



## Enzymatic activities in a moving bed membrane bioreactor for real urban wastewater treatment: Effect of operational conditions



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### ARTICLE INFO

#### Article history:

Received 21 March 2013

Received in revised form 26 July 2013

Accepted 20 September 2013

Available online 19 October 2013

#### Keywords:

Moving bed membrane bioreactor

Hydrolytic activities

Biofilm

Multivariate statistical analysis

### ABSTRACT

The microbial hydrolytic enzymatic activities (acid phosphatase, alkaline phosphatase and glucosidase) in a moving bed membrane bioreactor (MBMBR system) for real urban wastewater treatment were investigated. This study was conducted in both suspended biomass and attached biofilm present in a MBMBR system. For this, four experimental phases with 20% and 35% (v/v) carrier filling ratios (CFRs) were conducted, which combined two mixed liquor total suspended solid (MLTSS) concentrations (c.a. 2500 and 4500 mg/L) and two hydraulic retention times (HRTs) (10 and 24 h). Hydrolytic activities of acid phosphatase, alkaline phosphatase and  $\alpha$ -glucosidase in the mixed liquor (suspended biomass) were higher than in the biofilm (independent of the carrier concentration), during the four experiments, possibly due to better substrate diffusion in the mixed liquor. A redundancy analysis (RDA) was performed to evaluate the relationship between enzymatic activities and chemical oxygen demand (COD), biological oxygen demand at 5 days ( $BOD_5$ ), mixed liquor volatile suspended solid concentration (MLVSS), biofilm total solids (BTS), HRT, pH, CFR and temperature. According to the results obtained with the Monte Carlo permutation test, CFR, MLVSS, BTS, COD, temperature and HRT significantly contributed to the variation of enzymatic activities in the MBMBR.

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### 1. Introduction

The population in developed countries is constantly growing and consequently, the production of wastewater has become a huge environmental problem. Similarly, environmental legislation is becoming increasingly stringent concerning organic pollutant and nutrient (C, N and P) concentrations in wastewater effluent (Onnis-Hayden et al., 2011; Pal et al., 2012). In recent decades, to obtain a better quality of effluent, wastewater treatments based on conventional activated sludge have been improved by the development of new technologies.

Among these technologies, membrane bioreactor (MBR) systems, combine a conventional biological treatment of activated sludge and membrane filtration technology (Chang et al., 2011;

Yang et al., 2009). Membrane filtration replaces the clarification process and confers several advantages to the system compared to Conventional Activated Sludge Processes (CASPs) (Calderón et al., 2012a; Molina-Muñoz et al., 2010; Yang et al., 2009).

Alternatively, wastewater treatments based on biofilm processes, such as moving bed biofilm reactors (MBBRs), are emerging due to their efficiency in the removal of organic carbon, ammonium, nitrates, and nitrites (Di Trapani et al., 2010; Ødegaard, 2006). MBBRs are based on the addition of carriers to the activated sludge system. The carriers move freely in the bioreactor and are gradually colonised by the biomass. Thus, suspended and attached growth processes occur together (Chu and Wang, 2011; Martín-Pascual et al., 2011; Pal et al., 2012; Wang et al., 2005).

To improve the efficiency of MBRs and MBBRs systems further, a combination of biofilm processes and membrane filtration technology (MBMBR system) is a viable alternative, as reported by various authors in recent years (Ivanovic and Leiknes, 2008; Leiknes and Ødegaard, 2007; Ødegaard, 2006).

Microbial communities in a MBMBR system develop in different types of aggregates, such as suspended flocs or attached biofilms. The immobilisation of microorganisms promotes the development

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

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of slow-growing microorganisms, such as nitrifying bacteria, and can support an appropriate environment for both aerobic and anoxic microorganisms (Watanabe et al., 1992). Therefore, differences in the biological activity of the mixed liquor and the biofilm can occur. Consequently, in a heterogeneous biological system such as a MBMBR, the process of biodegradation might be enhanced, or at least altered.

In nature, the biodegradation process begins with the hydrolysis of macromolecules performed by extracellular microbial enzymes. This initial process is considered as the main rate-limiting step in organic matter degradation, since an important fraction of organic matter present in the influent must be hydrolysed by enzymes before it can be utilised by bacterial metabolism (Anupama et al., 2008; Chróst and Siuda, 2006; Guellil et al., 2001; Morgenroth et al., 2002). Furthermore, as reported Burgess and Pletschke (2008), the enzymes immobilised on extracellular polymeric substances (EPS), flocs or carriers are more stable. In this context, the first step to achieve a better characterisation of the biodegradation process involved in a MBMBR system is to measure biomass concentration and enzymatic activities in both suspended biomass (SB) and attached biofilm (AB).

The enzymatic activities of  $\alpha$ -glucosidase, acid phosphatase and alkaline phosphatase have an essential role in biological wastewater treatment, since carbohydrates comprise a large fraction of the organic matter and an important proportion of the total phosphorous is present in organic form. The study of these enzyme activities not only provides knowledge concerning the hydrolysis process, but can also help to optimise operational conditions to achieve a better biodegradation of sludge (Anupama et al., 2008; Li and Chróst, 2006; Molina-Muñoz et al., 2010).

