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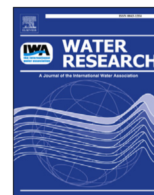

The ratio of metabolically active versus total Mycolata populations triggers foaming in a membrane bioreactor

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ABSTRACT

The abundance of total and metabolically active populations of Mycolata was evaluated in a full-scale membrane bioreactor (MBR) experiencing seasonal foaming, using quantitative PCR (qPCR) and retro-transcribed qPCR (RT-qPCR) targeting the 16S rRNA gene sequence. While the abundance of total Mycolata remained stable (10^{10} copies of 16S rRNA genes/L activated sludge) throughout four different experimental phases, significant variations (up to one order of magnitude) were observed when the 16S rRNA was targeted. The highest ratios of metabolically active versus total Mycolata populations were observed in samples of two experimental phases when foaming was experienced in the MBR. Non-metric multidimensional scaling and BIO-ENV analyses demonstrated that this ratio was positively correlated to the concentrations of substrates in the influent water, F/M ratio, and pH, and negatively correlated to temperature and solids retention time. It the first time that the ratio of metabolically active versus total Mycolata is found to be a key parameter triggering foaming in the MBR; thus, we propose it as a candidate predictive tool.

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1. Introduction

Since 1969, biological foaming has been reported worldwide in wastewater treatment plants (WWTPs) (de los Reyes, 2010a). This operational problem negatively influences plant performance and it is attributed to the excessive proliferation of filamentous micro-organisms (Seviour, 2010). Mycolic-acid containing actinobacteria (herein Mycolata) are often involved in the generation of biological foaming, mostly due to their cell surface hydrophobicity and their ability to excrete extracellular polymers which act as biosurfactants (de los Reyes, 2010a; Petrovski et al., 2011). Although several operation strategies have been devised to control this phenomenon in WWTPs, they are often ineffective and in practice the incidence of biological foaming is hard to predict (Blackall et al., 1991; Kragelund et al., 2009; Asvapathanagul et al., 2012).

To better cope with foaming with a more comprehensive understanding of its microbial background, many studies in the last decade have investigated the proliferation of Mycolata in WWTPs by different techniques, including conventional microscopy, quantitative fluorescence *in situ* hybridization (qFISH), or quantitative RNA membrane hybridization (Oerther et al., 2001; de los Reyes and Raskin, 2002; Parada-Albarracín et al., 2012). Quantitative PCR (qPCR) approaches have been recently introduced (Marrengane et al., 2011; Asvapathanagul et al., 2012), although focused only to the detection of *Gordonia*, since species from this genus (in particular *Gordonia amarae*) are often connected to foaming issues in WWTPs (de los Reyes, 2010b). However, a wide phylogenetic diversity of Mycolata occurs in WWTPs (Davenport et al., 2000), and foaming induced by genera other than *Gordonia* has been reported, particularly at temperatures below 20 °C (de los Reyes, 2010b; Maza-Márquez et al., 2015).

Studies robustly linking the changes of the operational and environmental conditions to the occurrence of foaming, the shifts in Mycolata abundance or their population dynamics contribute useful information for the prediction and control of foaming episodes in WWTPs; however, these approaches have seldom been attempted in the available literature (Frigon et al., 2006; Asvapathanagul et al., 2012; Maza-Márquez et al., 2015). In consequence, there is still a lack of knowledge about the role of

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Mycolata and the factors which trigger the generation of foam by these microorganisms.

In membrane bioreactors (MBRs), the adverse effects of biological foaming affect the stability of the process at several levels (Maza-Márquez et al., 2015). Nevertheless, very few studies are available focused on the identification and quantification of filamentous Mycolata in this type of WWTPs. In the present study, the abundance of total and metabolically active populations of Mycolata was evaluated in a full-scale MBR experiencing seasonal foaming, by means of qPCR and retrotranscribed qPCR (RT-qPCR) targeting the 16S rRNA gene sequence. The study spanned four different phases of operation in the MBR (Summer 2009, Autumn 2009, Summer 2010 and Winter 2012). Multivariate analyses of the biological data (non-metric multidimensional scaling, MDS, and BIO-ENV) were performed to evaluate the influence of operation parameters (hydraulic and solid retention times) and environmental conditions (fluctuations of temperature, pH, contaminant load of the influent wastewater and biomass accumulation) on the abundances of Mycolata populations in the MBR, in search of tools useful for the prediction and control of foaming in this kind of WWTPs.

2. Materials and methods

2.1. Description of the full-scale MBR experimental plant, operating conditions, and summary of plant performance

The MBR investigated in the study was described in detail previously (Gómez-Silván et al., 2013). It consisted of two bioreactors (BR): aerated BR (19.4 m^3), and anoxic BR (6.8 m^3) and a filtration tank (2 m^3) equipped with three ultrafiltration hollow-fiber membrane modules (0.034 μm nominal pore size) made of polyvinylidene fluoride (PVDF) (GE Water & Process Technologies, Fairfield, USA). A fourth tank (0.2 m^3) collected part of the treated water for the periodic backwashing of the membrane modules. The MBR was installed at the facilities of EDAR Sur-WWTP (EMASAGRA, Granada, Spain) and fed with urban wastewater taken from its pretreatment, after being filtered through a 1-mm brush screen.

