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Performance and bacterial community structure of a submerged biofilter subjected to high ammonium and high organic carbon concentrations

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ABSTRACT

A partial-nitritation biofilter was tested in order to investigate the effect of organic matter loading over its performance and bacterial community structure. Analyses were done after each of a two-step organic loading of 45 days each. Without organic matter addition, the partial-nitritation biofilter performed in nearly ideal conditions in terms of ammonium oxidation to nitrite. Under these conditions its bacterial community structure was dominated by *Nitrosomonas* bacteria. The first loading of organic matter caused a decrease in ammonium oxidation as well as decrease in relative abundance of *Nitrosomonas*. The COD and BOD_5 removal increased with the operation time up to 85% and 81%. For the second loading of organic matter the partial-nitritation biofilter achieved 91% and 97% in COD and BOD_5 removal at the end of the experimental period but ammonium oxidation efficiency was still inefficient. The changes in the bacterial community structure of the biofilter were more pronounced at the first organic loading step as suggested by α- and β-diversity analyses. In this way, the partial-nitritation biofilter could not achieve the desired coupled ammonium oxidation and organic matter removal efficiency.

1. Introduction

The need for the treatment of wastewater effluents with low organic carbon and high nitrogen concentrations caused the development of autotrophic nitrogen removal technologies (Ahn, 2006; Ali et al., 2016). Among them, the most popular are those based on the metabolism of anammox bacteria and so called anammox process, which have several advantages over other nitrogen removal technologies (Niu et al., 2016). The anammox process requires oxidation of half the influent ammonium to nitrite, named as partial nitritation, in order to trigger the metabolism of anammox bacteria (Kartal et al., 2004; Van Hulle et al., 2010).

The two-step anammox process is an anammox technology that attains autotrophic nitrogen removal in two different bioreactors by setting a partial-nitritation process upstream of an anammox bioreactor (Ke et al., 2015). The performance of the partial-nitritation process is of major importance for the nitrogen removal of this two-step technology.

Partial-nitritation has commonly been developed as a suspended growth process, but also this technology can operate in a biofilm process. It has been proven that partial-nitritation biofilters at lab-scale can achieve optimum performance under a hydraulic retention time (HRT) of 7 h (Gonzalez-Martinez et al., 2013), which is lower than for suspended growth processes (Mosquera-Corral et al., 2005; Milia et al., 2015). Therefore, the partial-nitritation biofilter is a technology for autotrophic nitrogen removal that offers advantages over suspended growth partial-nitritation systems and can compete with those.

Nevertheless, the effect of certain pollutants and environmental pressures over the partial-nitritation biofilter has not been thoroughly studied. One of the most critical parameters that can affect partial-nitritation systems is the input of organic matter, as it could cause an outcompetition of ammonium oxidizing bacteria by putative heterotrophic microorganisms (Rodriguez-Sanchez et al., 2014), therefore dropping the performance of partial nitritation.

The complete analysis of a bioprocess needs the characterization

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of the microbial communities developing in the bioreactor, mainly those belonging to *Bacteria* domain. In this sense, the exploration of bacterial communities thriving in bioreactors has been done for activated sludge systems, anaerobic digestion processes, constructed wetlands and anammox technologies, among other treatment systems (Gonzalez-Martinez et al., 2016a; Zhang et al., 2015; Rui et al., 2015; Gonzalez-Martinez et al., 2016b). For partial nitritation biofilters, the analysis of bacterial dynamics under the effect of common contaminants found in anaerobic digester supernatant, such as antibiotics and amino acids, was successfully conducted (Liang et al., 2011; Gonzalez-Martinez et al., 2014a, 2016c).

In this research, the effect of organic matter loading on a partial nitritation biofilter technology has been analyzed in terms of bioreactor's performance. Moreover, the bacterial community structure was studied and discussed using next generation sequencing techniques.

