



# Interlinkages between bacterial populations dynamics and the operational parameters in a moving bed membrane bioreactor treating urban sewage

P. Reboleiro-Rivas<sup>a,c,\*</sup>, J. Martín-Pascual<sup>b,c</sup>, J.A. Morillo<sup>c</sup>, B. Juárez-Jiménez<sup>a,c</sup>,  
J.M. Poyatos<sup>b,c</sup>, B. Rodelas<sup>a,c</sup>, J. González-López<sup>a,c</sup>

<sup>a</sup> Departamento de Microbiología, Facultad de Farmacia, Universidad de Granada, 18071 Granada, Spain

<sup>b</sup> Departamento de Ingeniería Civil, Universidad de Granada, 18071 Granada, Spain

<sup>c</sup> Instituto del Agua, Universidad de Granada, 18071 Granada, Spain

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## ABSTRACT

Bacteria are key players in biological wastewater treatments (WWTs), thus a firm knowledge of the bacterial population dynamics is crucial to understand environmental/operational factors affecting the efficiency and stability of the biological depuration process. Unfortunately, little is known about the microbial ecology of the advanced biological WWTs combining suspended biomass (SB) and attached biofilms (AB). This study explored in depth the bacterial community structure and population dynamics in each biomass fraction from a pilot-scale moving bed membrane bioreactor (MBMBR) treating municipal sewage, by means of temperature-gradient gel electrophoresis (TGGE) and 454-pyrosequencing. Eight experimental phases were conducted, combining different carrier filling ratios, hydraulic retention times and concentrations of mixed liquor total suspended solids. The bacterial community, dominated by *Proteobacteria* (20.9–53.8%) and *Actinobacteria* (20.6–57.6%), was very similar in both biomass fractions and able to maintain its functional stability under all the operating conditions, ensuring a successful and steady depuration process. Multivariate statistical analysis demonstrated that solids concentration, carrier filling ratio, temperature and organic matter concentration in the influent were the significant factors explaining population dynamics. Bacterial diversity increased as carrier filling ratio increased (from 20% to 35%, v/v), and solids concentration was the main factor triggering the shifts of the community structure. These findings provide new insights on the influence of operational parameters on the biology of the innovative MBMBRs.

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## 1. Introduction

The improvement of biological wastewater treatment (WWT) is a key goal to obtain treated water of high quality. The idea of combining suspended and attached biomass growth to improve the biological process on well-established WWT technologies represents an excellent option (Ivanovic and Leiknes, 2012). In this sense, the moving bed membrane bioreactors (MBMBRs), which incorporate biofilms to a regular membrane bioreactor (MBR), have

shown to be successful for the enhancement of organic pollutants and nutrients removal (Wang et al., 2010; Khan et al., 2011; Ivanovic and Leiknes, 2012; Di Trapani et al., 2015; Leyva-Díaz et al., 2015). Specifically, MBMBRs are based on the addition of freely moving carriers to the MBR to support biofilm development. Thus, in MBMBRs, the microorganisms grow both as suspended biomass (SB) and attached biofilm (AB) (Reboleiro-Rivas et al., 2013).

The biological WWT depends mainly on the presence of bacteria for the transformation of toxic substances, degradation of organic pollutants and removal of nutrients from urban/industrial effluents (Wells et al., 2011). Consequently, it is crucial to study the influence of the environmental/operational factors on bacterial diversity, since they affect the efficiency and stability of the biological process (Di Trapani et al., 2013; Biswas et al., 2014; Vanwonterghem et al., 2014). However, the identification of the microorganisms

*Abbreviations:* SB, suspended biomass; AB, attached biofilm; CFR, carrier filling ratio; BTS, biofilm total solids; BVS, biofilm volatile solids.

\* Corresponding author. Departamento de Microbiología, Facultad de Farmacia, Campus de Cartuja s/n, 18071 Granada, Spain.

E-mail address: [preboleiro@ugr.es](mailto:preboleiro@ugr.es) (P. Reboleiro-Rivas).

responsible for biotransformation processes in WWTs, as well as the interlinkages between microbial diversity and environmental/operational factors, are still challenging in the field of environmental engineering (Xia et al., 2010; Gómez-Silván et al., 2014).

Fingerprinting methodologies, such as temperature/denaturing gradient gel electrophoresis (TGGE and DGGE), have been used in recent studies on the characterization of microbial communities in WWTs (Pholchan et al., 2010; Calderón et al., 2013; De Vrieze et al., 2013; Gómez-Silván et al., 2014). These tools are useful for the study of population dynamics over time and the analysis of population responses to environmental/operational changes, enabling to process and compare a high number of samples (Gómez-Silván et al., 2014). However, only the dominant populations of the analyzed community are detected by these techniques, so less abundant but potentially important species are not revealed (Marzorati et al., 2008). The combination of Next Generation Sequencing (NGS) methods and TGGE is a valid option for a further and in-depth characterization of the bacterial community structure and population dynamics. Among the various NGS techniques, 454-pyrosequencing is widely applied to the study of microbial diversity in conventional activated sludge (CAS) and MBRs systems (Hu et al., 2012; Zhang et al., 2012; Kim et al., 2013; Ye and Zhang, 2013). Recently, 454-pyrosequencing has been applied to the study of bacterial community developed in SB and AB in moving bed biofilm reactors (MBBR) and fixed-film activated sludge (IFAS) systems (Kwon et al., 2010; Biswas et al., 2014). Nevertheless, few studies have linked the dynamics of bacterial populations to the fluctuations of environmental/operational parameters (Pholchan et al., 2010; Wells et al., 2011; Gómez-Silván et al., 2014). According to Liang et al. (2010), more information about the microbial community developed in advanced biological WWTs which combine suspended and attached growth is necessary for optimal design and operation. To the best of our knowledge, such investigations have not been performed in MBMBRs.

