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Biorreactores de lecho móvil y de membrana para el tratamiento de aguas residuales a bajo tiempo de retención hidráulico: estudio de la influencia en la eliminación de contaminantes

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Moving bed and membrane bioreactors for the treatment of wastewater at low hydraulic retention time: study of the influence on the removal of pollutants

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"Mucha gente pequeña, en lugares pequeños, haciendo
cosas pequeñas, pueden cambiar el mundo”
(Eduardo Galeano).

ABSTRACT

The constant increase in the emergence of emerging pollutants in wastewater, together with the increasingly demanding and restrictive regulations on wastewater disposal, makes it imperative, to redouble scientific research efforts in search of new systems of treatment of this type of compounds.

That is the reason why different physical-chemical, microbiological and respirometric analyzes were carried out during 2 years in a pilot plant located in the wastewater treatment plant (WWTP) Oeste in Granada, continuously fed with residual water from the primary decanting treatment of the plant, to which emerging contaminants such as Bisphenol A (BPA) and increasing concentrations of a series of drugs of different nature, such as ibuprofen, carbamazepine and ciprofloxacin, were added externally with the main purpose of analyzing the elimination capacity of said compounds in a biological membrane reactor (MBR) and in a hybrid system consisting of the sum of a mobile bed bioreactor and a membrane reactor (MBBR-MBR), working in cycles under different operational conditions, considering

low hydraulic retention time (HRT), with 6, 10 and 16 hours, mixed liquor suspended solids (MLSS) in the range of 3,400 to 7,800 mg/L and solids retention time (SRT) ranged from 6 to 24 days.

In the start-up of the BRM systems for the treatment of wastewater of municipal origin, in the general way, a positive effect of temperature on the kinetic behavior of the heterotrophic biomass is produced, with the consequent increase in the ratio of biological elimination of organic matter, reaching a compensated effect in those cases in which the operating conditions are especially optimal in MLSS (7,000 mg/L) and HRT (10 h).

In the same way and generally, in the presence of bisphenol A in the BRM system, the heterotrophic biomass not only proved not to suffer any type of inhibitory effect in terms of its behavior, but also in the opposite, with a rate of degradation of the substrate for the elimination of organic matter ($r_{su,H}$) of 190.22 $mgO_2/(L \cdot h)$and a minimum coefficient of decay (b_H), de 0.1304 day^{-1}, when the temperature was highest (31.1 °C).

As a consequence of the doping with ibuprofen, ciprofloxacin and carbamazepine, in increasing concentrations, they were observed

statistically significant differences in MLVSS, which indicated that there was a decrease in the biomass present in the MBR system. However, these differences were not observed in the organic matter removal as the increased cell growth rate cancelled out the increased cell decay rate due to the chemical stress caused by the addition of pharmaceuticals in the MBR system. Thus, it went from an organic matter degradation ratio of 86.27 $mgO_2/(L \cdot h)$ in the control cycle to values of 183.97 $mgO_2/(L \cdot h)$ and 192.88 $mgO_2/(L \cdot h)$ during the doping cycles. About the degradation of compounds of pharmaceutical origin, in the MBR system, all three compounds showed high elimination, especially at lower doping concentrations. The degradation rates were 0.0154, 0.0152 and 0.0160 $\mu gS/((\mu gS_{in}/L) \cdot h \cdot mgMLSS)$ for carbamazepine, ciprofloxacin and ibuprofen, the most biodegradable nature compound, with yields of 71.9, 88.7 and 94.7%, respectively, under the conditions tested. Interestingly, hybrid systems have a higher resistance to low temperatures but a lower drug elimination capacity.

Moreover, it was also proved that the structure of the bacterial community was affected mainly by the technology chosen at each moment, also affecting SRT and HRT, having allowed this research to improve our

knowledge about microbial communities in membrane-based technologies for the treatment of urban wastewater, highlighting the continued appearance of Tetrasphaera (1.1-19.2 % relative abundance) and highlighting the relevance of the phylotypes that are not commonly considered in the Intrasporangiaceae family.

Finally, we can say that throughout this investigation it has been showed that moving bed and membrane bioreactors technologies for the treatment of wastewater, working at low HRT, guarantee a high rate of elimination of conventional and emerging pollutants, (at least among those studied), showing a considerable ability to adapt and stabilize the sludge in situations of stress generated by incremental doping with different types and concentrations of drugs, being able to be a technically feasible alternative to the conventional treatment of activated sludge at the time of considering the possible extension of the biological treatment in a WWTP, that today is a widespread need that affects many plants around the world.

RESUMEN

El constante incremento en la aparición de contaminantes de tipo emergente en las aguas residuales, unido a las cada vez más exigente y restrictiva normativa en materia de vertidos, hace imprescindible, de cara al futuro, redoblar los esfuerzos de investigación científica en busca de nuevos sistemas de tratamiento de este tipo de compuestos.

Por ello, se realizaron diferentes análisis físico-químicos, microbiológicos y respirométricos durante 2 años en una planta piloto ubicada en la estación depuradora de aguas residuales (EDAR) Oeste de Granada, alimentada continuamente con agua residual procedente del tratamiento primario de la planta, a la cual se agregaron externamente contaminantes emergentes como el bisfenol A (BPA) y concentraciones crecientes de una serie de fármacos de diferente naturaleza, como ibuprofeno, carbamazepina y ciprofloxacina, con el propósito principal de analizar la capacidad de eliminación de dichos compuestos en un reactor biológico de membrana (BRM) y en un sistema híbrido compuesto por la suma de un reactor biológico de lecho móvil y un biorreactor de membrana

(RBLM-BRM), trabajando en ciclos bajo condiciones operativas diferentes, considerando un **bajo tiempo de retención hidráulico (TRH)**, con 6, 10 y 16 horas, sólidos suspendidos en licor mezcla (SSLM) en un rango de 3,400 a 7,800 mg/L y un tiempo de retención celular (TRC) que osciló entre 6 y 24 días.

En la puesta en marcha de sistemas BRM para el tratamiento de aguas residuales de origen municipal, de manera general se observó un efecto positivo de la temperatura en el comportamiento cinético de la biomasa heterótrofa, con el consiguiente incremento en cuanto al ratio de eliminación biológica de materia orgánica, llegando a compensarse dicho efecto en aquellos casos en los que las condiciones de operación resultaban especialmente óptimas en relación a SSLM (7,000 mg/L) y TRH (10 h).

Del mismo modo y de manera general, ante la presencia de bisfenol A en el sistema BRM, la biomasa heterótrofa no sólo demostró no sufrir ningún tipo de efecto inhibidor en cuanto a su comportamiento, sino que por el contrario mostró su mayor grado de actividad, con una tasa de degradación del sustrato para la eliminación de materia orgánica ($r_{su,H}$) de 190.22

$mgO_2/(L \cdot h)$ y un mínimo coeficiente de decaimiento (b_H), de 0.1304 d^{-1}, cuando la temperatura fue más elevada (31.1 °C).

Como consecuencia del dopaje con ibuprofeno, ciprofloxacina y carbamazepina, en concentraciones crecientes, se observaron diferencias estadísticamente significativas en SSVLM, lo que indicó que había una disminución en la biomasa presente en el sistema BRM. Sin embargo, estas diferencias no se observaron en la eliminación de materia orgánica, ya que la mayor tasa de crecimiento celular anuló la mayor tasa de descomposición celular debido al estrés químico causado por la adición de productos farmacéuticos en el sistema BRM. Así, se pasó de un ratio de degradación de materia orgánica de 86.27 $mgO_2/(L \cdot h)$ en el ciclo de control a valores de 183.97 $mgO_2/(L \cdot h)$ y 192.88 $mgO_2/(L \cdot h)$ durante los ciclos de dopaje. A cerca de la degradación de los compuestos de origen farmacéutico, en el sistema BRM, los tres compuestos presentaron una eliminación alta, especialmente en las concentraciones de dopaje menores. Las tasas de degradación fueron de 0.0154, 0.0152 y 0.0160 $\mu gS/((\mu gS_{in}/L) \cdot h \cdot mgSSLM)$ para carbamazepina, ciprofloxacina e ibuprofeno, el compuesto de naturaleza más biodegradable, con rendimientos de eliminación superiores al 71.9, 88.7 y 94.7%,

respectivamente, en las condiciones testadas. Curiosamente, los sistemas híbridos mostraron una mayor resistencia a las bajas temperaturas pero una menor capacidad de eliminación de los fármacos.

Los resultados de esta investigación han ayudado también a mejorar nuestro conocimiento sobre las comunidades microbianas en tecnologías basadas en membranas para el tratamiento de aguas residuales urbanas, destacando la continua aparición de Tetrasphaera (1.1-19.2 % abundancia relativa) y la relevancia de los filotipos que no son comúnmente considerados en la familia Intrasporangiaceae. También se demostró que la estructura de la comunidad bacteriana se vio afectada principalmente por la tecnología elegida en cada momento, influyendo también de una manera importante las condiciones de TRC y TRH.

Finalmente, a modo de conclusión, podemos decir que a lo largo de esta investigación se ha demostrado que las tecnologías de biorreactores de lecho móvil y de membrana para el tratamiento de aguas residuales que funcionan con un bajo TRH, garantizan una alta tasa de eliminación de contaminantes convencionales y emergentes (al menos entre los estudiados), mostrando una capacidad considerable para adaptar y

estabilizar el fango en situaciones de estrés generado por un dopaje incremental con diferentes tipos y concentraciones de fármacos, pudiendo ser una alternativa técnicamente viable al tratamiento convencional mediante fangos activados, a la hora de considerar la posible ampliación del tratamiento biológico de una depuradora de aguas residuales, que hoy es una necesidad generalizada que afecta a muchas plantas en todo el mundo.

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1. Problems of wastewater

In the last century, the continuous increase in population and industrialization has led to the degradation of numerous natural ecosystems such as rivers and oceans, largely as a result of the dumping of urban and industrial wastewater inadequately treated or even discharged without prior treatment (Chan et al., 2009).

Urban wastewater carries with it a series of pollutants, of which, on the one hand, organic matter, usually from domestic waters, stands out. These are compounds of a reducing nature and, therefore, are those that consume oxygen; on the other hand, these waters have elements of inorganic nature, of very different composition from nutrients such as nitrogen and phosphorus, to toxic and dangerous substances (Gómez and Hontoria, 2003).

At present, the treatment of wastewater, especially in areas with a dense population, has become an important environmental and economic

problem, in which all (citizens, companies, public administrations, etc.,) are involved and forced to collaborate.

In addition, the bigger and bigger social awareness in relation to this matter and the increase in normative pressure in Europe, with the objectives set by the Water Framework Directive 60/2000/CE, makes each more important the work of searching for alliances that allow us to advance in the development of new ways of dealing with the purification treatment of this type of compounds.

2. Emerging contaminants in wastewater: drugs and endocrine disruptors

As it will be explained in detail in Chapter number 3, the occurrence and fate of pharmaceutical residues in wastewater treatment and the environment has attracted increasing interest during the last years (Quintana et al., 2005).

Although pharmaceuticals have been present in water for decades, their levels in the environment have only recently begun to be quantified and acknowledged as potentially hazardous to ecosystems (Kolpin et al.,

2002; Fent et al., 2006; Jjemba, 2006). However, emerging substances have recently been detected in water resources worldwide, raising human and environmental health concerns (Hamza et al., 2016). Several studies in Europe and the United States have indicated that many of these compounds are present in the effluents of wastewater treatment plants, surface water, and groundwater (Puijjker and Mons, 2004).

In wide world sewages, it is becoming more and more frequent the presence of antibiotics as ciprofloxacin, antiepileptic drugs as carbamazepine and non-steroidal anti-inflammatory drugs (NSAIDs) as ibuprofen. That is the reason why this situation must be taken into account in order to design new wastewater treatment plants (WWTPs) and in already existing ones.

The main problem we have is that most wastewater biological treatments are unable to effectively remove pharmacologically active ingredients (Pomati et al., 2006). However, different conventional and alternative wastewater treatment processes and their combinations have been tested with regard to the removal of pharmaceuticals (Zupanc et al.,

2013). The efficiency of these technologies can depend on the characteristics of the pharmaceuticals.

The main impacts of pharmaceuticals on the biological treatment processes include a loss of organic matter removal efficiency, deflocculation with the consequent loss of biomass and respiration inhibition of the activated sludge (Love and Bott, 2000; Henriques et al., 2005). In light of this, the kinetic characterization of heterotrophic biomass constitutes an important tool to evaluate, control and predict the influence of pharmaceuticals on the process of organic matter removal.

On the other hand, as it´ll be commented in Chapter number 2, in the last years, new emerging pollutants have been identified due to their presence and persistence in the environment, such as endocrine disrupting compounds (EDCs), which are exogenous chemicals that alter the functioning of the endocrine system and have adverse effects on human health and the environment (Dorival-García et al., 2014; Rivero et al., 2014; Zielińska et al., 2016). Some of these negative effects are the reduction of

fertility, congenital malformations and appearance of cancer (Bonefeld-Jørgensen et al., 2007).

It is important to highlight that among the EDCs, bisphenol A (BPA) (2,2-(4,4- dihydroxydiphenyl) propane) is a monomer that is causing a major concern in the medical and scientific fields as it has more detrimental effects than other EDCs (Dorival-García et al., 2014; Omoike et al., 2013). BPA has estrogenic and anti-androgenic activities (Dorival-García et al., 2014; Birkett and Lester, 2003); it could cause reproductive and sexual dysfunctions and act as carcinogenic agent (Furuya et al., 2006; Stowell et al., 2006). In addition, BPA could also affect the neuroendocrine, behavioral and cognitive functions of individuals, as well as increasing the risk for cardiovascular disease, miscarriage and diabetes (Sugiura-Ogasawara et al., 2005; Takayanagi et al., 2006).

Due to de fact that BPA is one of the most produced chemicals in the world, (its production is higher than 3.2 million tons per year (EPA, 2005)), BPA is often detected in wastewater treatment plants (WWTPs) (Melcer and Klečka, 2011; Guerra et al., 2015) and it is a serious threat to aquatic

environment due to its extensive use, toxic properties and persistence since this compound is not readily biodegradable (Kang et al., 2006; Talsness et al., 2009; Limam et al., 2013).

As in the case of the pharmaceutical compounds discussed above, the main problem is that existing conventional treatments are not capable of removing this compound. However, there are few studies analyzing its influence on the biological processes of the different wastewater treatment technologies (Stasinakis et al., 2008).

To the best of our knowledge, there are no studies in the literature that report on the influence of BPA on the heterotrophic biomass concerning the organic matter removal that is carried out in an MBR system (Zielińska et al., 2016). Thus, it is necessary to widen between this study and other works consists of the fact that the present work has assessed the performance of the heterotrophic biomass of an MBR system under the presence of BPA, evaluating rates of organic matter removal, decay and biomass generation, and other studies have analyzed the kinetics and biodegradability of BPA in biological systems (Stasinakis et al., 2008) and

oxidation processes (Rivero et al., 2014), or investigated the removal of BPA in different wastewater treatment processes (Dorival-García et al., 2014; Zielińska et al., 2016; Guerra et al., 2015).

Respirometric method has been widely applied to model the microbial kinetics for wastewater treatment systems due to its high reproducibility and accuracy (Leyva-Díaz et al., 2013; Leyva-Díaz et al., 2017). In light of this, kinetic modeling can predict the organic substrate degradation rate under the influence of BPA, and constitutes a useful tool for designing and optimizing the biological process. Additionally, this information could be useful for the implementation and operation of MBR to treat wastewater containing BPA with a higher efficiency.

3. MBR and MBBR-MBR

In addition to trying to solve everything discussed above, it is also evident the need to deepen the search for the simplest and cheapest way to increase the treatment capacity of a multitude of under-dimensioned biological reactors as well as give solution to the increasing demand regarding the reuse of urban and industrial wastewater. For all this, it is

important to try to find new treatment systems that can be an alternative to conventional treatments and eliminate a greater number of compounds.

In Suez Water Spain, for example, we have a total of 627 wastewater treatment plants distributed throughout the Spanish geography, some of which are under-dimensioned, being necessary to compare the feasibility of carrying out a conventional extension versus the search for alternatives through another type of unconventional treatments.

Among the new technologies for biological treatment are membrane bioreactors and fixed biomass processes on a moving bed (Leyva-Díaz, 2015).

Generically, MBR can be defined as systems in which the biological degradation of effluents is integrated with membrane filtration (Cicek et al., 1998).

For its part, the objective of the MBBR systems is to ensure that the biomass develops in the form of a biofilm on support elements that, having a density lower than that of water, allows its movement freely in the mixed liquor.

The biofilm present in these supports allows microorganisms to perform their vital functions selectively allowing them to capture a greater concentration of nutrients, constituting in nature, a form of protected growth that allows the survival of bacteria in a hostile environment in which they find the fundamental needs for their development (Torres, 2007; Gómez et al., 2000).

The MBR systems can be easily combined with MBBR systems providing a mixed technology (MBBR-MBR). In recent years (Martín-Pascual, 2014), among others, they have carried out different studies integrating the mobile bed processes with membrane bioreactors, which have focused especially on the analysis of the improvement of yields in biological elimination of nitrogen and phosphorus with the use of a hybrid biomass system with biological flocs and moving bed, as well as in the analysis of the effect of introducing attached biomass into the behavior of the membrane bioreactor.

As it´ll be explained in detail along chapter 1, among the operational factors in the MBR process, temperature is one of the most important

(Monod, 1949). Temperature of mixed liquor varies due to seasonal and diurnal temperature changes, as well as mixing of hot industrial effluents with municipal wastewater (Arévalo et al., 2014; Grandclément et al., 2017).

These changes can affect the MBR performance through the influence on concentration of mixed liquor suspended solids (MLSS) and microbial kinetics. In light of this, microbial activity, reaction rate of the biological process occurring in MBR and other physicochemical properties could be influenced by temperature conditions (Calderón et al., 2012; Grandclément et al., 2017). Thus, the stability of an MBR system depends on temperature variability, which is related to sludge deflocculation and reduction of sludge metabolic activity.

4. Bacterial ecology in membrane bioreactors

It is widely accepted that biological wastewater treatment processes have an important microbiological factor defining their performance (Rodríguez et al., 2015). Indeed, it has been argued that the intrinsic microbial nature of wastewater treatment processes should be considered for their design (Cydzik-Kwiatkowska and Zielińska, 2016).

As it will be detailed later in chapter 5, the recently developed Anaerobic ammonium oxidation (Anammox) process provides a clear example of the importance of understanding microbial community interactions in optimized bioreactor design and operation (Said and Or, 2017). In practice however, wastewater treatment systems are usually designed under the influence of engineering considerations only (Gedalanga et al., 2013). Thus, a comprehensive characterization of microbial ecosystems in emerging wastewater treatment technologies is essential for future optimization of these bioprocesses.

In this context, the biotechnological potential of the Earth's microbiome is still largely unknown and underutilized and initiatives have been proposed in order to fully utilize its potential for biotechnological reasons, among which wastewater treatment becomes one of the most important (Strous and Sharp, 2018). Novel tools have been developed and are constantly evolving to provide new microbiological insights in the field of water treatment, such as the application of omics approaches in molecular biology (Eren et al., 2014). Such omics approaches allow extensive interrogation of microbial community dynamics within a bioprocess, which

can influence subsequent system design iterations (Schloss et al., 2009). One of the most promising molecular biology techniques for the investigation of microorganisms is based on Shannon entropy clustering of genetic sequences, known as oligotypes, achieving a very sensitive characterization of the microbiome within a sample beyond the traditional operational taxonomic unit (OTU) approach (Rognes et al., 2016). The characterization of oligotypes within a bioprocess could lead to a fine tuning of key operational conditions to enhance the systems performance.