In recent years, the effect of different operational parameters, including carrier filling ratio (CFR), on the efficiency of organic pollutant and nutrient removal in MBBRs and MBMBRs has been studied (Di Trapani et al., 2010; Hem et al., 1994; Martín-Pascual et al., 2011; Ødegaard, 2006; Wang et al., 2005; Yang et al., 2009). However, little attention has focussed on the analysis of biological activity and species composition variation in MBBRs (Pal et al., 2012) and, to the best of our knowledge, attempts to link enzymatic activity variations to changes of the different operational parameters have not been performed in a MBMBR system.

For these reasons, we investigated the evolution of  $\alpha$ -glucosidase, acid phosphatase and alkaline phosphatase activities in both SB and AB in a MBMBR system with a 20% and 35% (v/v) CFR under four different operational conditions. Furthermore, the AB contribution to the total enzymatic activities in the MBMBR was calculated. Finally, in order to understand the influence of physico-chemical parameters and CFR on the enzymatic activities, a multivariate statistical analysis was performed.

## 2. Materials and methods

### 2.1. Experimental set-up

#### 2.1.1. Pilot-scale experimental plant

To carry out this research, a pilot-scale experimental plant was configured as shown in Fig. 1. This consisted of one aerobic MBMBR system composed of two bioreactors: a moving bed bioreactor (MBBR) and a membrane bioreactor (MBR), where the biodegradation and clarification process, take place, respectively. The MBBR system consisted of a cylindrical tank with an operating volume of 358 L, where the carriers moved freely by aeration, whereas the MBR was composed of three ultrafiltration membrane modules of hollow fibre (Zenon®). The membranes were submerged in a sludge volume of 87 L under continuous aeration. Recirculation was performed, to maintain the same concentration

of total suspended solids in each reactor. Finally, the effluent was collected in a back-washing tank.

To approach real conditions as closely as possible, the MBMBR system was located in the municipal WWTP “Puente de los Vados” (Emasagra S.A., Granada, Spain). Specifically, the influent was pumped from the primary settler to the MBBR. The mean composition of wastewater was determined by standard methods (APHA, 2005). A control device was available to monitor the temperature in the MBBR.

#### 2.1.2. Carrier type and carrier filling ratio

The carrier K1, developed by AnoxKaldness (Norway), was used as a support material. Its use has been widely reported for different types of wastewater and appears to be one of the most commonly used in MBBRs (Ivanovic and Leiknes, 2008; Levstek and Plazl, 2009; Martín-Pascual et al., 2011; Ødegaard, 2006; Pal et al., 2012). The K1 carrier is a small cylinder of high-density polyethylene, with a cross inside and “fins” in the external surface and an effective surface area of 800 m<sup>2</sup>/m<sup>3</sup> (Ødegaard, 2006).

To achieve a good diffusion of substrates through the biofilm as reported by Levstek and Plazl (2009), two CFRs of K1 (20% or 35%, v/v) were used, and 3.8% or 6.65% of mixed liquor volume was displaced from the moving bed bioreactor, respectively. In agreement with Martín-Pascual et al. (2011), the units of carriers (K1) per m<sup>3</sup> were 1,030,000.

#### 2.1.3. Operating conditions

To perform a comparative study between a 20% and 35% (v/v) CFR, eight experimental phases (designated as experiments 1–8), were conducted in the MBMBR system. For this, four operational conditions with 20% (v/v) CFR (experiments 1–4) or 35% (v/v) CFR (experiments 5–8) were performed. Two different concentrations of Mixed Liquor Total Suspended Solids (MLTSS) and two Hydraulic Retention Times (HRTs) were combined: 10 h (experiments 1, 3, 6 and 7) or 24 h (experiments 2, 4, 5 and 8). Experiments with 20% (v/v) CFR were conducted in 2011, whereas those with 35% (v/v) CFR were conducted in 2012 (Table 1).

To evaluate the influence of the attached biofilm on the enzymatic activities, the MBMBR system was continuously operated throughout the study with either 20% or 35% (v/v) CFR. Furthermore, the experimental phases were conducted from lower to higher MLTSS concentrations (ca. 2500 mg/L to ca. 4500 mg/L).

Inflow rates of 45.5 and 18.96 L/h were used to maintain the same biomass concentration at a HRT of 10 h and 24 h, respectively. In addition, different purges of 35, 18, 20, 8, 24, 52, 25 and 8 L/day were required after stabilisation of the system in experiments 1–8, respectively. After completing each experimental phase, the membranes were cleaned with sodium hypochlorite (1 g/L) as previously reported by Poyatos et al. (2010).

To compare the nutrient biotransformation rates of the MBMBR system under different operational conditions, samples were collected during the steady period of each experiment. According to Calderón et al. (2012a), the stability of the MBMBR system was considered when the MLTSS concentration was constant.

### 2.2. Biofilm recovery

The evolution of microbial enzymatic activities in attached biofilm as well as Biofilm Total Solids (BTS) and Biofilm Volatile Solids (BVS) was studied by removing the biofilm from the supporting material, according to the methods described by Martín-Pascual et al. (2011). Fifty units of carriers with adhered biofilm were collected in sterile conditions, using a sieve-sampling device as recommended by Calderón et al. (2012b), from different areas of the MBMBR and were placed in flasks with 50 mL sterile saline

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