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Potential methane production and molecular characterization of bacterial and archaeal communities in a horizontal subsurface flow constructed wetland under cold and warm seasons

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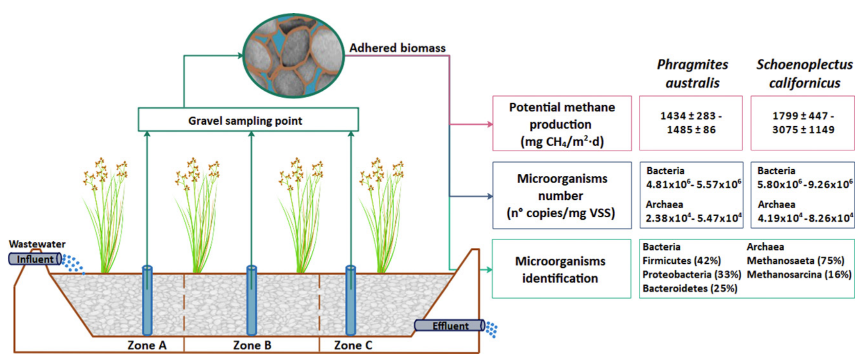
HIGHLIGHTS

- Potential methane production in a HSSF is influenced by organic matter content.
- The most representative bacteria found in HSSF was genera related to organic matter degradation under anaerobic conditions.
- The most important archaea genera found in HSSF was *Methanosaeta*.

GRAPHICAL ABSTRACT



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ABSTRACT

Organic matter removal in a horizontal subsurface flow constructed wetland (HSSF) treating wastewater is associated with the presence of bacteria and archaea. These organisms perform anaerobic microbial processes such as methanogenesis, which can lead to methane emissions. The aim of this study was to evaluate methane production and characterize the bacterial and archaeal communities found in HSSFs treating secondary urban wastewater during cold and warm seasons. The pilot system used in this study corresponds to four HSSFs, two planted with *Phragmites australis* (HSSF—Phr) and two planted with *Schoenoplectus californicus* (HSSF—Sch), the monitoring was carried out for 1335 days. Removal efficiencies for organic matter (biological and chemical oxygen demand) and total and volatile suspended solids were evaluated in each HSSF. Moreover, biomass from each HSSF was sampled during warm and cold season, and methane productions determined by Specific Methanogenic Activity assays$_{(maximum)}$ (SMA_m). In the same samples, the quantification and identification of bacteria and archaea were performed. The results showed that the degradation of organic matter (53–67% BOD_5 and 51–62% COD) and suspended solids (85–93%) was not influenced by seasonal conditions or plant species. Potential methane production from HSSF-Sch was between 20 and 51% higher than from HSSF-Phr. Moreover, potential methane production during warm season was 3.4–42% higher than during cold season. The quantification of microorganisms in HSSFs, determined greater development of bacteria (38%) and archaea (50–57%) during the warm season. In addition, the species *Schoenoplectus californicus* has a larger number of bacteria (4–48%) and archaea (34–43%)

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than *Phragmites australis*. The identification of microorganisms evidenced the sequences associated with bacteria belong mainly to Firmicutes (42%), Proteobacteria (33%) and Bacteroidetes (25%). The archaea were represented primarily by Methanosarcinales, specifically *Methanosaeta* (75%) and *Methanosarcina* (16%). The community structure of the methanogenic archaea in HSSFs did not change throughout the seasons or plant species.

1. Introduction

Horizontal subsurface flow constructed wetlands (HSSFs) have proven to be an efficient ecological technology for wastewater treatment. These systems are effective in the removal of total solids (80–95%) and organic matter (70–89% chemical oxygen demand (COD) and 74–94% biological oxygen demand (BOD_5)) from domestic wastewater (Trang et al., 2010; Vera et al., 2011, 2014).

The mechanisms of organic matter removal in HSSFs are associated with anaerobic microbiological processes, such as sulfate reduction, denitrification and methanogenesis, which account for 90–94% of processes (García et al., 2004). The prevailing conditions in HSSFs are anaerobic, with dissolved oxygen values usually lower than 2 mg/L, and redox potential between −400 to +200 mV (García et al., 2010). Specifically, methanogenesis occurs during the mineralization of organic matter in several sequential steps (hydrolysis, acidogenesis, acetogenesis and methanogenesis) (Zhang et al., 2012). If the methanogenic route is complete, the metabolic end products will be H_2, CO_2 and CH_4 (Vymazal and Kröpfelová, 2011; Mander et al., 2014).