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II - METHODOLOGY

II - METHODOLOGY

1. Bioreactor description

As will be described in depth throughout the following chapters, the research was carried out in a pilot plant located in the wastewater treatment plant (WWTP) Oeste in Granada (Spain), which was continuously fed with real urban wastewater from the primary settler.

During the research two different technologies were tested based on the use of a MBR. **Figure 1** shows a diagram of the pilot plant consisting of a cylindrical tank with 272 L of volume, connected to a rectangular tank of 78L capacity where four ZENON ZW-10 membrane modules were submerged. These modules are configured as hollow fiber with external filtration with a nominal surface area of 0.93 m^2, a nominal pore size of 0.04 μm and an absolute pore size of 0.1 μm.

A blower to provide the air scouring of the membrane under a constant flow of 200 L/h was installed. To maintain a homogeneous mixture in the system, MLSS was recirculated to the biological reactor from the membrane

tank with a constant flow of 90 L/h. During a first phase, the pilot plant was operated as a conventional MBR system. Upon completion the plant was completely cleaned and carriers were incorporated into the bioreactor to create a hybrid MBBR-MBR system which was operated under the same conditions to ensure that the results were comparable.

To set up the hybrid MBBR-MBR system, the biological reactor was filled with 35 % filling ratio of K1 carriers (Anoxkaldnes®). This carrier is made of high-density polyethylene and shaped into small cylinders (length 7 mm and diameter 10 mm) with a cross inside the cylinder.

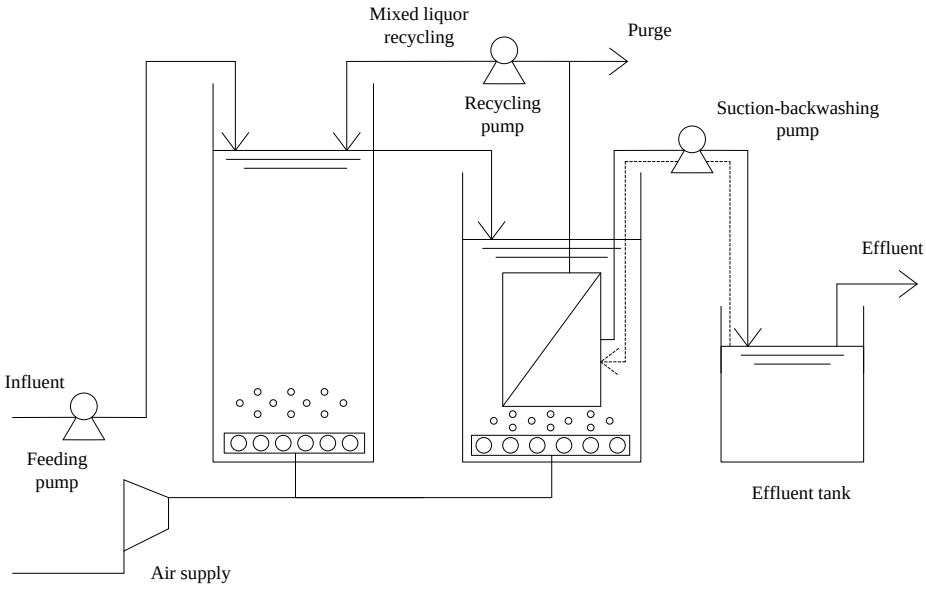

Figure 1. *Experimental pilot plant*

2. Bioreactor operation

The pilot plant was operating for 2 years under 8 different operational conditions. In the different cycles the key operational variables were: hydraulic retention time (HRT), with 6, 10 and 16 hours being tested, respectively; and the mixed liquor suspended solids (MLSS), in the range of 3400 to 7800 mg/L, to reflect the normal range of operation in the WWTP.

The solids retention time (SRT) ranged from 6 to 24 days, depending on the temperature, the purge flow rate for each cycle and the influent. The pilot plant was continuously fed with real urban wastewater in order to simulate an authentic process with a variable influent caused by the seasonal fluctuation. The same HRT and MLSS was maintained with the MBR and hybrid MBBR-MBR; however, the presence of biofilm in the bioreactor and the typical variation of temperature and influent characteristics caused a slight variation on the SRT during the research.

3. General analytical techniques

The analytical techniques, conditions and specific methodologies used throughout the investigation to achieve both the general objective and

each of the specific objectives, are specified in every detail in each of its corresponding chapters. For this reason, we will only describe now, in a superficial way, the main general methodologies used throughout the research and later and in the next section we will summarize the most relevant specific methodologies used in each of the different phases.

3.1. Physicochemical determinations

pH and conductivity were determined using a pH meter (Crison pH 25®) and a conductivity meter (Crison CM 35®). The temperature was measured continuously in the same biological tank of the pilot WWTP with a thermometer (LANGE LDOTM/SC 100). The chemical oxidation demand (COD), biological oxidation demand (BOD_5) and MLSS were determined according to the method of the American Public Health Association, the American Water Works Association and the Water Environment Federation (APHA, 2012). Total organic carbon (TOC) measurements were determined using a Formarcs HT TOC/TN Analyzer by oxidative combustion at 950 °C.

The concentration of the pharmaceutical studied (carbamazepine, ibuprofen and ciprofloxacin) in the MLSS of the system was measured by

chromatography using an Acquity UPLC System H-Class with Acquity UPLC BEHTM C 18 column (2.1 × 150 mm, 1.7 μm) at 40 °C.

3.2. Respirometric experiments

To analyze the degradation capacity and effect on the process, respirometric experiments, both exogenous and endogenous, were carried out on biomass samples taken from the MBR system in a BM-Advance respirometer according to Leyva-Díaz et al. (2013).

3.3. Statistical analysis

Among the different statistical tools used during the study and specified in each of the corresponding chapters, the following stand out: multivariable statistical analysis applying the software Canoco for Windows v. 4.5 (ScientiaPro, Budapest, Hungary), SPSS 20 for Windows , verification of the linear distribution of the model, provided by the detrended correspondence analysis (DCA), redundancy analysis (RDA) carried out as statistical method recommended by (Smilauer and Lepš, 2016), a least significant differences test (LSD test) was used to measure the differences between the results obtained for each cycle and an analysis of variance

(ANOVA) was used to assess the homogeneity of the variance and Monte Carlo test with 499 permutations used with a significance level of 0.05.

4. Specific techniques and conditions

Only as a synthesis, since later the characteristics of the different techniques used will be detailed in each chapter, in a general way we can say that to obtain the results that are shown in this document, different techniques have been carried out and in different conditions, depending on each case.

In this way, the analysis of the effect of seasonal temperature variations on the performance of a pilot-scale MBR on its heterotrophic kinetics in the start-up phase at the hydraulic retention time (HRT) values of 6 h and 10 h, and MLSS concentrations for the steady state of 4,000 mg/L and 6,000 mg/L, required some specific respirometric method and the influence of temperature on the biological process of organic matter removal was determined through the Arrhenius equation and Monod model, as will be detailed in chapter 1.

As will be explained in chapter 2, the kinetic study of the effect of bisphenol A on the rates of organic matter removal, decay and biomass generation in a membrane bioreactor, required of preparation of a BPA synthetic solution (97 %, CAS No: 80-05-7, MW: 228.29 g/mol), use of a respirometric method and use of stock solution of sodium acetate of 500 mg/L and three dilutions of 50, 80 and 100 %. The values of hydraulic retention time were (6-10 h), mixed liquor suspended solids (4,000-7,000 mg/L), temperature (12.1-31.1 °C), and sludge retention time (9.81-21.67 day).

As will be detailed in chapter 3, the study about the impact of this mixture of ciprofloxacin, carbamazepine and ibuprofen on a membrane bioreactor (MBR) system was carried out under a hydraulic retention time of 6 h, with 7.5 days of SRT, and an average value of MLSS of 4,551±534 mg/L. Under these conditions, three different dopings were performed in increasing concentrations of a mix of carbamazepine and ibuprofen (100, 1000 and 5000 μg/L) and ciprofloxacin (10, 100 and 500 μg/L).

As will be showed in chapter 4, about the effect of the biomass on the biodegradation capacity of a mix of pharmaceuticals (carbamazepine, ibuprofen and ciprofloxacin) in a membrane bioreactor, the MLSS levels were fixed at about 5,500 mg/L. The SRT for the MBR system varied from 11.23 days with 6 h of HRT to 21.67 days with 10 h of HRT. For the hybrid MBBR-MBR system, the SRT ranged from 6.02 days for 6 h of HRT to 23.89 days for 10 h of HRT. For the MBR system the temperature ranged from 21.5 to 12.6 °C for Cycles 1 and 2, respectively, and for the hybrid MBBR-MBR system the temperature oscillated from 28.1 to 17.6 °C for Cycles 3 and 4, respectively. The pharmaceutical compounds were incorporated continuously at a constant flow of 0.75 L/h with the real urban influent with a peristaltic pump.

As will be detailed in chapter 5, about the bacterial ecology in membrane bioreactor, operated under two different technologies (MBR and MBBR-MBR) at hydraulic retention time 6-16 h, sludge retention time 6-24 d and mixed liquor suspended solids 3400-7800 mg/L. DNA extractions at -20 °C from the biomass were carried on, with different PCR amplifications, taxonomic classifications of OTUs, analyses of similarity of samples and

correlation between OTUs, oligotyping analysis of OTUs of interest, and prediction of metagenome in the biological samples using specific softwares and methods.

5. References

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III - OBJECTIVES

III - OBJECTIVES

The filtration systems based on the use of membranes have been satisfactorily studied in wastewater of both urban and industrial origin operating at high hydraulic retention times (HRT) and solid retention time (SRT), achieving excellent results in removal of organic matter, nitrogen, drugs and emerging contaminants.

The main objective of this research is to analyze the behavior with two system, Moving bed biofilm bioreactor - Membrane bioreactor (MBBR-MBR) and Membrane bioreactor (MBR) for the treatment of wastewater to low HRT and their influence on the elimination of pollutants of various natures.

In this way it could be determined if the rehabilitation of existing treatment plants with medium load active sludge systems or those with low load that have experienced an increase in inlet flow in which the volume of the reactor does not allow to operate at high HRT could be carried out by incorporating a physical membrane separation system by converting the

purification process into a MBR, into a MBBR or even into a combination of both, without requiring modification of the biological reactor, ensuring an optimum effluent quality to be reused directly.

As secondary objectives of the present investigation, we could highlight the following:

1. Assess the effect of seasonal temperature variations on the performance of a pilot-scale MBR on its heterotrophic kinetics in the start-up phase at the HRT values of 6 h and 10 h, and MLSS concentrations for the steady state of 4,000 mg/L and 6,000 mg/L.

2. Analyze kinetic study of the effect of bisphenol A (BPA) on the rates of organic matter removal, decay and biomass generation in a MBR treating municipal wastewater at different values of HRT (6-10 h), MLSS (4,000-7,000 mg/L), temperature (12.1-31.1 °C), and SRT (9.81-21.67 day).

3. Provide a fundamental understanding of biodegradation capacity and microbial kinetics through the response of a MBR system treating urban wastewater that was doped continuously with three different

concentrations of a mixture of ciprofloxacin, carbamazepine and ibuprofen under a HRT of 6 h.

4. Analyze the effect of the biomass on the biodegradation capacity of a mixture of pharmaceuticals (carbamazepine, ibuprofen and ciprofloxacin) in a biorector with operational variables of 6 and 10 hours of HRT, the MLSS between 5,200 and 5,700 mg/L and the process (MBR or hybrid MBBR-MBR).

5. Analysis of important bacterial phylotypes to improve future design and operational parameters in MBR and hybrid MBBR-MBR systems, operated under different conditions of HRT (6, 10 and 16 h), SRT (6-10, 11-16, 20-24 days), and MLSS (from 3400 to 7800 mg/L).

IV - CHAPTER 1

Influence of Temperature on the Start-up of Membrane Bioreactor: Kinetic Study.

IV - CHAPTER 1

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1. Abstract

The start-up phase of a membrane bioreactor (MBR) for municipal wastewater treatment was studied to determine the effect of temperature on the organic matter removal and heterotrophic kinetics. The MBR system was analyzed at hydraulic retention times (HRTs) of 6 h and 10 h and temperature values varying between 11.5 °C and 30.1 °C. Arrhenius and Monod models were used to evaluate the effect of temperature on the biological process of organic matter removal. At the most favorable

conditions of HRT (10 h) and MLSS (6,000 mg/L) corresponding to phase 4, the effect of these variables dominated over the temperature. Heterotrophic biomass from phase 2 (HRT = 10 h, MLSS = 4,000 mg/L and T = 30.1 °C) had the highest values of COD degradation rate ($r_{su,H}$).

2. Introduction

Membrane bioreactor (MBR) systems have been widely used for the treatment of municipal and industrial wastewater (Wintgens et al., 2005). These systems improve the conventional activated sludge processes due to their higher effluent quality, smaller space and reactor requirements, increased volumetric loadings and lower sludge production rates (Oppenheimer et al., 2001; Poyatos et al., 2008; Wang et al., 2009).

Temperature is one of the most important operational factors affecting MBR process (Judd, 2011). Temperature of mixed liquor varies due to seasonal and diurnal temperature changes (Arévalo et al., 2014). In light of this, microbial activity, reaction rate of the biological process occurring in MBR and other physicochemical properties could be influenced by temperature conditions (Calderón et al., 2012; Grandclément et al., 2017).

The influence of temperature on the heterotrophic bacteria kinetics was evaluated through the Monod and Arrhenius models (Monod, 1949; Grandclément et al., 2017).

The aim of this study was to assess the effect of temperature variations on the performance of a pilot-scale MBR concerning its heterotrophic kinetics in the start-up phase at hydraulic retention time (HRT) values of 6 h and 10 h, and mixed liquor suspended solids (MLSS) concentrations for the steady state of 4,000 mg/L and 6,000 mg/L.

3. Materials and methods

A pilot-scale MBR was analyzed during the start-up periods corresponding to four operation phases (**Table 1**). Bioreactor was fed with municipal wastewater coming from the primary settler of Wastewater Treatment Plant of Puente de los Vados, located in Granada (Spain). MBR system was designed as an aerated cylindrical bioreactor of 272 L, as well as an external rectangular unit of 78 L which contained four vertically oriented submerged modules of hollow-fiber ultrafiltration membrane (ZW-10,

ZENON®). The membrane was flowing from the outside to the inner side by sucking. The total membrane area was 3.72 m^2.

Table 1. *Operation conditions and heterotrophic kinetic parameters, $\mu_{m,H}$, $K_{M,H}$, Y_H, b_H, for the different phases of start-up of MBR system. Y_H (yield coefficient for heterotrophic biomass), $\mu_{m,H}$ (maximum specific growth rate for heterotrophic biomass), $K_{M,H}$ (half-saturation coefficient for organic matter), b_H (decay coefficient for heterotrophic biomass).*

Phase	HRT (h)	MLSS (mg/L)	T (ºC)	Y_H (mgVSS/ mgCOD)	$\mu_{m,H}$ (h^{-1})	$K_{M,H}$ (mgO_2/L)	b_H (day^{-1})
1	6	4,000	14.2	0.4100±0.0502	0.0101±0.0024	8.0652±0.7662	0.0494±0.0074
2	10	4,000	30.1	0.9076±0.0976	0.1075±0.0205	30.7323±3.3806	0.2361±0.0307
3	6	6,000	22.9	0.6216±0.0784	0.0182±0.0020	13.6928±1.4377	0.1405±0.0139
4	10	6,000	11.5	0.4356±0.0314	0.0336±0.0040	24.5231±2.8202	0.0828±0.0091

Heterotrophic kinetic parameters and chemical oxygen demand (COD) degradation rate ($r_{su,H}$) were evaluated through a respirometric method according to Leyva-Díaz et al. (2013). The evolution of the dynamic oxygen uptake rate (R_S) was registered in dynamic respirometric experiments. Furthermore, an endogenous respiration test was also performed (Leyva-Díaz et al., 2013).

Thus, both respirometric tests facilitated the estimation of the maximum specific growth rate ($\mu_{m,H}$), substrate half-saturation coefficient

($K_{M,H}$), yield coefficient (Y_H) and decay coefficient (b_H) for heterotrophic biomass. The assessment of these parameters was carried out in six steps:

(1) Determination of the oxygen consumption (OC) through the integration of R_S, as shown in Eq. (1):

$$OC = \int_{t_0}^{t} R_S dt \quad (mgO_2/L) \tag{1}$$

(2) Estimation of Y_H according to Eq. (2) described by Helle (1999):

$$Y_H = \frac{S - OC}{S \cdot f_{CV}} \quad (mgVSS/mgCOD) \tag{2}$$

where S is the substrate concentration (mgO_2/L) and f_{CV} is a conversion factor (1.48 mgCOD/mgVSS).

(3) Evaluation of the substrate degradation rate (r_{su}) from R_S:

$$r_{su} = \frac{R_S}{1 - Y_H \cdot f_{CV}} \quad (mgO_2/(L \cdot h)) \tag{3}$$

(4) Assessment of the empirical specific growth rate (μ_{emp}) from the relation between the cell growth rate and r_{su}:

$$\mu_{emp} = \frac{Y_H \cdot R_S}{(1 - Y_H \cdot f_{CV}) \cdot X_H} \quad (h^{-1}) \qquad (4)$$

where X_H is the concentration of heterotrophic biomass (mgVSS/L)

(5) Estimation of $\mu_{m,H}$ and $K_{M,H}$ through the linearization of the Monod model:

$$\frac{1}{\mu_{emp}} = \frac{1}{\mu_{m,H}} + \frac{K_{M,H}}{\mu_{m,H}} \cdot \frac{1}{S} \quad (h) \qquad (5)$$

(6) Estimation of b_H according to Eq. (6) described by Ekama et al. (1986):

$$b_H = \frac{OUR_{end}}{1.42 \cdot X_T \cdot \left[1 - Y_H \cdot \left(1 - f_p\right)\right]} \quad (day^{-1}) \qquad (6)$$

where OUR_{end} is the endogenous oxygen uptake rate ($mgO_2/(L \cdot h)$), X_T is the total biomass concentration (mgTSS/L) and $(1 - f_p)$ is the fraction of volatile biomass (mgVSS/mgTSS).

The conversion of kinetic parameters to working temperature was carried out following Eq. (7) proposed by Metcalf and Eddy (2003):

$$r_T = r_{20} \cdot \theta^{(T-20)} \qquad (7)$$

where r_T and r_{20} symbolize the kinetic parameters at working temperature and 20 °C, respectively, θ is a fitting parameter with a value of 1.04 for MBR and T is the working temperature.

Furthermore, Arrhenius equation was used to fit the heterotrophic kinetic parameters as a function of temperature, as shown in Eq. (8):

$$Ln(r_T) = Ln(A) - \frac{E_a}{R} \cdot \frac{1}{T} \quad (8)$$

where A is the pre-exponential factor, R is the gas constant and E_a is the activation energy of the biological process.

