from the inlet, representing three zones: Zone A (the inlet zone), 0.65 m from the inlet; Zone B (the middle zone), 1.4 m from the inlet; and Zone C (the outlet zone), 2.25 m from the inlet. The superficial area of each zone was 1.5 m^2 (López et al., 2015).

The hydraulic loading rates were in the range 29.8–30.7 mm d^{-1}. The average applied OLR throughout the entire study was 5.8 ± 0.4 and 4.4 ± 0.1 g BOD_5 m^{-2} d^{-1} in the fall/winter (F/W) and the spring/summer (S/Sm) seasons, respectively.

The HSSF systems were monitored *in situ* monthly by considering the pH, temperature, ORP and DO. The measurements were carried out just before a feeding pulse. Each of these parameters was measured in every zone of the HSSF, *i.e.*, the entrance zone (Zone A), the middle zone (Zone B), and the exit zone (Zone C), except for DO, due to the lack of variation along the HSSF zones and the low concentrations measured, implying a significant error (García et al., 2004; López et al., 2015).

For HSSF monitoring, a physicochemical characterization was also considered. Samples were extracted from the influent and effluent and stored under refrigeration (4 °C). Influent and HSSF effluent samples were taken every 15d, starting from the 3rd year of operation (July, 2013, 755d) and ending in the 4th year of operation (December, 2015, 1980d), considering (F/W) and (S/Sm) seasons in the analysis.