ARTICLE IN PRESS
STOTEN-21804; No of Pages 9
Science of the Total Environment xxx (2017) xxx–xxx
Contents lists available at ScienceDirect
Science of the Total Environment
journal homepage: www.elsevier.com/locate/scitotenv

Unraveling the active microbial populations involved in nitrogen utilization in a vertical subsurface flow constructed wetland treating urban wastewater

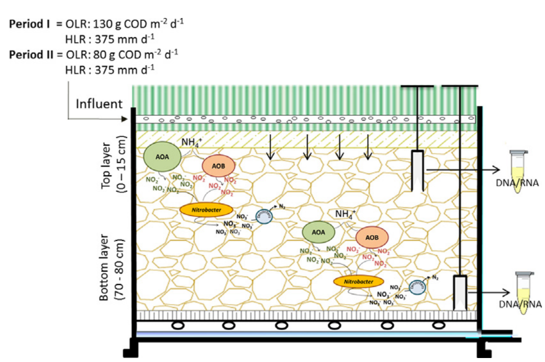
Catiane Pelissari [a,1], Miriam Guivernau [b,1], Marc Viñas [b], Samara Silva de Souza [c], Joan García [d], Pablo Heleno Sezerino [a], Cristina Ávila [d,e,*]

[a] GESAD - Decentralized Sanitation Research Group, Department of Sanitary and Environmental Engineering, Federal University of Santa Catarina, Trindade, Florianópolis, Santa Catarina 88040-900, Brazil
[b] GIRO Joint Research Unit IRTA-UPC, Research and Technology, Food and Agriculture (IRTA), Torre Marimon, E-08140, Caldes de Montbui, Barcelona, Catalonia, Spain
[c] INTELAB - Integrated Technologies Laboratory, Chemical and Food Engineering Department, Federal University of Santa Catarina, Trindade, Florianópolis, Santa Catarina 88040-900, Brazil
[d] GEMMA - Environmental Engineering and Microbiology Research Group, Department of Civil and Environmental Engineering, Universitat Politècnica de Catalunya-BarcelonaTech, c/ Jordi Girona, 1-3, Building D1, E-08034 Barcelona, Spain
[e] ICRA, Catalan Institute for Water Research, Scientific and Technological Park of the University of Girona, Emili Grahit, 101, E-17003 Girona, Spain

HIGHLIGHTS

- Active nitrifying & denitrifying microbial populations in a vertical flow wetland
- Ammonia-oxidizing bacteria (AOB) more abundant than archaea (AOA) at DNA level
- AOA populations exhibited higher resilience to physic-chemical shifts than AOB.
- *Nitrobacter* was the nitrite-oxidizer being active both at top and bottom layers.
- Active denitrifying bacteria detected in top and bottom layers in both campaigns

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 30 November 2016
Received in revised form 13 January 2017
Accepted 14 January 2017
Available online xxxx

Editor: D. Barcelo

ABSTRACT

The dynamics of the active microbial populations involved in nitrogen transformation in a vertical subsurface flow constructed wetland (VF) treating urban wastewater was assessed. The wetland (1.5 m^2) operated under average loads of 130 g COD m^{-2} d^{-1} and 17 g TN m^{-2} d^{-1} in Period I, and 80 g COD m^{-2} d^{-1} and 19 g TN m^{-2} d^{-1} in Period II. The hydraulic loading rate (HLR) was 375 mm d^{-1} and C/N ratio was 2 in both periods. Samples for microbial characterization were collected from the filter medium (top and bottom layers) of the wetland, water influent and effluent at the end of Periods I (Jun–Oct) and II (Nov–Jan). The combination of qPCR and high-throughput sequencing (NGS, MiSeq) assessment at DNA and RNA level of 16S rRNA genes and nitrogen-based functional genes (*amoA* and *nosZ*-clade I) revealed that nitrification was associated both with

* Corresponding author at: ICRA, Catalan Institute for Water Research, Scientific and Technological Park of the University of Girona, Emili Grahit, 101, E-17003 Girona, Spain.
E-mail address: cavila@icra.cat (C. Ávila).
[1] Both authors contributed equally to this manuscript.

http://dx.doi.org/10.1016/j.scitotenv.2017.01.091

Keywords:
Ammonia oxidizing bacteria
Ammonia oxidizing archaea
Metabolically-active populations
High organic load
High-throughput sequencing
Treatment wetland

ammonia-oxidizing bacteria (AOB) (*Nitrosospira*) and ammonia-oxidizing archaea (AOA) (*Nitrososphaeraceae*), and nitrite-oxidizing bacteria (NOB) such as *Nitrobacter*. Considering the active abundance (based in *amoA* transcripts), the AOA population revealed to be more stable than AOB in both periods and depths of the wetland, being less affected by the organic loading rate (OLR). Although denitrifying bacteria (*nosZ* copies and transcripts) were actively detected in all depths, the denitrification process was low (removal of 2 g TN m^{-2} d^{-1} for both periods) concomitant with NO_x-N accumulation in the effluent. Overall, AOA, AOB and denitrifying bacteria (*nosZ*) were observed to be more active in bottom than in top layer at lower OLR (Period II). A proper design of OLR and HLR seems to be crucial to control the activity of microbial biofilms in VF wetlands on the basis of oxygen, organic-carbon and NO_x-N forms, to improve their capacity for total nitrogen removal.

1. Introduction

Constructed wetlands (CW) are engineered systems designed to simulate the conditions that occur in natural systems to treat wastewater (Kadlec and Wallace, 2009). This technology is under continuous development worldwide as a sustainable alternative for decentralized wastewater treatment in small communities or remote areas, due to its low energy consumption, ease of operation and provision of ecosystem services, and it has been widely employed for the treatment of different types of wastewater (García et al., 2010).