In HSSFs, methane emissions into the atmosphere are the net result of production (methanogenesis) and consumption (oxidation). Methane production and emissions can vary in a wide range (from −375 to 36,792 mg $CH_4/m^2\cdot d$) (López et al., 2015; Corbella and Puigagut, 2015; de la Varga et al., 2015; Carballeira et al., 2017). Indeed, the emission rate is affected by a number of factors, including the redox condition, the amount of substrate, the type of plants, the temperature, the mobilization of CH_4, the microbiological community, and the characteristics of certain microbial groups (W. Wang et al. 2013a; Adrados et al., 2014; Niu et al., 2015; Maucieri et al., 2017).

Microbiological communities associated with anaerobic environments and methane production have been identified and quantified in natural wetlands (Lv et al., 2014), sediments (lakes, rivers and marine) (Zhang et al., 2015), artificial riverine wetlands (Ligi et al., 2014) and anaerobic reactors (Niu et al., 2015). However, there is still very little information about constructed wetlands (Adrados et al., 2014; Mander et al., 2014). The first investigations associated with bacterial and archaeal communities in constructed wetlands were carried out by Calheiros et al. (2009) and Adrados et al. (2014), identifying and quantifying only bacterial groups. These authors determined that the main phyla found in HSSFs were Proteobacteria (26%), Bacteroidetes (26%) and Firmicutes (15%). Moreover, He et al. (2014) found that β-Proteobacteria (55%) and γ-Proteobacteria (37%) were the dominant groups in vertical and horizontal constructed wetlands. However, the presence of archaeal communities in constructed wetland systems has not been extensively studied. In this line, it has been shown that microbial communities and methane emissions can be directly influenced by the plant species used (Y. Wang et al. 2013b; Zhang et al., 2018). Moreover, it has been determined that the dissolved oxygen of the rhizosphere is one of the most important factors affecting CH_4 flux and methanogenic communities (Zhang et al., 2018). However, Wang et al. (2016) have shown that the determining factor of microbial diversity in CW is seasonality. Even more, there appears to be no other studies that have investigated the effects of different plants and season of the year about potential methane production and microbiological composition, particularly in methanogenic community. The objective of this study was to evaluate potential methane production and to characterize the bacterial and archaeal communities found in HSSFs planted with two different species (*Phragmites australis* and *Schoenoplectus californicus*) under cold and warm seasons.

2. Material and methods

2.1. Pilot plant and sampling strategy

The constructed wetland pilot plant system was located in Hualqui (36°59′26.93″ south and 72°56′47.23″ west), Biobío Region, Chile. The HSSFs were fed with wastewater from a preliminary treatment that serves a rural community of 20,000 inhabitants.

The influent is primarily treated in sand trap-degreaser, septic tank and pumping tanks, and then uniformly distributed to the constructed wetlands by entering through a perforated distributor pipe (80 mm diameter) placed horizontally and perpendicular to the direction of flow (López et al., 2016). The HSSFs system consisted of four units, whose characteristics are presented in Table 1 and Fig. 1. Table 1 shows the

Table 1
Characteristics of HSSF units.

Characteristics	Unit	Value	
Support medium			
Type	–	Gravel	
Size	mm	19–25	
Porosity	–	0.4	
Geometric			
Surface area	m^2	4.5	
Length/width relation	–	2	
HSSF average height	m	0.57	
Water table height	m	0.4	
Bottom slope	m/m	0.05	
Total volume	m^3	1.8	
Active volume	m^3	0.73	
Control parameters			
Hydraulic loading rate	mm/d	Cold season	30.86 ± 10.68
		Warm season	27.47 ± 4,85
Hydraulic retention time	d	Cold season	6.10 ± 1.30
		Warm season	5.86 ± 2.13
Organic loading rate	$gBOD_5/m^2\cdot d$	Cold season	4.64 ± 1.30
		Warm season	4.74 ± 1.01
Operational conditions			
Temperature (constructed wetland)	°C	Cold season	12.07 ± 1.80
		Warm season	20.30 ± 3.27
ORP	mV	Cold season	−226.8 ± 50.4
		Warm season	−259.8 ± 53.3
Dissolved oxygen	mg/L	Cold season	0.47 ± 0.25
		Warm season	0.43 ± 0.39
Rainfall	$L/m^2\cdot d$	Cold season	3.10 ± 1.01
		Warm season	0.60 ± 0.22

ORP: Oxidation Reduction Potential; HSSF: horizontal subsurface flow constructed wetland; BOD_5: biological oxygen demand; cold season: average of fall/winter; warm season: average of spring/summer.