Ecological Engineering 64 (2014) 443–450

Contents lists available at ScienceDirect

Ecological Engineering

journal homepage: www.elsevier.com/locate/ecoleng

Study of nitrifying microbial communities in a partial-nitritation bioreactor

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ARTICLE INFO

Article history:
Received 2 September 2013
Received in revised form
30 December 2013
Accepted 1 January 2014
Available online 7 February 2014

Keywords:
CTO
Hydraulic retention time (HRT)
NxrA
Partial-nitritation
Submerged bed

ABSTRACT

The present study focused on the technical and biological characteristics of a bench-scale partial-nitritation bioreactor and established its operating parameters. In this manner 2 bench-scale submerged-bed bioreactor of 3 L were operated under identical conditions of pH, oxygen concentration and temperature but under different hydraulic retention time (9 and 12 h). This made it possible to study the influence of the hydraulic retention time (HRT) on the nitrification processes and on the nitrifying microbiota of the biofilms. Moreover, specific bacterial groups involved in the nitrification process, such as ammonium oxidizing (CTO) and nitrite oxidizing (nxrA) were investigated using a cultivation-independent approach based on PCR-TGGE fingerprinting. The results showed that the HRT may affect the nitrification processes of a partial-nitritation bioreactor using a synthetic wastewater containing 600 mg/L of ammonia. It was found that HRT of 12 h transformed 100% of the ammonium to nitrite. However, when the HRT was 9 h there was a significant reduction (35%) in ammonia converted. Cluster analysis of PCR-TGGE fingerprints showed significant differences in the profiles depending on the different HRT applied, especially on the ammonia oxidizing bacteria. The importance of this factor was confirmed by multivariate analysis. Phylogenetic analysis of bands sequences showed that CTO and nxrA sequences presented similarity to those present in the database and grouped in specific clusters. Our results suggested that changes in HRT can affect significantly the nitrifying microbial community and the performance of the partial nitritation system.

1. Introduction

One of the most serious ecological problems in the world is the proliferation of wastewater, since human activities have greatly accelerated and extended the natural cycles of nitrogen in the soil, water and atmosphere. In conventional wastewater treatment plants (WWTPs), nitrogen is often removed by the biological processes of nitrification and denitrification. Nitrification involves the oxidation of ammonia to nitrite and the subsequent oxidation of nitrite to nitrate under aerobic conditions, requiring 2.5 mol of oxygen per mol of NH_4–N. The nitrate generated is then denitrified to NO_2 in the presence of an organic carbon source to dinitrogen (Mosquera-Corral et al., 2005). However, this conventional process is not suitable for the treatment of effluents such us dewatering concentrate stream due to the toxic effects that occur, even to those microorganisms able to degrade it (Van Hulle et al., 2005). New and sustainable technologies are needed to comply with the stringent discharge standards (Van Loosdrecht et al., 2004). Thus in recent years more efficient and cost effective alternative systems have been developed for the removal of this nutrient, such us partial-nitritation Anammox technology.

The partial-nitritation process, is a technique in which nitrification is achieved with nitrite as the intermediate under stable process conditions, where only 50% of the ammonium in the influent is converted into nitrite. This system was described in detail by Hellinga et al. (1998), and its combination with the Anammox (Anaerobic Ammonium Oxidation) process has led to the development of a new technology of great interest in the treatment of effluents with a high content of N.

Nowadays, biological wastewater treatments are considered more effective and relatively inexpensive and have been widely adopted instead of the physicochemical processes. Thus, the study of microbial diversity in biological systems utilised for the treatment of urban or industrial wastewater represents one of the best

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http://dx.doi.org/10.1016/j.ecoleng.2014.01.009

ways to improve the efficiency of biological processes in the different wastewater treatment technologies. For this purpose, questions about community structure, activity and population kinetics have to be answered by means of molecular monitoring tools, which allow identifying and quantifying the microorganisms present in the WWTP. One of the major advances in the field of microbial ecology is the introduction of molecular biology techniques based on the in situ detection of nucleic acids (Ji et al., 2013). Several methods are available for assessing the abundance or diversity of bacterial communities in ecosystems, such as fluorescence in situ hybridisation (FISH) and fingerprinting methods (Sun et al., 2012). Denaturing and temperature gradient gel electrophoresis (DGGE/TGGE) are fingerprinting methods which yield extensive information about the diversity of microorganisms in their habitats, also allowing the taxonomic identification of community members (Muyzer, 1999). These data allow the monitoring of variations in the community profiles due to external factors. Both techniques were often used in recent studies on the ecology of biological processes in WWTPs, providing interesting new data in this area (Molina-Muñoz et al., 2009).