Vertical subsurface flow constructed wetlands (VF) are one of the configurations of subsurface flow CW which holds greater oxygen transfer capacity due to its design (unsaturated bed) and operational mode (intermittent feeding), and require a smaller land area compared to other types of CW operating without air induction (Cui et al., 2010). Given their large oxygen transfer capacity, VF wetlands are mainly employed for nitrification and removal of organic matter (Platzer, 1999).

It has been proven that the nitrification capacity of VF wetlands is directly related to the applied organic loading rate (OLR), since the excess of organic compounds can affect the oxidation of ammonia due to the competition of oxygen between heterotrophic and autotrophic organisms (Paredes et al., 2007; Saeed and Sun, 2011, 2012). On the other hand, the presence of biodegradable organic compounds seems to promote the growth of denitrifying organisms (Headley et al., 2005). In general, nitrogen removal is associated with nitrification of ammonia nitrogen followed by denitrification of nitrate. In this way, nitrogen transformation in VF wetlands is accomplished by ammonia-oxidizing bacteria (AOB), ammonia-oxidizing archaea (AOA), nitrite oxidizing bacteria (NOB) and to a lesser extent by denitrifying bacteria enriched in the biofilm of bed media, which are metabolically active depending on the specific environmental conditions.

Several studies have been conducted to elucidate the bacterial dynamics involved in the nitrogen cycle in VF wetlands. In a VF wetland operated under an OLR of 27 g COD m^{-2} d^{-1} *Nitrosomonas europaea*, *N. mobilis* and *Nitrosospira* were reported to be the dominant AOB in the bed media (Tietz et al., 2007). Guan et al. (2015) evaluated the influence of different bed media (sand, zeolite and gravel) in three VF wetlands (1.2 m^2) and showed that the bacterial community was significantly influenced by bed media type. However, *Nitrospira*, known as a NOB, was abundant in all units showing no influence of bed media type. Wu et al. (2016) using fluorescence in situ hybridization (FISH), reported that the growth of AOB and NOB in VF wetlands was enhanced by the use of intermittent aeration and of a specific bed media (sludge-ceramsite). Pelissari et al. (2016), observed how lower OLR and hydraulic loading rates (HLR) favored simultaneous nitrifying and denitrifying bacteria in two VF wetland microcosms (microcosm 1, 41 g COD m^{-2} d^{-1}, HLR of 72 mm d^{-1}; microcosm 2, 104 g COD m^{-2} d^{-1}, HLR of 170.5 mm d^{-1}). A study developed in a partially saturated VF at full-scale showed that nitrifying bacteria were present in the upper layers of the bed media (up to 34 cm depth), while denitrifying bacteria where identified from the intermediate layers downwards (Pelissari et al., 2017).Murphy et al. (2016), showed that after two weeks of no aeration in a mature areated VF wetland the nitrification process was resumed within two days, whereas, a newly VF wetland needed two months. These results exhibit that once resident nitrifying population is established, they become fairly robust.

Despite the progress achieved with modern molecular techniques, microbial dynamics involved in nitrogen transformation in VF wetlands are still unclear. Firstly, many of the microbiological studies carried out in CW refer to microbial abundance and do not demonstrate the active microbial abundance, which actually acts in the removal and transformation of nitrogen. Secondly, microbial processes in CW depend on environmental factors, properties of wastewater, bed media type and operational conditions of the treatment units (Meng et al., 2014).

It has been well established in the literature that the autotrophic oxidation of ammonia is not only limited to the bacteria domain, but it is also performed by archaea (Angnes et al., 2013; Konneke et al., 2005). Studies conducted in CW have demonstrated that bacterial diversity is greater than archaeal in VF wetlands and horizontal subsurface flow CW (HF) (Adrados et al., 2014), as well as in free water surface (FWS) wetlands (Fan et al., 2016). Zhi and Ji (2014) observed in a tidal flow CW that archaea were not dominant in the microbial community during the entire operational period. Oppositely, Sims et al. (2012) suggested that AOA were more persistent and abundant than AOB in natural wetland ecosystems, regardless of the seasonal period. Otherwise, in planted and unplanted HF wetlands, Paranychianakis et al. (2016) found that the abundance of AOA were lower than that of AOB, indistincly of plant species or period of time.

In spite of the knowledge gained in the above mentioned studies, the contribution fraction of ammonia oxidizers (AOA vs. AOB) and their *amoA* gene expression between kingdoms in VF wetlands is still unknown (You et al., 2009). In addition, there are no studies evaluating the effect of operational conditions at different OLR on nitrifying and denitrifying microbial populations. To our knowledge, the active microbial community (eubacteria and archaea) attached and involved in the nitrogen cycle of CW biofilms is scarcely assessed. The present study aims at gaining insight into the dynamics of active microbial populations during a nitrification-based process in a vertical flow constructed wetland treating urban wastewater under high OLR.

2. Materials and methods

2.1. Description of the wastewater treatment plant

This study was conducted in a VF wetland, which was part of a hybrid CW system treating urban wastewater from a nearby sewer. The hybrid system was comprised of a primary treatment performed by an Imhoff tank, followed by a VF wetland stage, a HF wetland, and a FWS wetland in series. The experimental treatment plant is set outdoors at the experimental facility of the GEMMA group (Department of Civil and Environmental Engineering of the Universitat Politècnica de Catalunya-BarcelonaTech, Spain) in a Mediterranean climate. It was commissioned in 2010, and up to the time of the current study the treatment system operated in a continuous mode under different organic and hydraulic loads over the years of operation (Ávila et al., 2013, 2014, 2016).