Therefore, the $r_{su,H}$ can be expressed depending on the temperature through the heterotrophic kinetic parameters, as well as the substrate and biomass concentrations, as indicated in Eq. (9):

$$r_{su,H} = \frac{\mu_{m,H} \cdot S \cdot X_H}{Y_H \cdot \left(K_{M,H} + S\right)} \quad (9)$$

4. Results and discussion

Table 1 shows the values of Y_H, $\mu_{m,H}$, $K_{M,H}$ and b_H. As observed in **Table 1**, the values of Y_H and b_H increased with temperature due to

maintenance energy requirements for biomass and increase of microbial activity (Pollice et al., 2007).

Figure 2 shows that the napierian logarithm of the heterotrophic kinetic parameters was correlated with the inverse of temperature, except for the values of phase 4, characterized by the lowest temperature (11.5 °C).

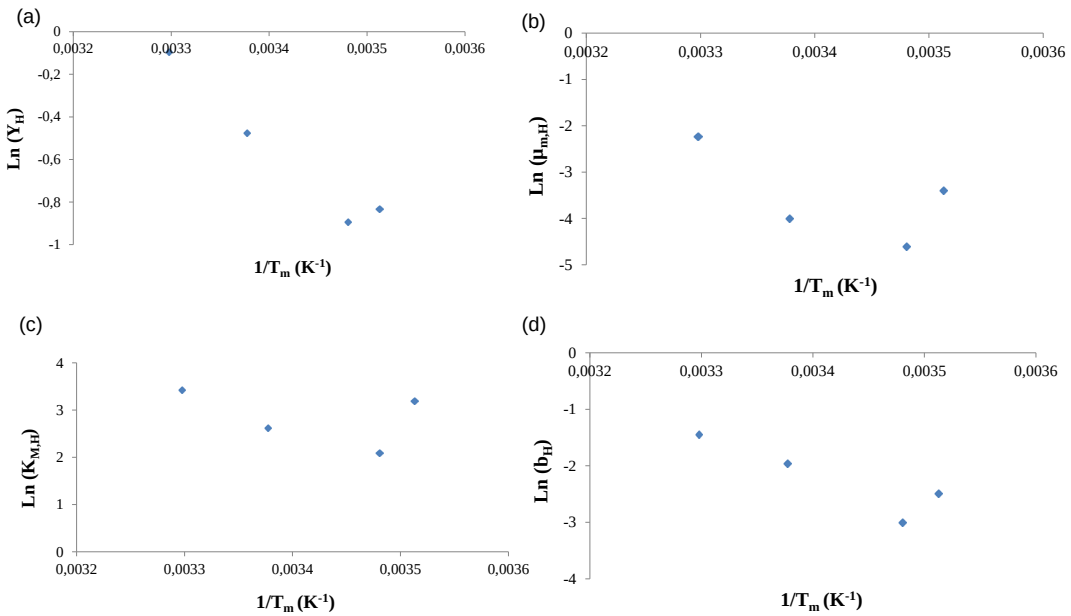

Figure 2. *Linear regression of the napierian logarithm of heterotrophic kinetic parameters, (a) Y_H, (b) $\mu_{m,H}$ (c) $K_{M,H}$, and (d) b_H, depending on the inverse of temperature using Arrhenius equation. Y_H (yield coefficient for heterotrophic biomass), $\mu_{m,H}$ (maximum specific growth rate for heterotrophic biomass), $K_{M,H}$ (half-saturation coefficient for organic matter), b_H (decay coefficient for heterotrophic biomass).*

The deviation of the values corresponding to phase 4 was probably due to the more favorable operation conditions of HRT and MLSS that characterized this phase (HRT = 10 h and MLSS = 6,000 mg/L), cancelling out the effect of temperature. This is supported by **Figure 3** as $\mu_{m,H}$, $K_{M,H}$, b_H are more positively correlated with HRT and mixed liquor volatile suspended solids (MLVSS) than temperature.

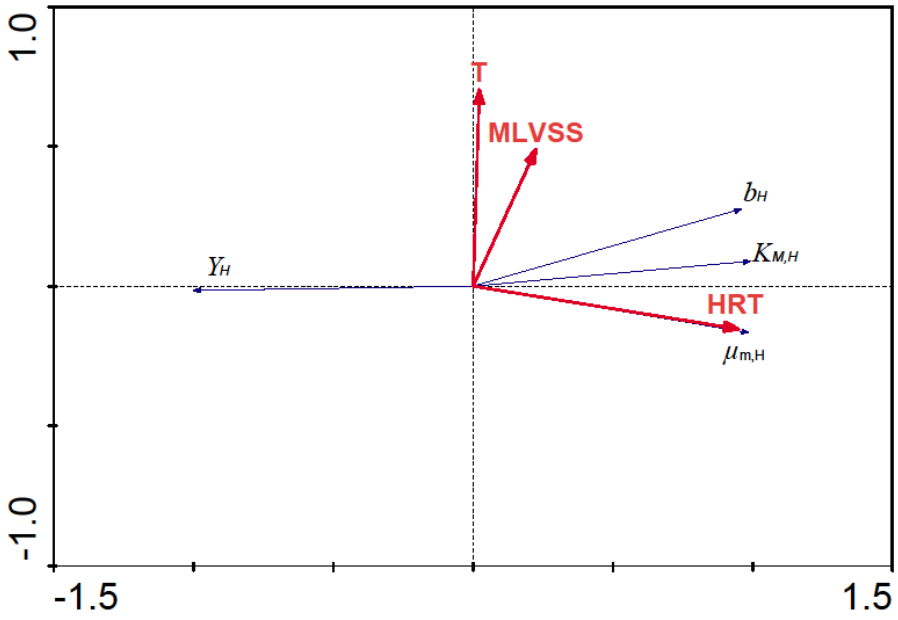

Figure 3. *Triplot diagram for RDA of the heterotrophic kinetic parameters, $\mu_{m,H}$, $K_{M,H}$, Y_H, b_H, in relation to the variables HRT, MLVSS and T. RDA (redundancy analysis), $\mu_{m,H}$ (maximum specific growth rate for heterotrophic biomass), $K_{M,H}$ (half-saturation coefficient for organic matter), Y_H (yield coefficient for heterotrophic biomass), b_H (decay coefficient for heterotrophic biomass), HRT (hydraulic retention time), MLVSS (mixed liquor volatile suspended solids), T (temperature).*

This effect was also observed when the $r_{su,H}$ was analyzed (**Figure 4**). Equation (10) was obtained to explain the evolution of $r_{su,H}$ depending on temperature, substrate concentration and heterotrophic biomass concentration:

$$r_{su,H} = \frac{1.16 \cdot 10^3 \cdot e^{\frac{-2939}{T}} \cdot S \cdot X_H}{1.08 \cdot 10^5 \cdot e^{\frac{-2563}{T}} + S} \quad (10)$$

As observed in **Figure 4**a, heterotrophic biomasses from phase 2 and phase 3, which were characterized by the highest temperatures showed the highest values for $r_{su,H}$. However, heterotrophic biomass corresponding to phase 4 had higher values of $r_{su,H}$ than heterotrophic bacteria from phase 1 in spite of its lower value of temperature (11.5 °C). This is explained as a consequence of the higher influence of HRT and MLSS compared with temperature. Furthermore, it should be noted that heterotrophic biomass required less time for organic matter oxidation during the start-up of phase 2 due to its higher $r_{su,H}$ (**Figure 4**a).

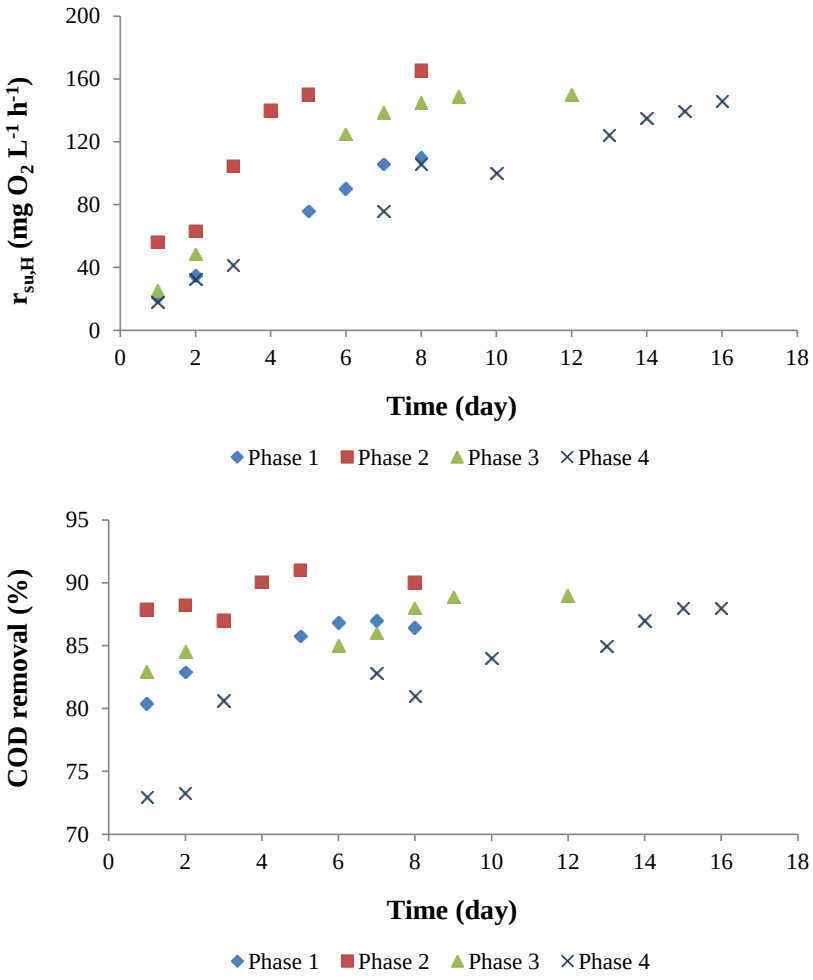

Figure 4. *(a) Evolution of COD degradation rate ($r_{su,H}$) obtained for heterotrophic biomass from MBR, and (b) COD removal during the four start-up phases. Phase 1: HRT = 6 h and MLSS = 4,000 mg/L; Phase 2: HRT = 10 h and MLSS = 4,000 mg/L; Phase 3: HRT = 6 h and MLSS = 6,000 mg/L; Phase 4: HRT = 10 h and MLSS = 6,000 mg/L. COD (chemical oxygen demand), HRT (hydraulic retention time), MLSS (mixed liquor suspended solids).*

This was in accordance with the COD removal efficiencies obtained in the four phases (**Figure 4**b). Heterotrophic biomass from phase 2 showed the highest COD removal, followed by the biomass from phases 3 and 4.

5. Conclusions

The kinetic behavior of heterotrophic biomass corresponding to phase 4 did not fit the Arrhenius model. This was probably due to the fact that the MBR worked at the most favorable operation conditions of HRT (10 h) and MLSS (6,000 mg/L), and the effect of temperature (11.5 °C) was cancelled out. This was confirmed by the higher values of $r_{su,H}$ and COD removal for phase 4 compared with those from phase 1 (HRT = 6 h, MLSS = 4,000 mg/L and T = 14.2 °C). Under the operation conditions of HRT = 10 h, MLSS = 4,000 mg/L and T = 30.1 °C that characterized phase 2, heterotrophic biomass showed the highest $r_{su,H}$, which implied less time to oxidize organic matter during the start-up.

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V - CHAPTER 2

Kinetic study of the effect of bisphenol A on the rates of organic matter removal, decay and biomass generation in a membrane bioreactor.

V - CHAPTER 2

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1. Abstract

This study analyzed the effect of bisphenol A (BPA) on the kinetic behavior of the heterotrophic biomass of a membrane bioreactor (MBR) treating municipal wastewater in order to determine possible variations in the rates of organic matter removal, decay and biomass generation. Four operation phases were analyzed in the steady state at different values of

hydraulic retention time (6-10 h), mixed liquor suspended solids (4,000-7,000 mg/L), temperature (12.1-31.1 °C), and sludge retention time (9.81-21.67 day). A respirometric procedure was used to model the kinetic behavior of heterotrophic biomass contained in the MBR in absence and presence of BPA, and a multivariable statistical analysis was applied to determine the effect of the variables on the substrate degradation rate for organic matter removal ($r_{su,H}$), decay coefficient for heterotrophic biomass (b_H), and net heterotrophic biomass growth rate ($r'_{x,H}$). The results showed that there was no inhibitory effect of BPA on heterotrophic biomass, with higher values of $r_{su,H}$ and $r'_{x,H}$, and lower values of b_H in presence of BPA. Heterotrophic biomass from phase 2 showed the highest values of $r_{su,H}$ (190.22 $mgO_2/(L \cdot h)$), b_H (0.1304 day^{-1}) and $r'_{x,H}$ (170.68 mgVSS/(L·h)) in presence of BPA due to its higher temperature (31.1 °C).

2. Introduction

In the last years, new emerging pollutants have been identified due to their presence and persistence in the environment, such as endocrine disrupting compounds (EDCs), which are exogenous chemicals that alter

the functioning of the endocrine system and have adverse effects on human health and the environment (Dorival-García et al., 2014; Rivero et al., 2014; Zielińska et al., 2014). Some of these negative effects are the reduction of fertility, congenital malformations and appearance of cancer (Bonefeld-Jørgensen et al., 2007).

Among the EDCs, bisphenol A (BPA) (2,2-(4,4-dihydroxydiphenyl)propane) is a monomer that is causing a major concern in the medical and scientific fields as it has more detrimental effects than other EDCs (Dorival-García et al., 2014; Omoike et al., 2013). BPA has estrogenic and antiandrogenic activities (Dorival-García et al., 2014; Birkett and Lester, 2003); it could cause reproductive and sexual dysfunctions and act as carcinogenic agent (Furuya et al., 2006; Stowell et al., 2006). BPA could also affect the neuroendocrine, behavioral and cognitive functions of individuals, as well as increasing the risk for cardiovascular disease, miscarriage and diabetes (Sugiura-Ogasawara et al., 2005; Takayanagi et al., 2006).

The production of BPA, one of the most produced chemicals, is higher than 3.2 million tons per year (Environmental Protection Agency, 2005). BPA is mainly used for the manufacturing of polycarbonate plastics and epoxy resins (Rivero et al., 2014; Plastics Europe, 2010; Geens et al., 2011), including plastic bottles, food cans, drinking and food containers, composite dental fillings and adhesives (Staples et al., 1998; Chiang et al., 2004; Guo and Ge, 2010).

As a result of its widespread use, BPA is often detected in wastewater treatment plants (WWTPs) (Melcer and Klečka, 2011; Guerra et al., 2015). Wastewater containing BPA is a serious threat to aquatic environment due to its extensive use, toxic properties and persistence since this compound is not readily biodegradable (Kang et al., 2006; Talsness et al., 2009; Viecelli et al., 2011; Limam et al., 2013). However, there are few studies analyzing its influence on the biological processes of the different wastewater treatment technologies (Stasinakis et al., 2008).

Membrane bioreactor (MBR) system constitutes a satisfactory treatment over conventional technologies; it can operate at higher biomass

concentration and sludge retention time (SRT), improving the effluent quality and disinfection capability, as well as reducing the footprint (Leyva-Díaz et al., 2014). To the best of our knowledge, there are no studies in the literature that report on the influence of BPA on the heterotrophic biomass concerning the organic matter removal that is carried out in an MBR system (Zielińska et al., 2014). Thus, it is necessary to widen knowledge of the effect of BPA on the biomass in relation to the organic matter removal and heterotrophic kinetics within the MBR. In this regard, it should be highlighted that the great difference between this study and other works consists of the fact that the present work has assessed the performance of the heterotrophic biomass of an MBR system under the presence of BPA, evaluating rates of organic matter removal, decay and biomass generation, and other studies have analyzed the kinetics and biodegradability of BPA in biological systems (Stasinakis et al., 2008) and oxidation processes (Rivero et al., 2014), or investigated the removal of BPA in different wastewater treatment processes (Dorival-García et al., 2014; Zielińska et al., 2014; Guerra et al., 2015).

Respirometric method has been widely applied to model the microbial kinetics for wastewater treatment systems due to its high reproducibility and accuracy (Leyva-Díaz et al., 2013; Leyva-Díaz et al., 2017). In light of this, kinetic modeling predicts the organic substrate degradation rate under the influence of BPA, and constitutes a useful tool for designing and optimizing the biological process. Additionally, this information could be useful for the implementation and operation of MBR to treat wastewater containing BPA with a higher efficiency.

The aim of this study is to analyze the effect of BPA on the heterotrophic biomass of an MBR system through the realization of respirometric tests that allow the assessment of its kinetic modeling. These experiments were carried out to evaluate the possible influence of shock additions of BPA on heterotrophic microorganisms in a respirometer with the objective of simulating an intrusion of BPA into an MBR system and evaluating the adaptive capacity of the biomass through possible changes in the rates of organic matter removal, decay and biomass generation.

3. Materials and methods

3.1. Bisphenol A and sodium acetate solutions

BPA was obtained from Sigma-Aldrich Co. (St. Louis, MO 63103 USA). A synthetic solution of BPA (97 %, CAS No: 80-05-7, MW: 228.29 g/mol) was prepared by dissolving 20 mg of this compound in 2 mL of methanol HPLC grade (Merck, Germany) (Dorival-García et al., 2014; Stasinakis et al., 2008). This solution was added to the mixed liquor to get a concentration of 20 mg/L of BPA in the respirometric assays.

Sodium acetate anhydrous was provided by AppliChem GmbH (Darmstadt, 64291 Germany). A stock solution of sodium acetate (99 %, CAS No: 127-09-3, MW: 82.03 g/mol) of 500 mg/L and three dilutions of 50, 80 and 100 % from this solution were prepared to evaluate the kinetic behavior of heterotrophic biomass. The sodium acetate concentration was expressed as chemical oxygen demand (COD) (Leyva-Díaz et al., 2013).

3.2. Description of the pilot plant of membrane bioreactor

Figure 5 shows the schematic diagram of the MBR system analyzed in this work. This pilot plant was located in Los Vados Wastewater Treatment Plant (WWTP) in Granada (Spain). MBR consisted of an aerated cylindrical bioreactor of 272 L, with an external rectangular unit of 78 L that had four submerged hollow-fiber ultrafiltration membrane modules (ZW-10, ZENON®) with a nominal pore size of 0.04 μm. Each membrane module had an area of 0.93 m^2. The bioreactor was fed with municipal wastewater coming from the primary settling tank of Los Vados WWTP. The operation mode for the membrane modules was cyclic by combining filtration and backwashing periods of 9.67 min and 0.33 min, respectively. The filtration through the membrane was carried out from the outside to the inner side by sucking. The recirculation from the membrane tank to the bioreactor allowed to maintain the working mixed liquor suspended solids (MLSS) concentration, and the waste sludge was purged from the system.