The following parameters were measured automatically in the MBR and registered continuously in a database: temperature, pH, and dissolved oxygen (DO) concentration; level of the tanks, transmembrane pressure, and flow rates. The datasets were collected and analyzed with the aid of the Active Factory v.9.2 software (Wonderware, Spain). The daily medium internal temperature (IT) and pH measured in the activated sludge of the MBR were calculated by the software. The MBR was operated in pre-denitrification mode, as previously described (Gómez-Silván et al., 2013). Air was supplied to the aerated BR with a fine bubble membrane diffuser, keeping automatically the dissolved oxygen (DO) concentration in the range of 0.5–1.6 mg/L. The filtration tank was also aerated to control membrane fouling and clogging.

Concentrations of suspended solids (total, iTSS; volatile, iVSS), total chemical oxygen demand (COD), and total biological oxygen demand at five days (BOD_5) were analyzed daily in influent and effluent (permeate) water. The influent and effluent water samples were automatically collected through the day using a time controller and a peristaltic pump, and were kept refrigerated at 4 °C until taken for analysis (Ruiz et al., 2011). Concentrations of total and volatile suspended solids (TSS and VSS, respectively) were also measured daily in the sludge of both BRs. All the analyses were done in accordance with the Standard Methods for the Examination of Water and Wastewater (APHA, 2005). The effluent water satisfied the EU regulation legal requirements for discharge to the environment (Directive 91/271/EEC, European Council, 1991) (Table S1).

The abundances of total and active populations of Bacteria and Mycolata in the activated sludge of the MBR were monitored by qPCR/RT-qPCR during four different phases of operation: Summer 2009, Autumn 2009, Summer 2010 and Winter 2012 (Sm09, Au09, Sm10, and Wn12, respectively). In each phase, different sets of hydraulic retention time (HRT) and solid retention time (SRT) were used, and markedly different average characteristics of the influent wastewater and temperature of the activated sludge were recorded (Maza-Márquez et al., 2015; summarized in Table S1). Two episodes of foaming, mainly affecting the anoxic BR, were observed, from 26/11/2009 to 22/12/2009 (end of phase Au09) and from 7/02/2012 to 18/02/2012 (phase Wn12). Previous studies concluded that seasonal foaming in the MBR was related to Mycolata phylotypes different from *G. amarae* (Parada-Albarracín et al., 2012; Maza-Márquez et al., 2015).

2.2. Activated sludge sampling for qPCR assays

Activated sludge samples were retrieved from the MBR in each experimental phase when steady-state conditions were reached (at least threefold the SRT since the start-up date, Maza-Márquez et al., 2015). Samples (200 ml) of activated sludge from both BRs (aerated and anoxic) were collected in sterile plastic containers, transported from the experimental plant to the laboratory, and preserved until nucleic-acid extraction, following previously described protocols (Gómez-Silván et al., 2014). Activated sludge samples (4 ml) were centrifuged (1 min, 14 × g) in a MiniSpin Plus table centrifuge (Eppendorf, Hamburg, Germany), and the supernatants were discarded. Two biological replicates were prepared for each DNA and RNA extraction. The pellets intended for DNA extraction were carried at 4 °C from the experimental plant to the laboratory and stored at −20 °C. The pellets intended for RNA extraction were resuspended in 1 ml of RNAprotect® Bacteria Reagent (Qiagen, Hamburg, Germany) and carried unrefrigerated from the experimental plant to the laboratory, then were precipitated again by centrifuging (1 min, 14 × g) in a MiniSpin Plus table centrifuge (Eppendorf, Hamburg, Germany), and the pellets stored at −20 °C after discarding the supernatants.

2.3. Nucleic acids extraction and purification

The extraction and purification of total DNA and RNA from the activated sludge samples were performed using methods previously described (Maza-Márquez et al., 2015). The FastDNA-2 ml SPIN Kit for Soil and the FastRNA Blue Kit (MP-Bio, Santa Ana, CA, USA) were used in combination with the FastPrep24 apparatus (MP-BIO, Santa Ana, CA, USA), following the manufacturer's instructions. The RNA samples were then digested using the rigorous procedure of TURBO DNA-*free* kit (Ambion®, Life Technologies Corporation, Carlsbad, CA, USA) and further purified with the aid of the RNA Cleanup protocol from RNeasy Mini Kit (Qiagen, Hamburg, Germany).

2.4. Reverse transcription of RNA to cDNA

Reverse transcription reactions were performed with Superscript III Reverse Transcriptase (Invitrogen, Life Technologies Corporation, Carlsbad, CA, USA), following the manufacturer's directions, and using 150–200 ng RNA in a final volume of 20 μl. The specific primers (Table 1) were supplied by Sigma Aldrich (St. Louis, MO, USA), and the dNTPs by Promega (Madison, USA). The quality and concentration of nucleic acids was measured using a NanoDrop ND-1000 Spectrophotometer (Thermo Scientific Waltham, MA USA).

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