2. Materials and methods

2.1. Bioreactor set-up and operation

Two partial-nitritation biofilters (named C and E) were operated in parallel. The bioreactors were of 5 L volume and a number of 4900 BioFlow 9 plastic carriers (Chen et al., 2014) were used as substratum for biomass attachment. The HRT under operation was of 7 h in correlation with (Gonzalez-Martinez et al., 2014b), with a synthetic wastewater feeding rate of 0.72 L h^{-1}. The temperature of the bioreactors was controlled to 32 ± 2 °C due to a thermostat displaced inside the bioreactor. The pH was controlled to remain stable at 7.5 ± 0.1. Aeration was provided at 1.5 mg L^{-1} by four air diffusers located at the bottom of the bioreactors. A schematic of the partial-nitritation biofilter set-up is offered in Fig. 1. Previously to the operation investigated in the study, activated sludge was used as inoculum to form attached biomass. During these 30 days of start-up period a synthetic wastewater resembling anaerobic digestion supernatant was used. For the experimentation, the bioreactors were fed with synthetic wastewater simulating anaerobic digestion supernatant with and without addition of organic matter during 90 days. The composition of the synthetic wastewater used in the experiment is shown in Table 1. Partial-nitritation biofilter C was operated under synthetic anaerobic digestion supernatant of constant composition as a control experiment. Partial-nitritation biofilter E was fed with anaerobic digestion supernatant synthetic wastewater with addition of organic matter. The organic matter loading in the partial-nitritation biofilter E subjected the system to around 0.048 and 0.1 g-TOC L^{-1} at the first and second organic loading steps, respectively, which are in the range of previous researches on organic matter effect over partial nitritation process (Mosquera-Corral et al., 2005).

2.2. Physic-chemical determinations

Characterization of the influent and effluent BOD_5, COD and suspended solids (SS) was done in accordance with the standard procedures used for determination of wastewater (APHA, 2012). The influent and effluent determination of nitrogenous compounds ammonium, nitrite and nitrate was done by ionic chromatography. The measurement of attached biofilm concentration was done following the procedure described in Gonzalez-Martinez et al. (2014a).

2.3. Collection of biological samples

200 mL of plastic carriers were collected for biological sampling at operational days 0, 45 and 90 from both partial-nitritation biofilters under study. The samples obtained were named as C0, C45, C90, E0, E45 and E90. The attached biomass was separated from the plastic carriers by sonicating the total carrier volume submerged in 0.9% NaCl solution and then centrifuging at 3000 rpm during 10 min at ambient temperature. The collected biomass was stored at −20 °C for DNA extraction.

2.4. DNA extraction and iTag sequencing

For each biological sample, 300 mg of biomass collected and stored at −20 °C was subjected to DNA extraction using the FastDNA SPIN Kit for soil extraction (MP Biomedicals, Solon, OH, USA) following the manufacturer's instructions. Extracted DNA was kept at −20 °C and sent to Research & Testing Laboratory (Lubbock, TX, USA) for iTag sequencing process. The primer pair 28F-519R (5′-GAGTTTGATCNTGGCTCAG-3′ and 5′-GTNTTACNGCGGCKGCTG-3′, respectively) (Fan et al., 2012) was used for the amplification of the hypervariable regions V1-V2-V3 of the 16S rDNA gene of *Bacteria*. iTag sequencing process (Degnan and Ochman, 2012) was done using the Illumina MiSeq equipment and the Illumina MiSeq reagent v3 kit. The PCR conditions for the high-throughput sequencing process were: 3 min at 94 °C, then32 cycles of: 30 s at 94 °C, 40 s at 60 °C, and 60 s at 72 °C; final elongation step of 5 min at 72 °C.

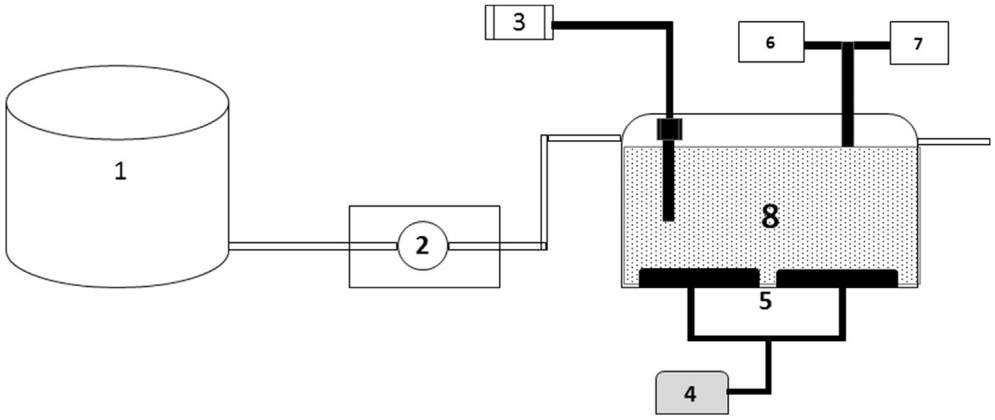

Fig. 1. Schematic of the pilot-scale partial-nitritation biofilter used in the 1) Synthetic wastewater tank; 2) Peristaltic pump; 3) Thermostat; 4) Air pump; 5) Oxygen diffusers (porous plates); 6) Tank of H2SO4 0.1 M for pH control; 7) Tank of NaOH 0.1 M for pH control and 8) Partial-nitritation bioreactor stuffed with BioFlow9 carriers.