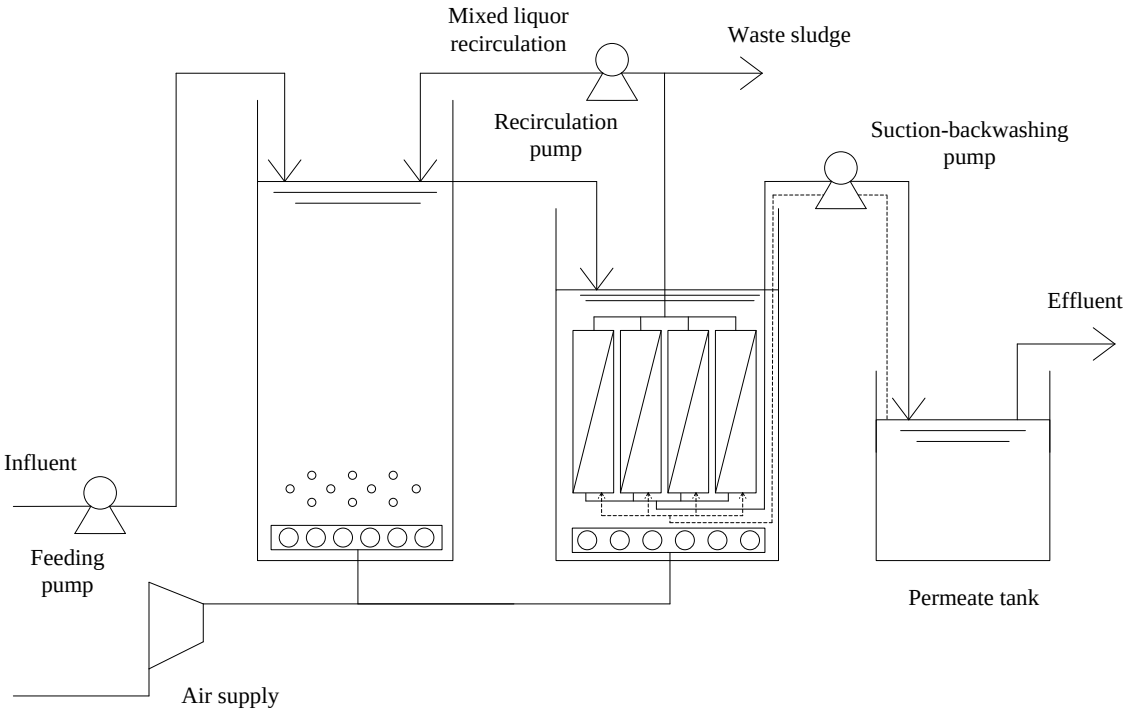

Figure 5. *Schematic diagram of the pilot plant of MBR for municipal wastewater treatment used in the study*

3.3. Operation conditions and activated sludge samples

Four operation phases were studied by varying the hydraulic retention time (HRT) (6 h and 10 h), and the MLSS concentration (4,000 mg/L and 7,000 mg/L). **Table 2** shows the values of HRT, MLSS, temperature and sludge retention time (SRT) of the different operation phases.

Table 2. *Operation conditions of HRT, MLSS, T and SRT for the steady state of the four operation phases. HRT (hydraulic retention time), MLSS (mixed liquor suspended solids), T (temperature), SRT (sludge retention time).*

Operation phase	HRT (h)	MLSS (mg/L)	T (ºC)	SRT (day)
1	6	4,000	18.6	9.81
2	10	4,000	31.1	16.87
3	6	7,000	20.8	11.23
4	10	7,000	12.1	21.67

Activated sludge samples were collected from the pilot plant of MBR during the steady state of the four operation phases. Two liters of mixed liquor were withdrawn from the bioreactor for each respirometric assay. The activated sludge was preconditioned by aerating it for 18 h at constant temperature of 20 °C to reach endogenous conditions in which any kind of substrate contained in the sample is consumed (Leyva-Díaz et al., 2013).

3.4. Kinetic modeling

The influence of BPA on the heterotrophic biomass was analyzed during the steady state of the four operation phases of the MBR. In light of this, heterotrophic kinetics was evaluated for each one of the stationary phases of MBR operation. The experiments were carried out to determine

the effect of shock additions of BPA on the performance of the heterotrophic biomass.

In this regard, the following kinetic parameters were evaluated through two different respirometric tests. The exogenous respiration experiment was performed to assess the maximum specific growth rate for heterotrophic biomass ($\mu_{m,H}$), half-saturation coefficient for organic matter (K_M) and yield coefficient for heterotrophic biomass (Y_H). The endogenous respiration test was carried out to assess the decay coefficient for heterotrophic biomass (b_H). The kinetic parameters for heterotrophic bacteria, as well as the substrate degradation rate for organic matter removal ($r_{su,H}$), in absence and presence of BPA, were evaluated through a respirometric method according to Leyva-Díaz et al. (Leyva-Díaz et al., 2013).

Once endogenous conditions were reached by the activated sludge sample of two liters, as explained in section 3.3, one liter of mixed liquor was transferred to the BMAdvance Respirometer to carry out the exogenous respiration test. The respirometer worked at 20.0±0.1 °C of temperature,

7.25±0.50 of pH, 0.906±0.001 L/min of air flow rate and 2,000 rpm of stirring rate. Apart from the mechanical stirring, a recirculation from the bottom to the top of the respirometer was carried out by a peristaltic pump in order to favor the homogenization of the mixed liquor. As indicated in section 3.1, a stock solution of sodium acetate at different dilutions was used to evaluate the heterotrophic kinetic parameters. When the basis line of dissolved oxygen (DO) was stabilized, the dynamic oxygen uptake rate (R_S) was registered for the three additions of sodium acetate, as shown in **Figure 6** for the operation phases 1, 2, 3 and 4 (Leyva-Díaz et al., 2013). The values of COD for the three additions of sodium acetate, and the MLSS and mixed liquor volatile suspended solids (MLVSS) concentrations were calculated from standard methods (APHA, 2012). The concentration of heterotrophic biomass (X_H) was evaluated by applying the fraction of heterotrophic biomass, which was estimated according to Metcalf (Metcalf and Eddy, 2003), to the MLVSS concentration.

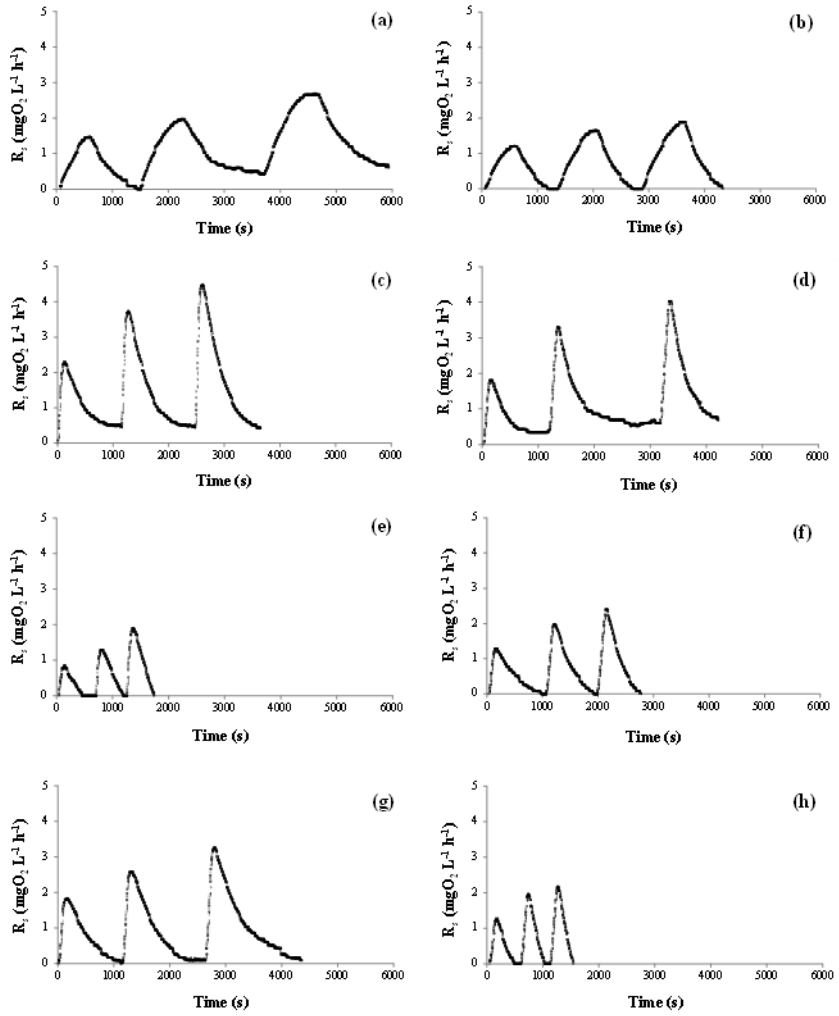

Figure 6. *Evolution of the dynamic oxygen uptake rate (R_s) in the respirometric experiments in absence and presence of bisphenol A for the determination of the kinetic parameters. (a) Phase 1 without BPA. (b) Phase 1 with BPA. (c) Phase 2 without BPA. (d) Phase 2 with BPA. (e) Phase 3 without BPA. (f) Phase 3 with BPA. (g) Phase 4 without BPA. (h) Phase 4 with BPA. (Phase 1: HRT=6 h, MLSS=4,000 mg/L, T=18.6 ºC, SRT=9.81 day; Phase 2: HRT=10 h, MLSS=4,000 mg/L, T=31.1 ºC, SRT=16.87 day; Phase 3: HRT=6 h, MLSS=7,000 mg/L, T=20.8 ºC, SRT=11.23 day; Phase 4: HRT=10 h, MLSS=7,000 mg/L, T=12.1 ºC, SRT=21.67 day).*

After this respiration test, the endogenous respirometric assay was carried out by leaving without aeration the mixed liquor. The evolution of

the static oxygen uptake rate (0UR) is shown in **Figure 7** for the different operation conditions.

In this way, the maximum specific growth rate for heterotrophic biomass in absence of BPA ($\mu_{m,H,n/BPA}$), half-saturation coefficient for organic matter in absence of BPA ($K_{M,n/BPA}$), yield coefficient for heterotrophic biomass in absence of BPA ($Y_{H,n/BPA}$) and decay coefficient for heterotrophic biomass in absence of BPA ($b_{H,n/BPA}$) were evaluated. Furthermore, the substrate degradation rate for organic matter removal in absence of BPA was also assessed ($r_{su,H,n/BPA}$).

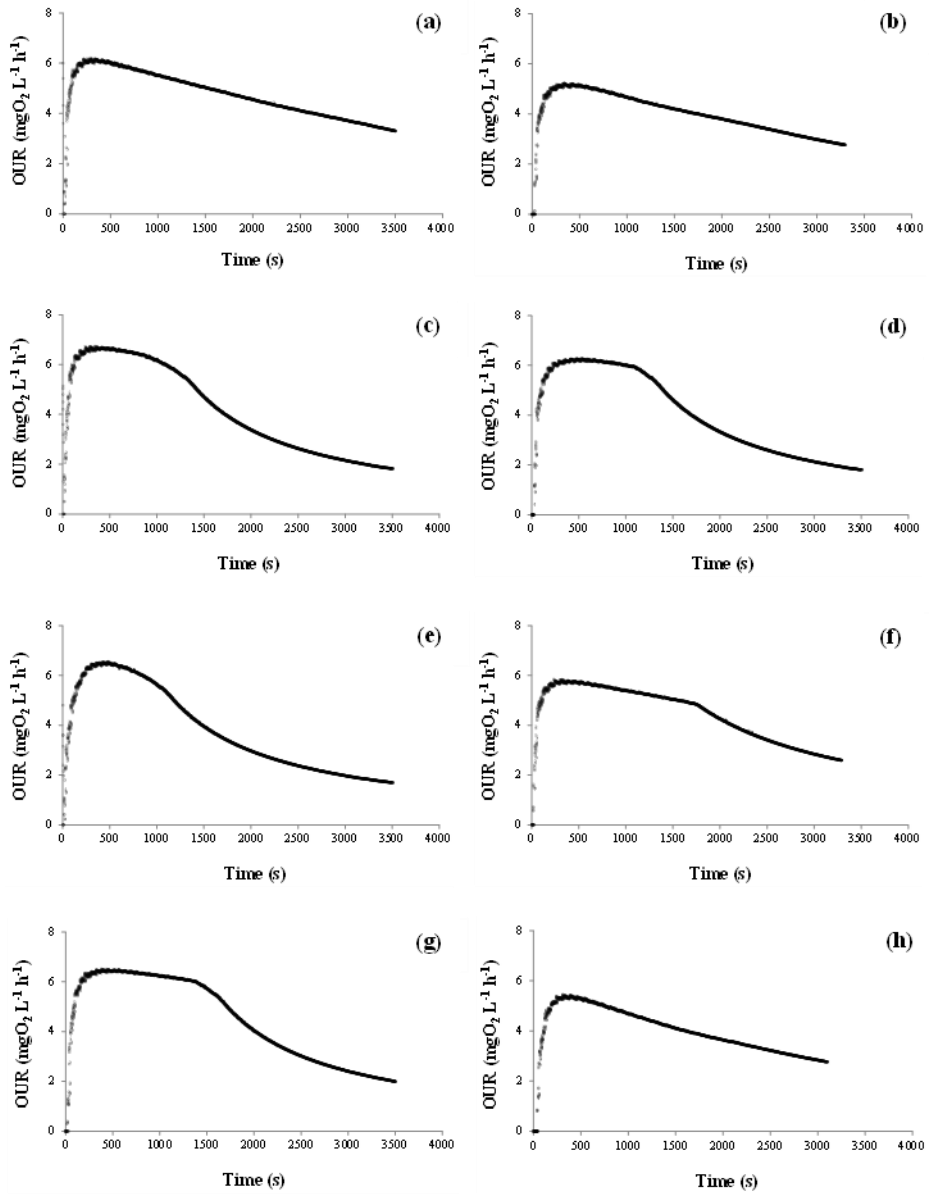

Figure 7. *Evolution of the static oxygen uptake rate (OUR) in the respirometric experiments in absence and presence of bisphenol A for the determination of the kinetic parameters. (a) Phase 1 without BPA. (b) Phase 1 with BPA. (c) Phase 2 without BPA. (d) Phase 2 with BPA. (e) Phase 3 without BPA. (f) Phase 3 with BPA. (g) Phase 4 without BPA. (h) Phase 4 with BPA. (Phase 1: HRT=6 h, MLSS=4,000 mg/L, T=18.6 ºC, SRT=9.81 day; Phase 2: HRT=10 h, MLSS=4,000 mg/L, T=31.1 ºC, SRT=16.87 day; Phase 3: HRT=6 h, MLSS=7,000 mg/L, T=20.8 ºC, SRT=11.23 day; Phase 4: HRT=10 h, MLSS=7,000 mg/L, T=12.1 ºC, SRT=21.67 day).*

Having evaluated the heterotrophic kinetics in absence of BPA, the remaining liter of activated sludge sample was transferred to the BM-Advance Respirometer. A solution of BPA was prepared, as indicated in section 3.1, and added to the respirometer. The exogenous respiration test in presence of BPA was initiated when the basis line of DO was stabilized after the addition of BPA. This test was carried out in a similar way to that previously explained for heterotrophic kinetics in absence of BPA. The evolution of R_S in presence of BPA is shown in **Figure 6** for the operation phases 1, 2, 3 and 4. After the finalization of this test, the endogenous respiration experiment was performed to determine the effect of BPA on the OUR (**Figure 7**).

In light of this, the maximum specific growth rate for heterotrophic biomass in presence of BPA ($\mu_{m,H,BPA}$), half-saturation coefficient for organic matter in presence of BPA ($K_{M,BPA}$), yield coefficient for heterotrophic biomass in presence of BPA ($Y_{H,BPA}$) and decay coefficient for heterotrophic biomass in presence of BPA ($b_{H,BPA}$) were evaluated. Furthermore, the substrate degradation rate for organic matter removal in presence of BPA was also assessed ($r_{su,H,BPA}$).

The heterotrophic kinetic parameters, in absence or presence of BPA, were estimated as follows:

1) Numerical integration of R_S to calculate the oxygen consumption (OC) for each addition of sodium acetate, as indicated in Eq. (1):

$$OC = \int_{t_i}^{t_f} R_S \, dt \quad (mgO_2L^{-1}) \tag{1}$$

2) Application of Helle equation (Helle, 1999), Eq. (2), to estimate Y_H:

$$Y_H = \frac{S - OC}{S \cdot f_{CV}} \quad (mgVSS\, mgCOD^{-1}) \tag{2}$$

where S is the substrate concentration (mgO_2/L) and f_{CV} is a conversion factor (1.48 mgCOD/mgVSS).

3) Determination of the empirical specific growth rate (μ_{emp}) from the relation between the biomass growth rate and substrate degradation rate, according to Eq. (3):

$$\mu_{emp} = \frac{Y_H \cdot R_S}{(1 - Y_H \cdot f_{CV}) \cdot X_H} \quad (h^{-1}) \tag{3}$$

where X_H is measured as (mgVSS/L).

4) Linearization of the Monod model to assess $\mu_{m,H}$ and K_M (Monod, 1949), as observed in Eq. (4):

$$\frac{1}{\mu_{emp}} = \frac{1}{\mu_{m,H}} + \frac{K_{M,H}}{\mu_{m,H}} \cdot \frac{1}{S} \quad (h) \tag{4}$$

5) Application of Ekama equation (Ekama et al., 1986), Eq. (5), to estimate b_H:

$$b_H = \frac{OUR_{end}}{1.42 \cdot X_T \cdot \left[1 - Y_H \cdot \left(1 - f_p\right)\right]} \quad (day^{-1}) \tag{5}$$

where $0UR_{end}$ is the endogenous oxygen uptake rate ($mgO_2/(L \cdot h)$), X_T is the total biomass concentration (mgTSS/L) and $(1 - f_p)$ is the fraction of volatile biomass (mgVSS/mgTSS).

6) Application of Metcalf equation (Metcalf and Eddy, 2003), Eq. (6), to evaluate the kinetic parameters at working temperature:

$$r_T = r_{20} \cdot \theta^{(T-20)} \tag{6}$$

where r_T and r_{20} symbolize the kinetic parameters at working temperature and 20 °C, respectively, θ is a fitting parameter with a value of 1.04 for MBR, and T is the working temperature.

7) Evaluation of $r_{su,H}$ depending on Y_H, $\mu_{m,H}$, and K_M, as well as the substrate and biomass concentrations, as shown in Eq. (7):

$$r_{su,H} = \frac{\mu_{m,H} \cdot S \cdot X_H}{Y_H \cdot (K_M + S)} \quad (7)$$

8) Assessment of net heterotrophic biomass growth rate ($r'_{x,H}$) based on the biomass growth rate and biomass decay rate, as indicated in Eq. (8):

$$r'_{x,H} = \frac{\mu_{m,H} \cdot S}{K_M + S} \cdot X_H - b_H \cdot X_H \quad (8)$$

3.5. Statistical analysis

The effect of the environmental variables HRT, MLVSS, T and SRT on the species data $r_{su,n/BPA}$, $r_{su,BPA}$, $b_{H,n/BPA}$ and $b_{H,BPA}$ was evaluated by a multivariable statistical analysis applying the software Canoco for Windows v. 4.5 (ScientiaPro, Budapest, Hungary).

This software also allowed to determine the variables which had the highest influence on the different heterotrophic biomasses analyzed (Smilauer and Lepš, 2016). After the verification of the linear distribution of the model, provided by the detrended correspondence analysis (DCA), a

redundancy analysis (RDA) was carried out as statistical method recommended by Lepš and Šmilauer (Smilauer and Lepš, 2016). Monte Carlo test with 499 permutations was used with a significance level of 0.05.