The biofilm technologies for wastewater treatment are an alternative to the suspended growth activated sludge process (Schlegel and Koeser, 2007). Researchers have been trying to correlate the microbial community structures of biofilms with the performance of wastewater treatment, and reported the link between them and the efficiencies of nitrification, denitrification and phosphorus removal. Thus knowledge of the microbial community's composition involved in the biofilm processes and the influence of operating conditions on their structure is regarded as crucially important for the optimisation of nutrient removal rates on submerged fixed bed bioreactor systems and to implement control strategies. Determining the identity of microorganisms responsible for specific biotransformation processes in complex environments remains one of the major challenges in environmental microbiology and environmental engineering (Calderón et al., 2012). The aim of our study was to analyse the effect of HRT on the performance and community structure of ammonium oxidizing bacteria and nitrite oxidizing bacteria in a partial-nitritation bioreactor assembled as a fixed bed biofilm reactor.

2. Materials and methods

2.1. Operating conditions of the bench-scale partial-nitritation bioreactor

Two partial-nitritation bioreactors bench-scale plants were constructed as a submerged bed with PVC carriers (Bioflow 9®) with a volume of 3 L. The Bioflow 9® with filigree structure shows a very high specific surface area of 800 m^2/m^3. It is mostly used for wastewater with low organic load. A schematic diagram of the experimental plants is shown in Fig. 1.

The operating conditions in the bioreactors (i.e. HRT, pH, dissolved oxygen concentration and temperature) were monitored continuously during the whole operational period to ensure that they remained constant. Four 15 cm porous plates at the bottom of the vessel supplied oxygen from an air pump to maintain a constant concentration of 1.5 mg/L. All the experimental work was performed at pH 7.5 and temperature of 35 °C thanks to an adjustable thermostat.

The partial-nitritation bioreactors were inoculated with the same mixed liquor from an aerobic reactor located in the Los Vados urban wastewater treatment plant (Granada, Spain). The mixed liquor was recirculated for three days until the appearance of a biofilm on the surface of the plastic carriers and acclimated under defined laboratory conditions for 5 days (Kaewpipat and Grady, 2002). During acclimation the partial nitritation bioreactors were fed with synthetic wastewater.

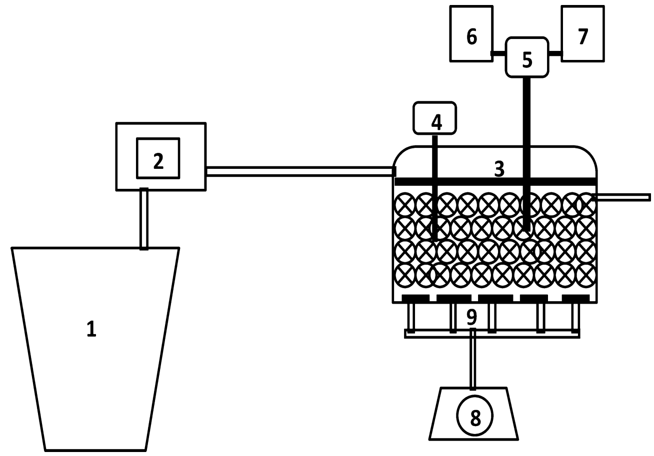

Fig. 1. Diagram of the bench-scale partial nitrification (Partial-SHARON) bioreactor used in the experiments.

The synthetic wastewater (Mosquera-Corral et al., 2005) used in this research simulated leachate from an anaerobic digester, since it contained a high concentration of ammonium and was low in organic matter. The chemical composition of the synthetic wastewater can be observed in Table 1.

To prepare the synthetic wastewater, 24 L of distilled water were poured inside a 60 L tank along with the exact quantity of chemical compounds that made up the synthetic sewage medium. Later others 25 L of distilled water were added and all components were then mixed and dissolved. The influent was continuously fed into the bioreactors by a peristaltic pump (Watson Marlow s-520), that pumped the synthetic wastewater at different flow rates.

2.2. Physico-chemical parameters

The physico-chemical parameters analysed were the following: pH, dissolved oxygen concentration, temperature and nitrogen concentration in its various forms (ammonium, nitrite, and nitrate). All samples for ammonium, nitrite and nitrate determination were taken every 24 h for 30 days.

Under constant conditions, two experiments in parallel were performed at different HRT (9 and 12 h) with a view to analysing the evolution of nitrogen concentration in the bioreactors and also the microbial diversity in the biofilms. During both experiments the pH was constant at 7.5 (Van Hulle et al., 2005; Tao et al., 2012). This parameter was continuously measured in the bioreactors using a pH meter (ORION). The equipment was adjusted automatically with buffer solutions of pH 4.0 and 7.0. On the other hand, the Oxygen was supplied by means of air gasification through the liquid phase using porous plates to obtain small air bubbles kept

Table 1
Chemical composition of the synthetic wastewater in g/L used in the experiments.

Chemical	g/L
$(NH_4)_2SO_4$	2.35
$NaHCO_3$	3.25
$CaCl_2$	0.30
KH_2PO_4	0.07
$MgSO_4$	0.02
$FeSO_4 \cdot 7H_2O$	0.009
H_2SO_4	0.005
EDTA	0.006