This work explore in depth the bacterial populations dynamics in a pilot-scale MBMBR treating municipal sewage with 20% and 35% (v/v) of carrier filling ratio (CFR) under four different sets of operational conditions. The bacterial community structure and population dynamics were investigated in both the SB and AB fractions by TGGE fingerprinting and 454-pyrosequencing methodologies. Finally, multivariate analysis (redundancy analysis, RDA) was used to evaluate the influence of environmental/operational parameters on the bacterial community structure and MBMBR performance.

## 2. Material and methods

### 2.1. Pilot-scale MBMBR experimental plant and operating conditions

The experimental MBMBR plant (Fig. S1) and the operating conditions were previously detailed in Reboleiro-Rivas et al. (2013). In essence, the MBMBR consisted of two bioreactors: a hybrid moving bed bioreactor (HMBBR), in which the carriers K1 (Anox-Kaldnes, Norway) moved freely by aeration in an operating volume of 358 L, and a MBR composed of three ultrafiltration membrane modules of hollow fiber (ZW-10, Zenon®) submerged in 87 L of operating volume under continuous aeration. The membrane modules have a nominal membrane surface area of 0.93 m<sup>2</sup>, a nominal pore size of 0.04 micron and 0.1 micron of absolute pore size. The specific surface area of carrier K1 is determined to be 139 m<sup>2</sup>/m<sup>3</sup> with an effective surface area for biofilm growth of 100 m<sup>2</sup>/m<sup>3</sup> at the 20% of filling ratio. While, at the 35% of filling ratio, the specific surface area of K1 is 243 m<sup>2</sup>/m<sup>3</sup> with an effective surface area for biofilm growth of 175 m<sup>2</sup>/m<sup>3</sup> (Pal et al., 2012). Air

was supplied by a diffuser placed on the bottom of both HMBBR and MBR bioreactors. Dissolved oxygen concentration was kept in a range from 1.5 to 2 mg/L throughout the experimental set. Sludge recirculation from MBR to HMBBR was performed to maintain the biomass concentration in the HMBBR. Finally, the effluent was collected in a back-washing tank. The MBMBR was installed in the municipal WWT plant “Puente de los Vados” (Emasagra S.A., Granada, Spain). The urban sewage was pumped from the outlet of the primary settler to the HMBBR.

The MBMBR was operated under different CFRs (20% or 35%), hydraulic retention times (HRT, 10 h or 24 h) and concentrations of mixed liquor total suspended solids (MLTSS, ca. 2500 mg/L or 4500 mg/L), throughout eight experimental phases (designated as experiments 1–8). Four experimental phases were performed with 20% CFR (experiments 1–4) or 35% CFR (experiments 5–8), and the same combinations of HRT and MLTSS were established for both CFRs (20% and 35%) (Table 1A).

Biological oxygen demand at 5 days (BOD<sub>5</sub>), chemical oxygen demand (COD), MLTSS, and concentrations of mixed liquor volatile suspended solids (MLVSS) were analyzed daily, according to Standard Methods for the Examination of Waste and Wastewater (APHA et al., 2005). Biofilm total and volatile solids (BTS and BVS) concentrations were calculated according to Plattes et al. (2006). pH was measured using a Crison pH 25 pH-meter (Crison instruments S.A., Barcelona, Spain). Temperature was monitored by a control device available in the HMBBR. The means ± standard deviation (SD) values of MLTSS, MLVSS, BTS, BVS, temperature and pH, as well as the BOD<sub>5</sub> and COD removal rates measured in the MBMBR for each experiment, are summarized in Table 1(B and C). In terms of biotransformation of organic matter (COD and BOD<sub>5</sub> removal), a high quality effluent was obtained in all the experiments according to the European Legislation for effluent discharge to the environment (91/271/CEE, 1991).

### 2.2. Biofilm growth measurement

To measure BTS and BVS concentrations, 50 units of biofilm-colonized carriers were collected from different sites in the HMBBR and placed in sterile flasks with 50 mL of saline solution water (0.9% NaCl). Subsequently, the biofilm was removed from the carriers as previously described (Reboleiro-Rivas et al., 2013). For DNA extraction, 5 biofilm-colonized carrier units were placed in sterile flasks with 5 mL of saline solution water (0.9% NaCl), vortexed for 1 min, sonicated for 3 min, and the resultant biofilm suspensions were collected by centrifuging for 5 min at 3000 × g. This process was repeated twice.

### 2.3. Analysis of bacterial community by TGGE

To obtain detailed information about the longitudinal evolution of the bacterial community in each experiment, the SB and AB fractions were sampled weekly. The samples were named according to the experiment (1–8), the sample type (SB or AB) and the sampling week in each experiment. E.g., “1 SB2” designates samples of experiment 1, retrieved from the SB fraction, during the second sampling (second week) of this experiment.

#### 2.3.1. DNA extraction, PCR amplifications of partial bacterial 16S rRNA gene and TGGE

Genomic DNA from ca. 250 mg of SB and AB samples was extracted using the FastDNA® SPIN Kit for Soil and the FastPrep® 24-Instrument (MPBiomedicals, Germany), according to the manufacturer's indications. A two-step PCR approach was performed using universal primers (fd1/RD1) to amplify the 16S rRNA gene, and subsequently the V3-hypervariable region of the 16S rRNA

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