4. Results and discussion

4.1. Dynamic and static oxygen uptake rates: R_S and OUR

The exogenous respiration tests for the four operation phases of the MBR system are included in **Figure 6**. The respirometric assays in absence of BPA had a higher duration than those obtained in presence of this compound for the operation phases 1 and 4 (**Figure 6**a-b, **Figure 6**g-h). Specifically, the exogenous respirometric tests lasted for 5,938 s and 4,350 s in absence of BPA for phases 1 and 4, respectively, and these experiments lasted for 4,325 s and 1,544 s in presence of BPA for phases 1 and 4, respectively. However, in phases 2 and 3, the presence of BPA increased the duration of the respirometric test, 4,214 s and 2,772 s, respectively, in relation to the experiments without BPA, 3,650 s and 1,743 s, respectively. Therefore, the presence of BPA at lower temperatures, 18.6 °C for phase 1 and 12.1 °C for phase 4 (**Table 2**), decreased the duration of the respirometric test; the

operation at higher temperatures in presence of BPA, 31.1 °C for phase 2 and 20.8 °C for phase 3 (**Table 2**), increased the time required by heterotrophic biomass to degrade the organic matter substrate.

In general, the BPA reduced the three maximum values of R_S of each respirometric test. This occurred in all operation phases with the exception of phase 3, which could be due to the fact that working at high biomass concentration (7,000 mg/L) compensated the effect of BPA for this phase.

It should be highlighted that, independently of the presence of BPA, the duration of the respirometric assays decreased from phase 1 to phase 3. This was probably due to the fact that phase 2 worked at more favorable operation conditions (HRT = 10 h, T = 31.1 °C and SRT = 16.87 day) than phase 1 (HRT = 6 h, T = 18.6 °C and SRT = 9.81 day), as indicated in **Table 2**. The reduction of duration from phase 2 to phase 3 could be based on the higher biomass concentration corresponding to phase 3 (7,000 mg/L) compared with phase 2 (4,000 mg/L), as shown in **Table 2**. However, the lowest operation temperature (12.1 °C) of phase 4 cancelled out the most

favorable operation conditions regarding HRT (10 h), MLSS (7,000 mg/L) and SRT (21.67 day) (**Table 2**).

Figure 7 shows the endogenous respiration tests for the four operation phases of the MBR system. It should be noted that the presence of BPA reduced the maximum value of OUR, i.e. the endogenous oxygen uptake rate (OUR_{end}), in all operation phases. OUR_{end} was reduced from 6.099 to 5.147 $mgO_2/(L \cdot h)$ for phase 1, from 6.670 to 6.222 $mgO_2/(L \cdot h)$ for phase 2, from 6.501 to 5.764 $mgO_2/(L \cdot h)$ for phase 3, and from 6.453 to 5.364 $mgO_2/(L \cdot h)$ for phase 4.

The different trends for R_S and OUR were considered in order to evaluate the heterotrophic kinetic parameters through the mathematical procedure that was described by the Eqs. (1) to (8), as indicated in section 3.4.

4.2. Heterotrophic kinetic modeling

The kinetic parameters for heterotrophic biomass in absence and presence of BPA at the different operation conditions are indicated in **Table 3**.

Table 3. *Kinetic parameters for the characterization of heterotrophic biomass in absence and presence of bisphenol A (BPA) for the four operation phases of the MBR system. $Y_{H,n/BPA}$ (yield coefficient for heterotrophic biomass in absence of BPA), $\mu_{m,H,n/BPA}$ (maximum specific growth rate for heterotrophic biomass in absence of BPA), $K_{M,n/BPA}$ (half-saturation coefficient for organic matter in absence of BPA), $b_{H,n/BPA}$ (decay coefficient for heterotrophic biomass in absence of BPA), $Y_{H,BPA}$ (yield coefficient for heterotrophic biomass in presence of BPA), $\mu_{m,H,BPA}$ (maximum specific growth rate for heterotrophic biomass in presence of BPA), $K_{M,BPA}$ (half-saturation coefficient for organic matter in presence of BPA), $b_{H,BPA}$ (decay coefficient for heterotrophic biomass in presence of BPA).*

Parameter	Operation phase			
	1	2	3	4
Absence of BPA				
$Y_{H,n/BPA}$ (mg VSS/mg COD)	0.5486±0.0672	0.9423±0.0918	0.6809±0.0732	0.4447±0.0544
$\mu_{m,H,n/BPA}$ (h^{-1})	0.0052±0.0004	0.1398±0.0127	0.0239±0.0030	0.0147±0.0016
$K_{M,n/BPA}$ (mg O_2/L)	2.4166±0.2109	49.2654±3.7686	6.1931±0.5486	10.7284±0.9816
$b_{H,n/BPA}$ (day^{-1})	0.0752±0.0089	0.1358±0.0098	0.0924±0.0099	0.0389±0.0019
Presence of BPA				
$Y_{H,BPA}$ (mg VSS/mg COD)	0.5776±0.0581	0.9676±0.0797	0.6625±0.0611	0.4801±0.0618
$\mu_{m,H,BPA}$ (h^{-1})	0.0108±0.0015	0.2621±0.0211	0.0299±0.0017	0.0193±0.0025
$K_{M,BPA}$ (mg O_2/L)	5.1695±0.3496	80.1991±6.3125	14.9616±1.0986	7.2516±0.6482
$b_{H,BPA}$ (day^{-1})	0.0715±0.0058	0.1304±0.0106	0.0871±0.0065	0.0354±0.0027

The amount of hetrotrophic biomass produced per substrate oxidized was higher in presence of BPA ($Y_{H,BPA}$) than in absence of BPA ($Y_{H,n/BPA}$), with the exception of phase 3 (**Table 3**). Specifically, the increase of $Y_{H,n/BPA}$ values was 2.61-7.37 %, for phase 2 and phase 4, respectively, higher in presence of BPA.

Regarding the maximum specific growth rate, the values of $\mu_{m,H,BPA}$, in presence of BPA, were higher than those obtained in absence of BPA ($\mu_{m,H,n/BPA}$), according to **Table 3**. The values of $\mu_{m,H,BPA}$ exceeded those obtained without BPA in 51.85 % for phase 1, 46.66 % in phase 2, 20.07 % in phase 3 and 23.83 % in phase 4. This implied that the heterotrophic biomass required less time to oxidize organic matter in presence of BPA for all operation conditions (**Table 2**). The same trend could be observed for $K_{M,BPA}$ and $K_{M,n/BPA}$ in **Table 3**. In this regard, the values of $K_{M,BPA}$ were higher than $K_{M,n/BPA}$, with the exception of phase 4, probably due to the operation at the lowest temperature (12.1 °C). The increase percentages of $K_{M,BPA}$ in relation to $K_{M,n/BPA}$ were 53.25 % for phase 1, 38.57 % for phase 2 and 58.61 % for phase 3.

The effect of these variations in kinetic parameters that characterize the heterotrophic biomass in the MBR system are included in the values of $r_{su,H,n/BPA}$ and $r_{su,H,BPA}$. In this regard, **Figure 8**a shows the values of $r_{su,H,n/BPA}$ and $r_{su,H,BPA}$ in absence and presence of BPA, respectively, for the four operation phases. It must be highlighted that the substrate degradation rate was increased in presence of BPA, with increases of 46.51 % for phase

1, 24.39 % for phase 2, 12.68 % for phase 3 and 21.95 % for phase 4. Therefore, organic matter was degraded faster in presence of BPA than in absence of this compound. Heterotrophic biomass corresponding to phase 2 had the highest $r_{su,H}$ regardless of the presence of BPA, which could be due to the highest operation temperature (31.1 °C) and high HRT (10 h), according to **Table 2**. The values of $r_{su,H}$ for phase 3 were lower than those obtained in phase 2 since the temperature, HRT and SRT were also lower, i.e. 20.8 °C, 6 h and 11.23 day, respectively, in spite of its higher value of MLSS (**Table 2**). Heterotrophic biomass subjected to the operation conditions of phase 4 showed lower values of $r_{su,H}$ than those corresponding to phase 3, probably due to its lower temperature (12.1 °C). However, these values were higher than those obtained in phase 1 despite the fact that the temperature of phase 1 was higher (18.6 °C). This could be due to the more favorable operation conditions of phase 4 regarding HRT (10 h), MLSS (7,000 mg/L) and SRT (21.67 day) in relation to those characterizing phase 1 (HRT = 6 h, MLSS = 4,000 mg/L, SRT = 9.81 day).

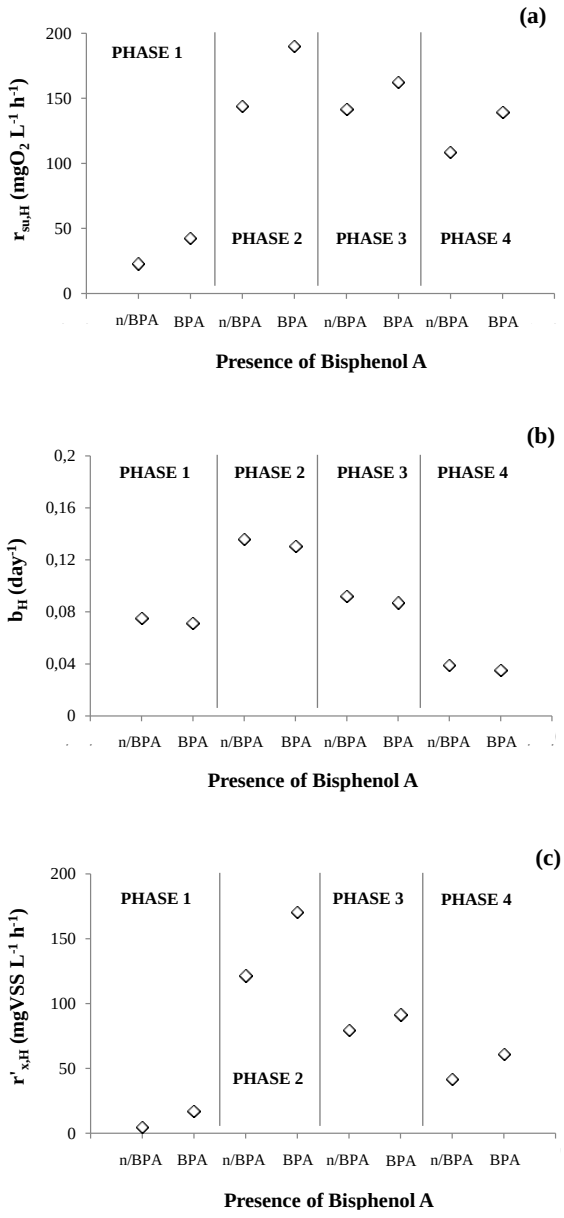

Figure 8. *Substrate degradation rate for organic matter removal ($r_{su,H}$) (a), decay coefficient for heterotrophic biomass (b_H) (b), and net heterotrophic biomass growth rate ($r'_{x,H}$) (c) depending on the presence of bisphenol A for the four operation phases (Phase 1: HRT=6 h, MLSS=4,000 mg/L, T=18.6 ºC, SRT=9.81 day; Phase 2: HRT=10 h, MLSS=4,000 mg/L, T=31.1 ºC, SRT=16.87 day; Phase 3: HRT=6 h, MLSS=7,000 mg/L, T=20.8 ºC, SRT=11.23 day; Phase 4: HRT=10 h, MLSS=7,000 mg/L, T=12.1 ºC, SRT=21.67 day). For the horizontal axis, "n/BPA" means absence of BPA and "BPA" means presence of BPA.*

Figure 8b shows the values of b_H in absence and presence of BPA. The decay rate for heterotrophic biomass was higher in phase 2 due to its higher temperature (31.1 °C), followed by phase 3 (20.8 °C), phase 1 (18.6 °C) and phase 4 (12.1 °C). It must be pointed out that the presence of BPA reduced the biomass decay rate for heterotrophic biomass as $b_{H,BPA}$ values were lower than $b_{H,n/BPA}$, with reduction percentages of 4.84 % for phase 1, 3.91 % for phase 2, 5.67 % for phase 3 and 9.17 % for phase 4 (**Table 3**). This implied lower quantity of biomass oxidized per day in presence of BPA. This was supported by the lowest values for OUR_{end}, as shown in **Figure 7**.

Ferro Orozco et al. analyzed the kinetic behavior of BPA-acclimated activated sludge for the degradation of BPA in an aerobic laboratory-scale (4.5 L) activated sludge reactor with partial biomass recirculation that operated at HRT of 48 h, SRT of 30 day, temperature of 20 °C and MLSS ranging from 3,700 to 4,500 mgTSS/L (Ferro Orozco et al., 2016). These authors obtained values of μ_m of 0.061 h^{-1}, b_H of 0.06 day^{-1} and Y_H of 0.57, which were in the ranges shown in this research (**Table 3**), i.e. 0.0108-0.2621 h^{-1} for $\mu_{m,H,BPA}$, 0.0354-0.1304 day^{-1} for $b_{H,BPA}$, and they were lower for Y_H (0.7105-1.4320, as a result of multiplying $Y_{H,BPA}$ by f_{CV}).

Figure 8c shows the values of $r'_{x,H}$ in absence and presence of BPA, with a similar trend to that observed for $r_{su,H}$. The highest biomass growth rate occurred in phase 2, which showed the highest values of HRT and T (**Table 2**), followed by phase 3, phase 4 and, finally, phase 1. Thus, at highest temperature (phase 2), $r'_{x,H}$ had the highest value and more organic matter was oxidized to CO_2. The net cell growth rate for heterotrophic biomass increased in presence of BPA, i.e. 72.48 % for phase 1, 28.75 % for phase 2, 13.11 % for phase 3 and 31.25 % for phase 4, which corroborated the highest values for Y_H in presence of BPA.

In this regard, Stasinakis et al. studied the biodegradation of BPA in presence of a readily biodegradable compound as sodium acetate by respirometric tests (Stasinakis et al., 2008). These authors observed that there was no inhibitory effect of BPA on heterotrophic microorganisms, concluding that a simultaneous detoxification process occurred in its biodegradation. This supported the results obtained in the present work, with higher values of $r_{su,H}$ and $r'_{x,H}$, and lower values of b_H in presence of BPA.

Dorival-García et al. studied an MBR pilot plant with a total bioreactor volume of 474 L, HRT of 12 h, SRT of 32 day and MLSS varying between 8,500 and 9,300 mg/L (Dorival-García et al., 2014). They added 893 mgBPA day^{-1} and this working concentration of BPA had no acute toxic effects and did not interfere with the biological activity in the bioreactor, which also supported the behavior of heterotrophic biomass corresponding to the present work (**Figure 8**). In this regard, the slight toxicity of BPA on bacteria was also supported by other authors (Groshart et al., 2015; Zhang et al., 2007). According to Dorival-García et al., the biomass acclimatization to BPA improved its biodegradation potential, assuming an increase of specific heterotrophic biomass capable of degrading BPA (Dorival-García et al., 2014). This could contribute to the increase of $r'_{x,H}$ that was observed in the present study. Chen et al. found that the higher diversity of microorganisms in an MBR system facilitated the acclimating procedure to BPA in relation to a conventional activated sludge reactor, allowing bacteria in MBR to biodegrade BPA (Chen et al., 2008; Luxmy et al., 2000).

4.3. Multivariate statistical analysis

The results of the multivariable statistical analysis for organic matter removal in absence and presence of BPA are observed in **Figure 9**. The triplot diagram for the MBR system shows the effect of HRT, MLVSS, T and SRT (variables) on $r_{su,H,n/BPA}$, $r_{su,H,BPA}$, $b_{H,n/BPA}$ and $b_{H,BPA}$ (species). The interactions between the different operation conditions, such as the influence of temperature on SRT, and the effect of them on the response of the system could be analyzed through the use of Canoco software. In this regard, bearing in mind the length and the angles between the different vectors represented in **Figure 9**, the influence of each variable can be independently analyzed on the different species.

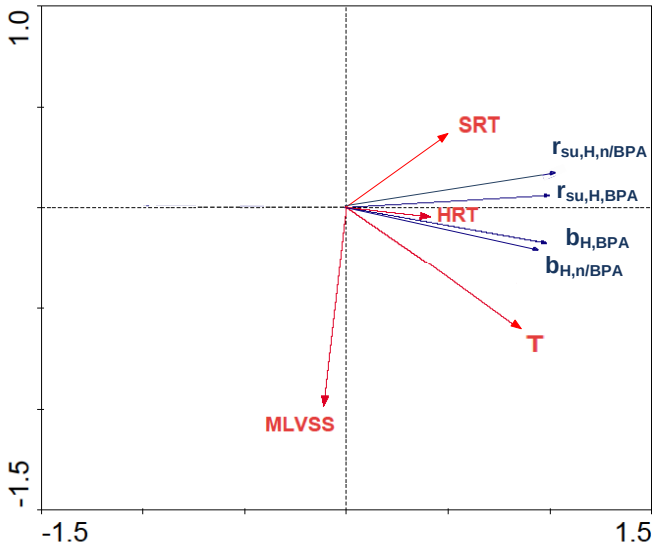

Figure 9. *Triplot diagram for RDA of the substrate degradation rate for organic matter removal in absence and presence of bisphenol A (BPA),* $r_{su,n/BPA}$ *and* $r_{su,BPA}$*, respectively, and decay coefficient for heterotrophic biomass in absence and presence of BPA,* $b_{H,n/BPA}$ *and* $b_{H,BPA}$*, respectively, in relation to the variables HRT, MLSS, T and SRT in the membrane bioreactor (MBR) system. RDA (redundancy analysis), HRT (hydraulic retention time), MLSS (mixed liquor suspended solids), T (temperature), SRT (sludge retention time).*

Regarding the temperature, this variable presented a positive correlation with the substrate degradation rate for organic matter removal and the decay coefficient for heterotrophic biomass in absence and presence of BPA ($r_{su,H,n/BPA}$, $r_{su,H,BPA}$, $b_{H,n/BPA}$ and $b_{H,BPA}$). Moreover, temperature showed a higher influence on the species of the system than the rest of variables due to the higher length of its vector. In general, the higher the temperature was, the higher the $r_{su,H}$, b_H and $r'_{x,H}$ were, as shown in **Figure 8**.

The HRT and SRT had also a positive correlation with the $r_{su,H,n/BPA}$, $r_{su,H,BPA}$, $b_{H,n/BPA}$ and $b_{H,BPA}$ although their effect on the species was lower than in the case of temperature due to the lower length of their vectors. This supported that the values of $r_{su,H}$ and $r'_{x,H}$ were higher for phase 4 than those obtained for phase 1 in spite of the lower value of T (12.1 °C), whose effect was cancelled out by the HRT and SRT. Finally, the MLVSS concentration had almost no influence on the $r_{su,H}$ and b_H since the angles between these vectors were of approximately 90º. This explained that the values of $r_{su,H}$, b_H and $r'_{x,H}$ for phase 2 (MLSS = 4,000 mg/L) were higher than those corresponding to phases 3 and 4 (MLSS = 7,000 mg/L), according to **Figure 8**, prevailing the effect of T, HRT and SRT.

5. Conclusions

Based on the kinetic results in presence of bisphenol A (BPA) obtained in a respirometer for the heterotrophic biomass contained in a membrane bioreactor (MBR) with the aim of simulating an intrusion of BPA into an MBR system at four different operation phases, the following conclusions were drawn:

- The substrate degradation rate for organic matter removal ($r_{su,H}$) increased in presence of BPA, with rising percentages of 46.51 % for phase 1, 24.39 % for phase 2, 12.68 % for phase 3 and 21.95 % for phase 4, which supposed a consumption of organic matter faster than in absence of BPA. Heterotrophic biomass belonging to operation phase 2 (HRT = 10 h, T = 31.1 °C) showed the highest $r_{su,H}$ in absence of BPA (143.84 $mgO_2/(L \cdot h)$) and in presence of BPA (190.22 $mgO_2/(L \cdot h)$). A similar trend was observed for the net heterotrophic biomass growth rate ($r'_{x,H}$).
- The presence of BPA decreased the decay rate for heterotrophic biomass, implying lower quantities of biomass oxidized per day, with values of 0.0715 day^{-1} for phase 1, 0.1304 day^{-1} for phase 2, 0.0871 day^{-1} for phase 3 and 0.0354 day^{-1} for phase 4.

In a nutshell, the presence of BPA increased the substrate degradation rate for organic matter removal and the net heterotrophic biomass growth rate, and decreased the decay rate for heterotrophic biomass. Thus, the heterotrophic biomass of the MBR was not inhibited by the presence of BPA, showing an adaptive capacity to enhance the rates of organic matter removal

and biomass generation, and to reduce the decay rate. The temperature was the variable with the highest influence on the rates of organic matter removal, decay and biomass generation.

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VI - CHAPTER 3

Impact of ciprofloxacin, carbamazepine and ibuprofen on a membrane bioreactor system: Kinetic study and biodegradation capacity.

VI - CHAPTER 3

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1. Abstract

This study analyzed the effects of carbamazepine, ciprofloxacin and ibuprofen on the behavior of a membrane bioreactor (MBR) system treating urban wastewater that was doped continuously with three different concentrations of this mix of pharmaceuticals under a hydraulic retention time of 6 h.

The degradation capacity of these chemicals and the heterotrophic kinetics regarding the organic matter removal were evaluated in the control and doping cycles.

Although the MLSS decreased drastically, these differences were not observed in the organic matter removal as the increase of cell growth rate cancelled out the increase in cell decay rate due to the chemical stress caused by the addition of pharmaceuticals, as shown by the increased organic matter degradation rate from 86.27 $mgO_2/(L \cdot h)$ in the control cycle to values within the limits 183.97 $mgO_2/(L \cdot h)$ and 192.88 $mgO_2/(L \cdot h)$ in doping cycles. The degradation rates were 0.0154, 0.0152 and 0.0160 $\mu gS/((\mu gS_{in}/L) \cdot h \cdot mgMLSS)$ for carbamazepine, ciprofloxacin and ibuprofen, respectively, involving removal yields higher than 71.9, 88.7 and 94.7 % for carbamazepine, ciprofloxacin and ibuprofen, respectively, at the three different concentrations tested. Therefore, MBR technology can be used as a reliable process to significantly reduce this mix of pharmaceuticals without reducing its organic matter removal capacity.

2. Introduction

The occurrence and fate of pharmaceutical residues in wastewater treatment and the environment has attracted increasing interest during the last years (Quintana et al., 2005). Although pharmaceuticals have been present in water for decades, their levels in the environment have only recently begun to be quantified and acknowledged as potentially hazardous to ecosystems (Kolpin et al., 2002; Fent et al., 2006; Jjemba, 2006). However, emerging substances have recently been detected in water resources worldwide, raising human and environmental health concerns (Hamza et al., 2016). Several studies in Europe and the United States have indicated that many of these compounds are present in the effluents of wastewater treatment plants, surface water, and groundwater (Puijjker and Mons, 2004). This becomes a new concern which must be considered in the design of new wastewater treatment plants (WWTPs) and in already existing ones.

According to Stuart et al., the primary entry routes for pharmaceuticals into the environment include human excretion, veterinary usage and the disposal of unused products (Stuart et al., 2012). It is estimated that 30–90 %

of antibiotics used in veterinary medicine are excreted from the bodies of animals as active substances (Chen et al., 2006). The contamination of water with pharmaceutical residues is an emerging environmental concern in Europe (European Union, 2013). Most wastewater biological treatments are unable to effectively remove pharmacologically active ingredients (Pomati et al., 2006). However, different conventional and alternative wastewater treatment processes and their combinations have been tested with regard to the removal of pharmaceuticals (Zupanc et al., 2013). The efficiency of these technologies can depend on the characteristics of the pharmaceuticals. According to Hamza et al., there are different pharmaceutical sub-groups depending on usage: i) antibiotics (e.g. amoxicillin, chloramphenicol, ciprofloxacin, norfloxacin, and erythromycin); ii) antidepressant (e.g. venlafaxine); iii) antiepileptic drugs (e.g. carbamazepine, gabapentin, and primidone); iv) non-steroidal anti-inflammatory drugs (NSAIDs, e.g. ibuprofen, naproxen, diclofenac, and acetaminophen); v) cytostatic drugs (e.g. cyclophosphamide, and ifosfamide); vi) illicit drugs (e.g. cocaine, and amphetamines); and vii) hormones (e.g. 17α-estradiol, 17β-estradiol, estrone, estriol, and ethinylestradiol) (Hamza et al., 2016). Many of these

compounds are suspected or potential endocrine-disrupting chemicals (Boyd et al., 2003). Moreover, a mix of these compounds can be found in the wastewater; the toxicity of pharmaceuticals can be influenced by additive and synergistic effects (Daughton and Ternes, 1999; Pomati et al., 2004).

Biological wastewater treatment can be obtained by adopting novel treatment technologies that may prove more efficient and less time consuming (Zupanc et al., 2013). The membrane bioreactor (MBR) is a process in which a conventional biological system is coupled with the membrane process, which has been widely studied and applied on full scale in wastewater treatment (Hosseinzadeh et al., 2016). MBRs can achieve excellent effluent qualities with respect to pathogens, suspended solids, dissolved organic carbon and nitrogen (Yang et al., 2009). According to Kruglova et al., using the MBR process in wastewater treatment could enhance the removal of emerging micropollutants (Kruglova et al., 2016). One of the most important advantages versus conventional technologies is the prolonged acclimatization of organisms due to long SRTs. This is suitable for the removal of emergent substances, as the complete retention of bacterial flocs prevents slow-growing microorganisms (Hai et al., 2011).

The main impacts of pharmaceuticals on the biological treatment processes include a loss of organic matter removal efficiency, deflocculation with the consequent loss of biomass and respiration inhibition of the activated sludge (Love and Bott, 2000; Henriques et al., 2005). In light of this, the kinetic characterization of heterotrophic biomass constitutes an important tool to evaluate, control and predict the influence of pharmaceuticals on the process of organic matter removal. To the best of our knowledge, kinetic modelling of MBR systems under the effect of pharmaceuticals is a novel field that has not been extensively studied. Thus, this study can provide a fundamental understanding of microbial kinetics through analysis of the response to different pharmaceuticals, which is a key aspect for stable and efficient operation of wastewater treatment processes (Kraigher et al., 2008).

The aim of the present research was to study the effect occasioned by the addition of a mix of three pharmaceuticals (carbamazepine, ciprofloxacin and ibuprofen) on the behavior of an MBR system treating urban wastewater for 6 h of HRT and to assess the degradation capacity of these mixed-chemicals by the system. Furthermore, evaluation of the effect

of these pharmaceuticals on the heterotrophic kinetics in the organic matter removal process was carried out to analyze the capacity of the bioreactor to adapt when different concentrations of pharmaceutical mixes are introduced to the biological process.

3. Materials and methods

3.1. Experimental set-up

The pilot scale WWTP used to carry out the research was situated in the WWTP Oeste in Granada (Spain). The experiment was performed with a submerged MBR, schematized in the diagram of Figure 10.

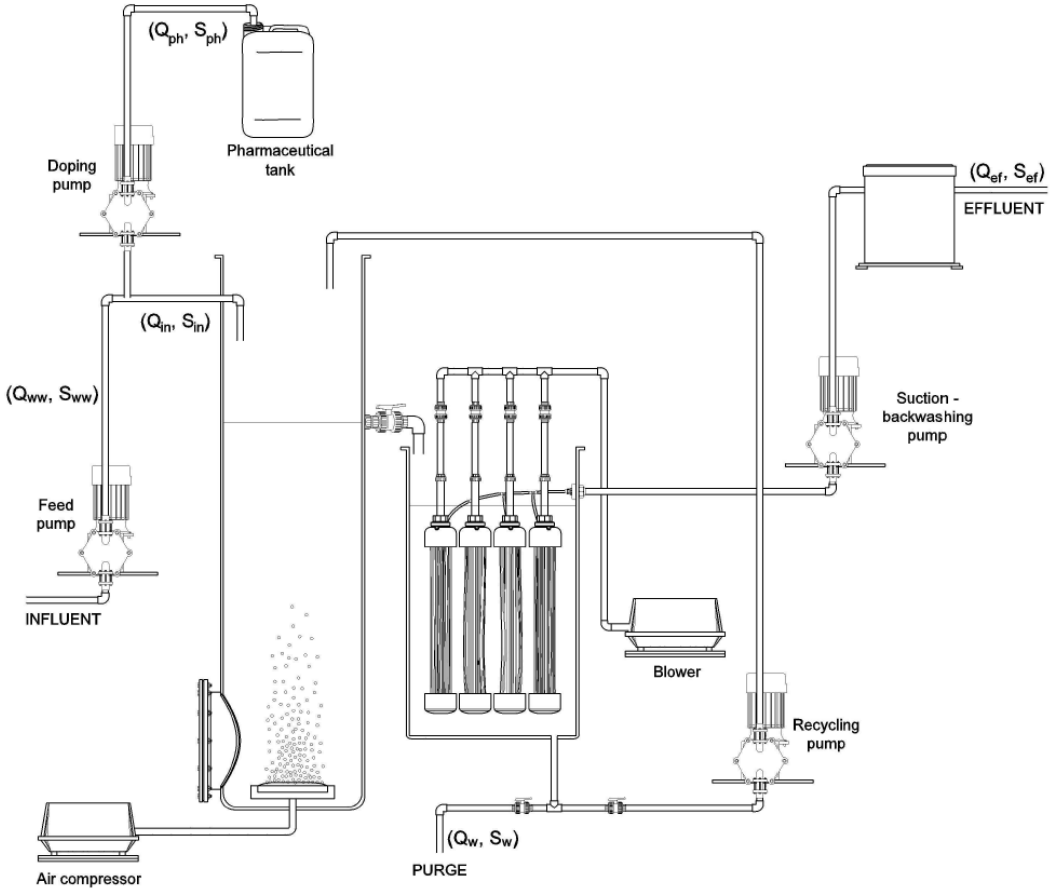

Figure 10. *Schematic diagram of the pilot plant used in the present research.*

The experimental system consisted of an aerated cylindrical bioreactor with a volume of 272 L in which biological treatment is carried out, with an attached rectangular tank with a 78 L capacity in which four ultrafiltration membrane modules ZW-10 (ZENON®) were submerged. These modules were hollow fiber type (outside-in filtration). They have a unitary surface of

0.93 m^2, so the total area of the membrane was 3.72 m^2 with a nominal pore size of 0.04 μm and an absolute pore size of 0.1 μm.

The influent of the pilot plant was supplied by the feed pump from the primary settler of the WWTP. In order to maintain a constant concentration of about 4,500 mg/L of MLSS in the bioreactor, a recycling pump was used to return sludge to the bioreactor, about 165 % of the influent (90 L/h), with a purge of flow (43.97 L/d) being maintained.

In order to introduce the pharmaceutical into the influent continuously, a hole was drilled in the inlet tube. The pharmaceutical tank was daily feed with the different concentrations of pharmaceutical according the condition tested. A peristaltic pump with a fixed flow (0.75 L/h) was used to introduce the doping from the pharmaceutical tank into the inlet tube. The concentrations in the auxiliary tank of each pharmaceutical at different dopings are shown in **Table 4**.

Table 4. *Time required to ensure the availability of the pharmaceuticals in the bioreactor and concentrations of carmazepine, ciprofloxacin and ibuprofen used in each test during the research.*

Cycle	Carbamazepine (µg/L)		Ciprofloxacin (µg/L)		Ibuprofen (µg/L)		Time required (h)
	Bioreactor	Pharmaceutical Tank	Bioreactor	Pharmaceutical Tank	Bioreactor	Pharmaceutical Tank	
Dop 1	100	7,603.3	10	761.3	100	7,603.3	26.04
Dop 2	1000	76,033.3	100	7,603.3	1000	76,033.3	26.04
Dop 3	5,000	380,166.7	500	38,016.7	5,000	380,166.7	25.36

3.2. Operation conditions

To reach the aim of the research, the pilot plant was working constantly at the hydraulic retention time (HRT) and sludge retention time (SRT) for 28 days, needing 9 days to reach the required biomass concentration. During the first nine days, the HRT was constant; however, no purge was done until the desired concentration was reached. The HRT was 6.0 h and the SRT was 7.5 days, with an average value of MLSS of 4,551±534 mg/L. Under these conditions, three different dopings were performed in increasing concentrations of a mix of carbamazepine, ciprofloxacin and ibuprofen to

analyze the degradation capacity and effect on the process. The compounds tested were selected according to their consumption, their diversity in terms of physico-chemical characteristics and the availability of analytical methods. The concentration of each pharmaceutical used is shown in **Table 4**. The range of concentrations of each pharmaceutical was selected according to the data obtained by different authors who studied these compounds in wastewater, such as Quintana et al. (2005), Gros et al., 2007; Rosal et al. (2010); Dorival-García et al. (2013); Rivera-Utrilla et al. (2013) and Peña-Álvarez and Castillo-Alanís (2015). Once the range was chosen, three increasing concentrations were used to check the pilot plant.

An important aspect to consider for the test was the selection of time to ensure that the required concentration was available for the microorganisms as a consequence of the dilution effect in the bioreactor. For this reason, a non-steady compound balance was used to predict the evolution in the bioreactor.

At the beginning, concentration of pharmaceuticals in the bioreactor (S_{BR}) change with the doping time. The evolution of each pharmaceutical

concentration ($\frac{dS_{BR}}{dt}$) in the volume of bioreactor (V) considering two inlet flows (urban wastewater influent flow (Q_{WW}) and pharmaceutical flow, Q_{Ph}) and two outlet flows (purge flow (Q_W) and effluent flow (Q_{Ef})) is shown in Equation (1):

$$V\frac{dS_{BR}}{dt} = Q_{WW} * S_{WW} + Q_{Ph} * S_{Ph} - Q_{Ef} * S_{Ef} - Q_W * S_W \qquad (1)$$

Given that $S_{WW} \approx 0$ in comparision to S_{Ph}, and pharmaceutical are soluble compounds ($S_{BR} = S_{Ef} = S_W$), Equation (1) can be simplified as Equation (2):

$$\frac{dt}{V} = \frac{dS_{BR}}{Q_{Ph} * S_{Ph} - S_i * (Q_{Ef} + Q_W)} \qquad (2)$$

The time required (t) to ensure the constant concentration required of pharmaceutical from the initial concentration (S_{in}) in the bioreactor is defined by Equation (3), and the values for each doping are shown in **Table 4**:

$$t = \frac{-V}{Q_{Ef} + Q_W} * Ln\left(\frac{Q_{Ph} * S_{Ph} - S * (Q_{Ef} + Q_W)}{Q_{Ph} * S_{Ph} - S_{in} * (Q_{Ef} + Q_W)}\right) \qquad (3)$$

Once the concentration of pharmaceutical was ensured, the pilot plant was working under each condition for three days in order to simulate punctual exposure to these pharmaceuticals and to determine their degradation capacity and effect on the organic matter removal of the system.

3.3. Physical and chemical determinations

The samples for analytical determination were taken daily at three points (feed tank, biological reactor and permeate). MLSS, COD and BOD_5 were determined according to the APHA (APHA, 2012). TOC measurements were determined using a Formarcs HT TOC/TN Analyzer by oxidative combustion at 950 °C.

Ammonium, nitrites, and nitrate ions were determined by ionic chromatography using a conductivity detector (Methrom). The separation and dilution of the anions was carried out on a Metrosep A supp5 column using a solution of carbonate/biocarbonate as the eluent, and sulfuric acid as a regenerate. A Metrosep C 4 column was used to separate and dilute the cations with a solution of dipicolinic acid as the eluent, and distilled water as the regenerate. The carbamazepine, ciprofloxacin and ibuprofen

concentrations were measured by chromatography using an Acquity UPLC System H-Class with Acquity UPLC BEH™ C18 column (2.1 x 150 mm, 1.7 μm) at 40 °C.

The pH and conductivity were determined using a pH meter (Crison pH 25®) and a conductivity meter (Crison CM 35®), respectively. Temperature was taken continuously in the pilot plant with a thermometer (LANGE LDOTM / sc100).

3.4. Degradation rate assessment

The analytical method for the pharmaceutical measurements was based on optimized and validated method reported by Martín et al. and Garrido et al. (Martín et al., 2015; Garrido et al., 2016). After the filtration of the samples (1.2 μm), dissolved fraction was determined by solid phase extraction, and solid fraction was treated by sonication assisted extraction. The extraction of the pharmaceuticals from the dissolved fraction was carried out in Oasis HLB SPE cartridges, using methanol:water (5:95 v/v) acidified to pH 2. The extraction of the analyzed compounds from the solid fraction was performed after the lyophilization of the samples; samples were

extracted with methanol acidified with 5 % of acetic acid. The supernatants obtained from the extraction were treated by dispersive solid-phase extraction, using C18 sorbent and analyzed by liquid chromatography-triple quadrupole mass spectrometry (LC-QqQ-MS/MS), using methanol:water (50:50, v/v).

Analytical method was validated by the determination of extraction process recovery, which was assessed by comparing the peak areas of the analyte in samples spiked before and after extraction (solutions A and B, respectively), according to R(%) = (A/B) · 100. Blank corrections were applied to signals obtained from spiked samples.

To calculate the degradation rate (DR_i) of each pharmaceutical tested (carbamazepine, ciprofloxacin and ibuprofen) by the system, the compound balance shown in Equation (4) was used:

$$Q_{In} * S_{In} = Q_{Ef} * S_{Ef} + Q_W * S_W + V\frac{dS_{BR}}{dt} + DR_i * X \quad (4)$$

Considering that there are no variations in pollutants in the bioreactor ($\frac{dS_i}{dt} = 0$) after the dilution time, the DR_i can be obtained with Equation (5):

$$DR_i = \frac{1}{X}\left(Q_{In} * S_{In} - Q_{Ef} * S_{Ef} - Q_W * S_W\right) \quad (5)$$

3.5. Kinetic study

Respirometric experiments, both exogenous and endogenous, were carried out on biomass samples taken from the MBR system in a BM-Advance respirometer according to Leyva-Díaz et al. (J. Leyva-Díaz et al., 2013). These tests allowed the estimation of the kinetic parameters for heterotrophic bacteria, i.e. maximum specific growth rate ($\mu_{m,H}$), half-saturation coefficient (K_M), yield coefficient (Y_H) and decay coefficient (b_H). The decay coefficient for heterotrophic biomass, b_H, was evaluated from the decay coefficient for the global biomass (k_d) through the application of Equation (6) proposed by Ekama et al. (Ekama et al., 1986):

$$b_H = \frac{k_d}{1 - Y_H\left(1 - f_p\right)} \quad (6)$$

where $(1 - f_p)$ is the fraction of volatile biomass.

The substrate degradation rate for organic matter as BOD_5 ($r_{su,H}$) was assessed according to Leyva-Díaz et al. (Leyva-Díaz et al., 2014).

3.6. Statistical analysis

The data obtained were analyzed using SPSS 20 for Windows. A least significant differences test (LSD test) was used to measure the differences between the results obtained for each cycle. An analysis of variance (ANOVA) was used to assess the homogeneity of the variance, with a significance level of 5 % (p-value < 0.05). As the dataset was smaller than 2,000 elements, normality tests were undertaken using the Shapiro-Wilk test.

4. Results and discussion

4.1. Influent characterization

The average values of the physical chemical characteristics of the influent under the four tested conditions are shown in **Table 5**. The pH ranged between 6.61 and 8.38 on average and the conductivity between 977 and 1501 μS/cm. The average suspended solids in the effluent were 108±16, 108±12, 115±8 and 109±8 mg/L to cycle 0, 1, 2 and 3, respectively, changing between 83 and 135 mg/L. The average organic matter values in each cycle are also shown in **Table 5**. TOC, COD and BOD_5 values ranged between 72

and 220 mg/L, 224 and 579 mg/L and 250 and 480 mg/L, respectively, with the lowest values corresponding to the rainy days in the city. The fluctuations of organic matter regarding TOC, COD and BOD_5 are mainly caused by the daily variations of organic loading rate of the influent coming from the primary settler of WWTP Oeste.

Table 5. *pH, conductivity, total suspended solids (TSS) and organic matter concentration (TOC, COD and BOD_5) of the influent during the experiments.*

Cycle	pH	Conductivity (µS/cm)	TSS (mg/L)	VSS (mg/L)	TOC (mg/L)	COD (mg/L)	BOD_5 (mg/L)
Control	7.68±0.39	1254±211	108±16	96±14	158±43	464±124	368±84
Dop 1	7.86±0.17	1700±154	108±12	90±18	200±17	530±43	427±32
Dop 2	7.95±0.14	1495±169	115±8	81±25	178±22	500±57	383±31
Dop 3	7.99±0.03	1722±98	109±8	81±29	186±14	500±31	370±85

In light of this, the differences observed are typical from real urban wastewater in relation to the daily fluctuations, as shown by statistical analysis (J. Leyva-Díaz et al., 2013). The ANOVA test showed that no statistically significant differences were detected in the influent characterization, so the differences produced in the behavior of the process must be caused by the addition of pharmaceuticals. The average data of the

doping cycles were obtained from the three days maintained under stable conditions, without considering the previous period to ensure the availability of the compounds since the concentrations of the pharmaceutical were not stable.

4.2. Biological system performance

During the doping cycles not differences were observed in the pH and conductivity of the biomass getting 7.63±0.38, 7.48±0.60, 7.90±0.16 and 1.91±0.11 of pH and 1,191±142, 1,478±41, 1349±112 and 1.406±15 μS/cm of conductivity both to the cycles 0, 1, 2 and 3 respectively. The ANOVA test showed no statistically significant differences in these data; however, the concentration of biomass showed an important change once the doping was done, i.e. cycles 1, 2 and 3 showed statistically significant differences with cycle 0 but not itself. The MLSS decreased from 4,552±535 mg/L in cycle 0 to around 3 g/L in the rest of the cycles (3,003±217, 3,216±163 and 3,381±252 mg/L to cycle 1, 2 and 3, respectively); however, the fixed solids remained relatively constant, around 600 mg/L. This was observed in the fixed rate, which increased from approximately 13 % in the control cycle to values close

to 19 % in the rest of experimental phases. Considering the above, the introduction of the pharmaceutical could increase the decay of microorganisms in the bioreactor due to its antimicrobial activity (Kraigher et al., 2008). The average temperature during the control cycle was 17.2±2.3 °C while in the doping cycles were 20.5±1.7, 18.9±1.7 and 21.4±0.4 °C, respectively.

With respect to the organic matter consumption, in spite of the biomass reduction, the organic matter removal remained relatively constant, as shown in **Table 6**.

Table 6. *Organic matter removal efficiency of the process measured as TOC, COD and BOD_5 during the experiments.*

Cycle	TOC (%)	COD (%)	BOD_5 (%)
Control	84.70±5.93	90.93±3.39	96.02±0.26
Doping 1	85.23±2.36	90.67±2.15	97.34±0.20
Doping 2	83.78±1.72	89.70±1.37	96.93±0.56
Doping 3	86.85±3.86	90.31±2.14	97.34±0.80

Continuous exposure to the antibiotic group of pharmaceuticals can lead to the emergence of resistant strains of bacteria with attendant health concerns (Zhang et al., 2009). In light of this, despite the biomass decay, the

biomass remaining in the MBR increased the organic matter degradation rate (as explained in the section "Kinetic modeling") due to the stress situation caused to the microbial community under an important dosage of pharmaceutical mix. The organic matter removal rates are in accordance with those obtained in a previous study by the authors Rodríguez et al., who operated with the same configuration and module membrane type, and showed higher COD efficiencies operating under a similar MLSS concentration but with a three times higher HRT (95.60 %) (Rodríguez et al., 2012). Obviously, the reduction of HRT implies a decrease of the organic matter consumption (Martín-Pascual et al., 2015). The reduction of MLSS also produces lower organic matter degradation; however, in this case, the MLSS reduction as a consequence of the pharmaceutical impact was not due to the microbial activity increase, as shown in the kinetic study (**Table 8**).

4.3. Pharmaceutical degradation

The concentration of each pharmaceutical in the effluent and the efficiencies of the system to remove the pharmaceutical mix tested are shown in **Table 7**. The three compounds presented a high removal,

especially at the lowest concentrations. In the same way of organic matter consumption, the efficiency of the system to remove pharmaceutical was affected by the average temperature of the biomass. The efficiency of the system was slightly lower in doping 2 as a consequence of the lower temperature in relation to the other dopings. However, considering that the pharmaceuticals have rather different physico-chemical characteristics, their removal during treatment is expected to be diverse. Both ibuprofen and ciprofloxacin were not detected in the effluent; for this reason, the removal efficiency was almost total. Interestingly, in many cases, pharmaceutical loads increased during the wastewater treatment, resulting in a removal efficiency above 100 %, due to fluctuating sorption and desorption of the pharmaceuticals to organic matter (Langenhoff et al., 2013). Thus, the removal observed during the process could be due to sorption onto sludge and not to biological transformation. However, the yield obtained with carbamazepine was slightly lower, with an average efficiency ranging from 71.9 to 92.9 %. This is in accordance with the results obtained by Wijekoon et al. in an investigation about emerging substances in MBR (Wijekoon et al., 2013). Wijekoon et al. observed that the hydrophilic emerging substances

exhibited removal efficiencies ranging from 80 % to 100 % with the exception of carbamazepine among other four substances (Wijekoon et al., 2013). The removal efficiencies obtained by Fernandez-López et al. in wastewater treatment plants in the region of Murcia (Spain) for carbamamacepine ranged from 27 % to almost total elimination in the effluent (Fernández-López et al., 2016). The removal efficiency of ciprofloxacin varied from 88.7 % to almost total removal.

Table 7. *Average removal efficiency and degradation rate of carbamazepine, ciprofloxacin and ibuprofen for concentrations obtained during the research. N.D: not detected values with the analytical procedure used.*

Cycle	Carbamazepine				Ciprofloxacin				Ibuprofen			
	Influent	**Efluent**	**Removal**	**$DR_{Carbamazepine}$**	**Influent**	**Efluent**	**Removal**	**$DR_{Ciprofloxacin}$**	**Influent**	**Efluent**	**Removal**	**$DR_{Ibuprofen}$**
	(µg/L)	(µg/L)	(%)	µg/(h*mgMLSS)	(µg/L)	(µg/L)	(%)	µg/(h*mgMLSS)	(µg/L)	(µg/L)	(%)	µg/(h*mgMLSS)
Doping 1	100	16.5±12.8	83.5±21.8	1.54±0.05	100	N.D.	≈100.0	0.18±0.02	100	N.D.	≈100.0	1.84±0.13
Doping 2	1,000	281.0±144.3	71.9±14.4	12.60±2.63	1,000	11.3±6.1	88.7±6.1	1.52±0.05	1,000	52.8±10.1	94.7±1.0	16.48±1.18
Doping 3	5,000	356.8±204.0	92.9±4.1	75.81±6.68	5,000	39.4±22.0	92.1±7.8	7.61±1.29	5,000	82.3±20.6	98.4±0.4	80.13±5.71

The highest efficiencies were reached for ibuprofen (higher than 94.7 %), due to ibuprofen isomers being easily degradable and not accumulating at higher concentrations (Quintana et al., 2005; Monteiro and Boxall, 2010). In the literature, other technologies such as aerobic granular sequential

bioreactor or activated sludge have been used to remove ibuprofen. Using a granular sequential bioreactor, Zhao et al. reported an ibuprofen removal efficiency of 45 % (Zhao et al., 2015).

However, in activated sludge municipal wastewater treatment, removal was above 90 % for ibuprofen (Ternes, 1998; Buser et al., 1999; Metcalfe et al., 2003; Quintana and Reemtsma, 2004). Joss et al. tested the removal of pharmaceuticals with a primary (PRIM) and secondary sludge of the MBR and in CAS obtaining removal efficiencies for ibuprofen above 90 % (Joss et al., 2005). However, no removal was seen for carbamazepine, under SRT between 10 and 25 days in CAS and between 16 and 75 days in the MBR process. The biomass behavior in an MBR technology is similar to a CAS process, except for physical separation, so both technologies can be efficient for the removal of ibuprofen.

The degradation rate of each pharmaceutical for the different concentrations are also shown in **Table 7**. Independent of the compound, the rate increases with initial concentration, as shown by Fig. 2; however, an approximation could be made by fitting the data obtained considering the

initial concentration. The correlation obtained for each fitting was 0.9988, 0.9999 and 0.9999 for carbamazepine, ciprofloxacin and ibuprofen, respectively. From these fittings, the relative degradation rates from the initial concentration of each pharmaceutical (S_{in}) were 0.0154 µg/((µgS_{in}/L)·h·mgMLSS) for carbamazepine, 0.0152 µg/((µgS_{in}/L)·h·mgMLSS) for ciprofloxacin and 0.0160 µg/((µgS_{in}/L)·h·mgMLSS) for ibuprofen. These results are in accordance with the removal efficiencies reported and show that the MBR process is a reliable process for the removal of these pharmaceuticals.

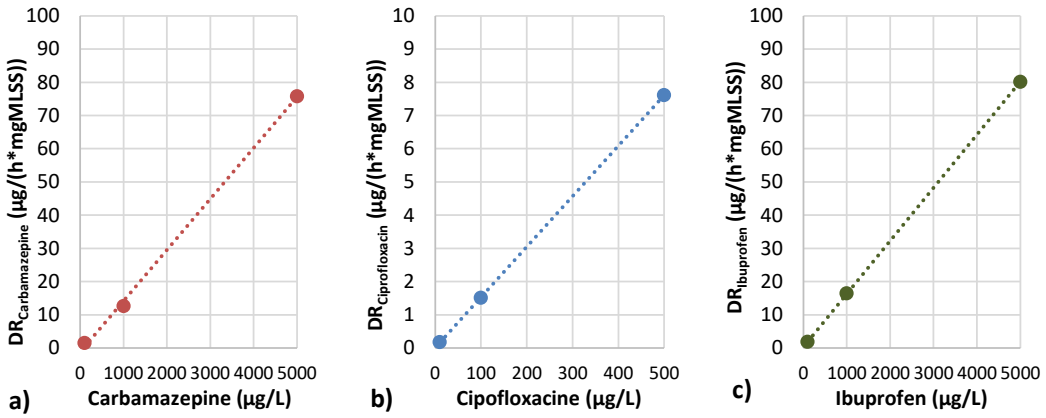

Figure 11. *Degradation rate (DR) of carbamazepine (a), ciprofloxacin (b) and ibuprofen (c) in relation to the initial concentration of this compound.*

4.4. Kinetic modeling

Kinetic parameters for heterotrophic bacteria are shown in **Table 8**. As can be observed in **Table 8**, the values of Y_H were similar in the different cycles whereas the biomass had higher values of $\mu_{m,H}$ and K_M under the addition of different pharmaceuticals than in the control phase. The effect of these variations in kinetic parameters that characterize the heterotrophic biomass in the MBR system are included in the values of $r_{su,H}$ for the control and doping cycles.

Table 8. *Kinetic parameters for the characterization of heterotrophic biomass. Y_H (yield coefficient), $\mu_{m,H}$ (maximum specific growth rate), K_M (half-saturation coefficient), $r_{su,H}$ (substrate degradation rate), b_H (endogenous or decay coefficient).*

Cycle	Parameter				
	Y_H (mgVSS/mgCOD)	**$\mu_{m,H}$ (1/h)**	**K_M (mgO_2/L)**	**$r_{su,H}$ mgO_2/(L·h)**	**b_H (1/day)**
Control	0.6037	0.0145	8.2666	86.27	0.0741
Doping 1	0.5982	0.0519	18.7052	192.88	0.1116
Doping 2	0.6119	0.0537	18.1815	184.77	0.0911
Doping 3	0.5895	0.0447	20.9444	183.97	0.0933

When the mix of carbamazepine, ciprofloxacin and ibuprofen was added to the MBR system in growing concentrations, the heterotrophic biomass showed higher substrate degradation rates for organic matter,

which varied between 183.97 $mgO_2/(L \cdot h)$ and 192.88 $mgO_2/(L \cdot h)$ (**Table 8**). These values almost doubled the $r_{su,H}$ of the control cycle in absence of pharmaceuticals. This involved that the cell growth rate was also higher for the biomass under the effect of pharmaceuticals as the values of Y_H were similar in the four cycles.

Furthermore, the endogenous or decay coefficients for the biomass under the pharmaceuticals effect were higher than that obtained in the control cycle without these chemicals. Thus, percentages in the range of 9.11 % and 11.16 % of the total quantity of heterotrophic biomass were oxidized per day in the doping cycles, while the percentage of biomass oxidized was 7.41 % in the control cycle (**Table 8**).

Therefore, the cell growth rate and the cell decay rate increased under doping 1, doping 2 and doping 3, so the substrate consumption was compensated, which supports the similar removal efficiencies of organic matter as COD and BOD_5 both in the control and doping phases (**Table 6**). In light of this, in spite of the synergy derived from the use of three pharmaceuticals, the effect of the addition of growing concentrations from

doping 1 cycle to doping 3 cycle, and the extended period of time during the doping cycles (9 days), the biomass became stabilized and the organic matter degradation rate and cell decay rate for heterotrophic bacteria remained constant. In light of this, the biomass that remained in the MBR system cancelled out the loss of biomass present, which was probably due to the chemical stress caused by the introduction of pharmaceuticals in the system.

Aubenneau et al. obtained similar results as they did not observe any modification of COD removal under exogenous respiration (Aubenneau et al., 2010). This could be supported by two assumptions, i.e. the presence of a different bacterial community or a change in the metabolic pathways of the substrate (Kraigher et al., 2008; Wang et al., 2008). Kraigher et al. observed the loss of several groups of bacteria in the presence of pharmaceutical micropollutants, which caused some groups to become more dominant (Kraigher et al., 2008). Topalova et al. obtained similar results to those shown by Kraigher et al. (Topalova et al., 1999; Kraigher et al., 2008). Regarding antibiotics, such as ciprofloxacin, Bouki et al. stated that the environmental conditions in wastewater treatment processes are

suitable for the proliferation of antibiotic resistant bacteria, which may transfer resistance genes to non-resistant bacteria (Bouki et al., 2013).

Moreover, Aubenneau et al. demonstrated that endogenous respiration, potentially assimilated to the maintenance requirement, increased after the addition of carbamazepine (Aubenneau et al., 2010). This was in accordance with the higher values of b_H obtained in this study in the doping cycles. Aubenneau et al. stated that these results could be due to the acclimatization of bacterial populations to the presence of carbamazepine, which induced chemical stress (Aubenneau et al., 2010). This could change the environment and modify the exchanges and transfers through the biological membrane, involving higher maintenance requirements.

5. Conclusions

Given the results obtained in a Membrane Bioreactor pilot plant treating real urban wastewater at 6 h of HRT, 7.5 days of SRT and 4,552 ± 535 mg/L of MLSS under three different shock of pharmaceutical (carbamazepine, ciprofloxacin and ibuprofen) with increasing concentrations, the following conclusions were made:

- The organic matter removal capacity was not affected by the addition of pharmaceuticals, i.e. the TOC, COD and BOD_5 removal rates did not present statistically significant differences (pvalue < 0.05). The TOC, COD, and BOD_5 removal rates were higher than 83.78±1.72, 89.70±1.37 and 96.02±0.26 %, respectively.
- The removal rates for ibuprofen, ciprofloxacin and carbamazepine were higher than 94.7, 88.7 and 71.9 %, respectively, under the three different concentrations tested.
- The degradation rates were 0.0154 $\mu gS/((\mu gS_{in}/L)\cdot h\cdot mgMLSS)$ for carbamazepine, 0.0152 $\mu gS/((\mu gS_{in}/L)\cdot h\cdot mgMLSS)$ for ciprofloxacin and 0.0160 $\mu gS/((\mu gS_{in}/L)\cdot h\cdot mgMLSS)$ for ibuprofen.
- There were statistically significant differences in MLSS, which indicated that there was a decrease in the biomass present in the MBR system. However, these differences were not observed in the organic matter removal as the increased cell growth rate cancelled out the increased cell decay rate due to the chemical stress caused by the addition of pharmaceuticals in the MBR system. This showed that the COD and BOD_5 removal was similar in the control phase and the

doping cycles. Therefore, despite the synergistic effect caused by the use of three pharmaceuticals, the influence of adding growing concentrations of them and the effect of the extended period of time during doping cycles, the biomass became steady, as shown by stabilization of the organic matter degradation rate (183.97-192.88 $mgO_2/(L \cdot h)$ and the decay coefficient (0.0911- 0.1116 day^{-1}) after the increase from the control cycle.

Considering the above, the MBR process can be used as a reliable technology to remove most of these pharmaceuticals at 5, 0.5 and 5 mg/L of carbamazepine, ciprofloxacin and ibuprofen, respectively, without reducing the organic matter removal capacity.

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VII - CHAPTER 4

Effect of the biomass on the biodegradation capacity of a mix of pharmaceuticals (carbamazepine, ibuprofen and ciprofloxacin) in a membrane bioreactor.

VII - CHAPTER 4

This chapter, "Effect of the biomass on the biodegradation capacity of a mix of pharmaceuticals (carbamazepine, ibuprofen and ciprofloxacin) in a membrane bioreactor" is in process of being published.

1. Abstract

A membrane bioreactor (MBR) system and a hybrid moving bed biofilm reactor-membrane bioreactor (MBBR-MBR) system with a 35 % filling ratio were used to remove pharmaceutical compounds treating real urban wastewater in a semi-technical pilot plant. The operational variables were hydraulic retention time (6 and 10 hours) and the technology (MBR and hybrid MBBR-MBR). The mixed liquor suspended solids changed between 5,200 and 5,700 mg/L and the temperature varied between 12.6 °C and 28.1 °C simulating real conditions. The plant received a shock with a mix of pharmaceutical compounds (carbamazepine, ibuprofen and ciprofloxacin) to study its behaviour under different operational. The removal of ibuprofen, carbamazepine and ciprofloxacin was above 83.7, 48.6

and 10.6 %, respectively, regardless of the process and the operative variables; these removal rates increased when temperature increased. In addition, the hybrid MBBR-MBR system, presented a higher relative degradation rate than that of the conventional MBR system.

2. Introduction

Most human activities produce wastewaters of varied composition, reflecting the range of contaminants released by various sources; one of them is the effluent from domestic from domestic sources that does not usually contain hazardous substances, but there are growing concerns about emerging pollutants including commonly used medications that, even at low concentrations, may have long-term impacts (WWAP, 2017). At the European level there is no by-law that regulates the dumping of pharmaceuticals into the environment. Directive 2013/39/EU, amending Directives 2000/60/EC and 2008/105/EC, as regards priority substances in the field of water policy, says that, as a priority, it is necessary to identify the causes of pollution and treat the emissions of pollutants at the source, in the most efficient way in economic and environmental terms.

Compounds of emerging concern (CECs) consist of a growing variety of both anthropogenic and natural substances (Luo et al., 2014a). CECs are compounds present in the environment at trace concentrations of ng/L to μg/L (Luo et al., 2014a; He et al., 2016; Grandclément et al., 2017). These pollutants are unknown or unrecognised; their presence in the environment does not have to be new, although concern about the effects they may cause in the environment. They may have subtle detrimental effects on aquatic organisms or public health are new, due to their intrinsic properties, such as often high polarity and persistence (Sipma et al., 2010). These pollutants include pharmaceuticals, personal care products, industrial chemicals, pesticides, polycyclic aromatic hydrocarbons, as well as metallic trace elements (Grandclément et al., 2017). The main sources of CECs and their metabolites in aquatic environments are wastewater treatment plants (WWTPs) (Al Qarni et al., 2016; Park et al., 2017). The purpose of wastewater treatment is to remove pollutants found in wastewater to acceptable levels before discharging the treated effluent into the environment, to prevent human health issues and ecological hazards arising from untreated wastewater (Hamza et al., 2016). Different systems are used to remove the

CECs, both physical-chemical and biological. Most of the CECs can be degraded using advanced oxidation processes such as ozonation, UV/$H2O_2$, O_3/H_2O_2, and Fenton/photo-Fenton reactions (Katsoyiannis et al., 2011; Ibáñez et al., 2013; Liu et al., 2016). The biological systems that have been researched to remove the CECs are, among others, membrane bioreactor (MBR), conventional activated sludge (CAS), or moving bed biofilm reactor (MBBR) systems (Clara et al., 2005; Radjenović et al., 2009; Sipma et al., 2010; Tambosi et al., 2010; Casas et al., 2015; Calero-Díaz et al., 2017).

However, several studies have shown that the considerable number of pharmaceuticals detected in the effluents of various WWTPs confirmed that CAS treatment is inefficient for full removal of these recalcitrant products from wastewater (Casas et al., 2015).

In the last two decades, the MBR process has become an alternative to CAS processes in terms of the removal of pharmaceutical products, as well as conventional pollutants such as organic matter and nutrients, during wastewater treatment (Park et al., 2017). MBR technology has a series of advantages, such as superior effluent quality, absolute control of solids and

hydraulic retention times (HRTs), and smaller volume and footprint (Dialynas and Diamadopoulos, 2012), with exceptional quality of improvement of its effluent, and it is capable of meeting the most stringent water quality requirements (Stephenson et al., 2000).

The activated sludge systems develop biomass that grows suspended on the mixed liquor; nevertheless, other technologies exist, such as MBBR that develops the growth of biomass attached to surfaces (Rodriguez-Sanchez et al., 2018). MBBR is a simple yet effective and compact technology that consists of a process tank in which carriers are immersed and gradually colonised by the attached biomass on the protected internal surface, where the carriers move around the bioreactor freely and are kept in the tank by a sieve arrangement without the necessity for sludge recycling (Luo et al., 2014a).

The aim of this research was to analyse the removal capacity of ibuprofen (100, 1000 and 5000 μg/L), carbamazepine (100, 1000 and 5000 μg/L) and ciprofloxacin (10, 100 and 500 μg/L) with a biological process based on MBR technology under different HRTs (6 and 10 h) and to compare

the effect of including hybrid biomass incorporating carrier in the bioreactor (a hybrid MBBR-MBR system). For this purpose, the biodegradation rate of each pharmaceutical was calculated to compare the effect of the operative variables.

3. Material and methods

3.1. Pilot plant

The research was carried out in a pilot-scale plant situated in the WWTP Oeste of Granada (Spain). The semi-technical plant was continuously fed with real wastewater in order to simulate a real process. In order to evaluate the direct effect of the biofilm, two different processes were studied in the pilot plant: firstly, a conventional MBR system under two different HRTs and then a hybrid MBBR-MBR system at the same HRT. For the configuration of the hybrid MBBR-MBR system in the biological reactor, Anoxkaldnes® K1 carriers were introduced at a 35 % filling ratio.

The semi-technical pilot plant, showed in **Figure 12**, consists of a biological reactor of 272 L volume and a membrane tank with a volume of 78 L, in which four modules of ultrafiltration membranes are submerged.

The membrane units used were ZENON® ZW-10, which were configured as hollow fibres with external filtration and a nominal surface area of 0.93 m^2, a nominal pore size of 0.04 μm and an absolute pore size of 0.1 μm.

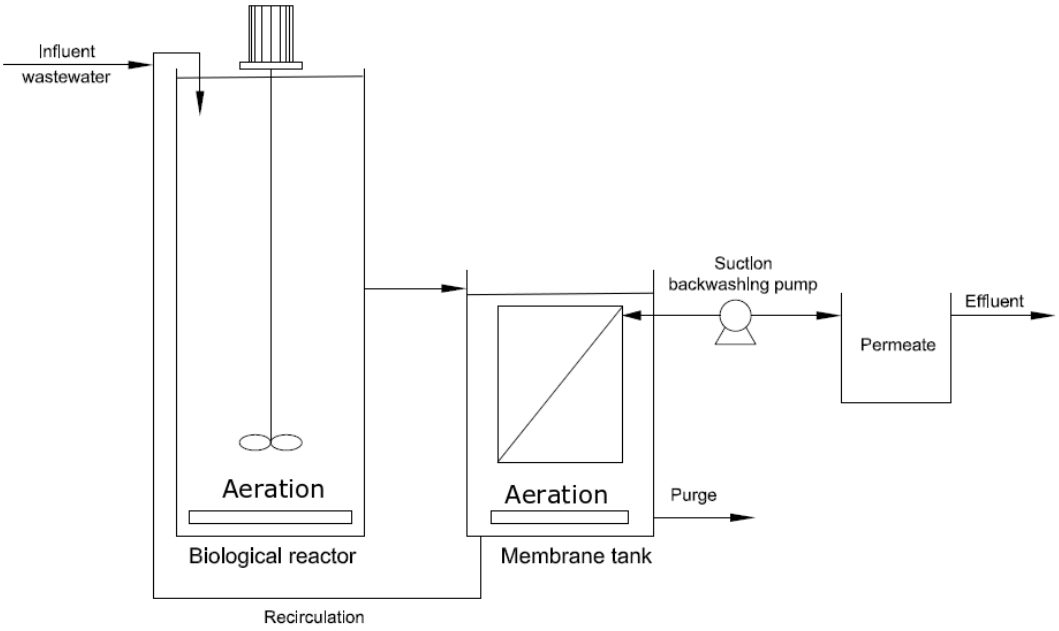

Figure 12. *Schematic diagram of the semi-technical pilot plant*

In order to maintain the concentration of mixed liquor suspended solid (MLSS) in perfect mix in the bioreactor, the sludge was returned from the MBR to the bioreactor with a pump.

For each condition of HRT and process, three different doping mixes were carried out with increasing concentrations of the pharmaceutical. The

pharmaceuticals used were ibuprofen, carbamazepine and ciprofloxacin because they are some of the most common pharmaceutical compounds found in wastewater. The initial concentrations of pharmaceuticals in the system were, for carbamazepine and ibuprofen, 100, 1000 and 5000 μg/L and for ciprofloxacin 10, 100 and 500 μg/L. These concentrations were selected according to literature (Camacho-Muñoz et al., 2009; Rosal et al., 2010; Tambosi et al., 2010; Rivera-Utrilla et al., 2013), and who detected concentrations of pharmaceutical compounds lower than those used in this study. Higher concentrations were used to analyse their effect in the behaviour of the system, to simulate a shock and to analyse the effect of including biofilm in the biomass to improve the behaviour of the biomass versus the impacts of the pharmaceutical compounds.

3.2. Operational conditions

The operational conditions are shown in **Table 9**. During the four cycles, both the MBR system and the hybrid MBBR-MBR system were tested with different HRTs (6 and 10 h). The MLSS levels were fixed at about 5,500 mg/L, a typical value in the operation of an MBR for energy consumption

through aeration and membrane fouling (Yigit et al., 2008; Sun et al., 2011). The pilot plant was fed with real urban wastewater, which was conditioned by environmental and population changes and the purge was maintained to keep the solid retention time (SRT) constant in the process; so MLSS were kept between 5,200 and 5,700 mg/L during the research.

Table 9. *Operational conditions for the two cycles of the MBR system and the two cycles of the hybrid MBR-MBBR system.*

Cycle	HRT Hours	Filling ratio (%)	MLSS mg/L	SRT Days	Temperature ºC
1	6	0	5,642.73±577.94	11.23	21.5±3.1
2	10	0	5,332.83±304.06	21.67	12.6±1.8
3	6	35	5,772.51±492.09	6.02	28.1±2.7
4	10	35	5,285.12±279.94	23.89	17.6±2.7

The SRT varied according to the cycle and the system, although between the two systems under the same conditions it was similar. The SRT for the MBR system varied from 11.23 days with 6 h of HRT to 21.67 days with 10 h of HRT. For the hybrid MBBR-MBR system, the SRT ranged from 6.02 days for 6 h of HRT to 23.89 days for 10 h of HRT. The SRT was affected by the temperature of the process, the SRT being high when the temperature was low in the process. For the MBR system the temperature ranged from

2.5 to 12.6 °C for Cycles 1 and 2, respectively, and for the hybrid MBBR-MBR system the temperature oscillated from 28.1 to 17.6 °C for Cycles 3 and 4, respectively. The temperature was imposed because the plant was located outdoors and was affected by changes in the environment, making the data more representative.

The pharmaceutical compounds were incorporated continuously at a constant flow of 0.75 L/h in the real urban influent with a peristaltic pump. To ensure that the mix of pharmaceuticals was available for the biomass, 26.4, 44.2, 25.9 and 44.2 hours were needed to ensure that the concentration required was reached for Cycles 1, 2, 3 and 4, respectively.

To model the process, the degradation rate (DR_i) of each pharmaceutical concentration (S) in the volume of bioreactor (V) taking as inlet flows the urban influent flow with pharmaceutical (Q_{In}) and to outlet flows (purge flow (Q_B) and effluent flow (Q_{Ef})) was calculated throughout the balance shown in Eq. 1:

$$Q_{In} * S_{In} = Q_{Ef} * S_{Ef} + Q_B * S_B + V\frac{dS_B}{dt} + DR_i * X \quad (1)$$

Where concentration of pharmaceuticals in the influent (S_{In}), in the effluent (S_{Ef}) and in the bioreactor (S_b)

Considering the biomass is in steady state, no variations of compounds in the bioreactor ($\frac{dS_i}{dt} = 0$), the DR_i can be determined by Eq. 2:

$$DR_i = \frac{1}{X}(Q_{In} * S_{In} - Q_{Ef} * S_{Ef} - Q_B * S_B) \quad (2)$$

3.3. Physical and chemical determinations

The analytical determinations were made daily from the feed tank, biological reactor, membrane tank and permeate. pH and conductivity were determined using a pH meter (Crison pH 25®) and a conductivity meter (Crison CM 35®). The temperature was measured continuously in the same biological tank of the pilot WWTP with a thermometer (LANGE LDOTM/SC 100). The chemical oxidation demand (COD), biological oxidation demand (BOD_5) and MLSS were determined according to the method of the American Public Health Association, the American Water Works Association and the Water Environment Federation (APHA, 2012). Total

organic carbon (TOC) measurements were determined using a Formarcs HT TOC/TN Analyzer by oxidative combustion at 950 °C.

The concentration of the pharmaceutical (carbamazepine, ibuprofen and ciprofloxacin) in the MLSS of the system was measured by chromatography using an Acquity UPLC System H-Class with Acquity UPLC BEHTM C 18 column (2.1 × 150 mm, 1.7 μm) at 40 °C.

3.4. Statistical analysis

The statistical analysis of the data was performed using SPSS 20 for Windows. Analysis of variance (ANOVA) was used to measure the differences between the results of doping mix obtained with the MBR system and hybrid MBBR-MBR system and the different conditions in the systems. The confidence interval was 95 % (p-value < 0.05).

4. Results and discussion

4.1. Influent characteristics

The pilot plant was fed continuously with real urban wastewater taken from the influent of the real bioreactor of the WWTP where the pilot plant

was located. The influent had COD, BOD_5 and TOC values that did not present statistically significant differences during the two years of the study. The concentrations in the influent were 561.25 ± 101.78 mgO_2/L COD, 329.32 ± 80.43 mgO_2/L BOD_5 and 182.84 ± 40.55 mg/L TOC. The pH and conductivity of the influent also did not vary statistically and for the four cycles they were similar, with values of pH 7.72 ± 0.22 and conductivity 1172 μS/cm ± 181 μS/cm.

Since there were no statistically significant differences in the influent of the bioreactor, the variations in the behaviour that took place when the pharmaceutical compounds were introduced were due to the operative variables (HRT, temperature and operative system) and the pharmaceutical concentration.

4.2. Pharmaceutical compound removal

Table 10 shows the removal rates of each pharmaceutical included in the doping mix (carbamazepine, ibuprofen and ciprofloxacin) separately. Both, MBR and hybrid MBBR-MBR, removed pharmaceuticals at high rates,

higher than 60 %, although ciprofloxacin in Doping 1 for Cycle 4 had a very low removal rate (about 11 %).

Table 10. *Removal performance of pharmaceuticals carbamazepine, ibuprofen and ciprofloxacin separately.*

Carbamazepine removal (%)			
Cycle	**Doping 1**	**Doping 2**	**Doping 3**
1	84.1[a] 8.5	88.9[a]±0.8	94.8[b]±0.4
2	67.5[a]±4.9	90.2[a]±2.3	
3	92.2[a]±5.0	90.7[a]±6.4	90.6[a]±2.3
4	48.6[a]±36.9	92.8[a]±0.5	97.8[b]±0.2
Ibuprofen removal (%)			
Cycle	**Doping 1**	**Doping 2**	**Doping 3**
1	≈100[a]	≈100[b]	97.0[a]±1.4
2	≈100[a]±-	97.2[a, b]±2.5	
3	83.7[a]±28.2	95.9[a, b]±1.2	96.6[a]±3.9
4	88.5[a]±19.8	89.3[a]±5.0	91.6[a]±0.9
Ciprofloxacin removal (%)			
Cycle	**Doping 1**	**Doping 2**	**Doping 3**
1	87.8[b]±8.5	89.4[b, c]±5.7	83.8[a]±7.9
2	61.4[b]±10.2	92.1[c]±5.7	
3	31.2[a]±18.4	62.2[a]±1.5	81.7[a]±3.9
4	10.6[a]±10.8	77.1[b]±7.4	78.0[a]±2.4

Numerous authors (Bernhard et al., 2006; Luo et al., 2014a, Luo et al., 2015) removed more ibuprofen and ciprofloxacin than carbamazepine in MBR systems at both pilot and laboratory scale. This is due to the fact that ciprofloxacin remains in the sludge due to adsorption, since the mixed liquor remains in the membrane tank (Arya et al., 2016; Cecconet et al., 2017).

It is important to take into account the synergistic effect on living beings that introducing a mixture of pharmaceutical compounds into the environment can have. According to the study by Cleuvers (2003), the toxicity of pharmaceutical compounds is such that effect of individual substances in the aquatic environment is very unlikely to be harmful; although it must be borne in mind that considerable combination effects can occur, causing an environmental risk from the residues of the pharmaceutical compounds (Cleuvers, 2003). For compounds with different mechanisms of action, the additive effects may be unexpected and the combined effects may be less (subtractive) or more (synergistic) than the sum of the effects of each compound alone (Galus et al., 2013).

In general, ibuprofen, which is one of the most common pharmaceutical compounds found in water, and more biodegradable, with numerous processes achieving more than 90 % removal rates (Zwiener and Frimmel, 2000; Clara et al., 2005; Luo et al., 2014a), reached the objective of high removal rates in both systems used in this research. At the lowest doping level, which did not show statistically significant differences between the different cycles under study, it was almost completely removed

by the MBR system. With an MBR system, Bernhard et al. (2006) removed 98.8 ± 0.7 % of ibuprofen with an interval of 7 to 10 h of HRT (Bernhard et al., 2006); Clara et al. (2005) also removed 99 % with an MBR system (Clara et al., 2005). However, for the hybrid MBBR-MBR system the removal rates were lower than with the MBR system: above 83 % and 88 % for Cycles 3 and 4, respectively. In the case of Doping 2, there were statistically significant differences between Cycles 1 and 4. In Cycle 1, no ibuprofen was detected in the effluent and in Cycle 4 the removal rate was 89 %, being the highest removal rate with the MBR system, which worked at an HRT of 6 h and a temperature above 20 °C. With a hybrid MBBR-MBR system, Luo et al. (2015) removed 93.7 % of ibuprofen with a very low initial concentration (5 μg/L) in a synthetic water (Luo et al., 2015). Likewise, the removal rates for the cycles that worked with the MBR system were higher than with hybrid MBRR-MBR system, at 5–7 %. However, for Doping 3, there were no statistically significant differences. This may have happened because the statistical analyses were performed with less data because the data of Doping 3 in Cycle 2 were not available, since the system was inhibited by the shock of pharmaceuticals mix combined with the low temperatures of

this cycle. In Cycle 2, the MBR system was depleted due to the low temperatures that existed in the plant. For the hybrid system, the hybrid biomass resisted the low temperatures in the environment, giving removal rates above 90 %.

On the other hand, within the pharmaceutical mix used in doping, carbamazepine is not as degradable as ibuprofen. Carbamazepine removal efficiency in others work using MBR ranged between 0 and 34 % (García-Gómez et al., 2016). Dopings 1 and 2 did not show statistically significant differences; however, Doping 3 did present statistically significant differences between Cycles 1 and 4 with respect to Cycle 3. In this case, the removal rates were somewhat lower than in the case of ibuprofen. For Doping 1, the removal rates ranged from 48 % for Cycle 4 to 92 % for Cycle 3. For Doping 2, the removal rates were very similar for the four cycles; all above 89 % for carbamazepine removal. For Doping 3, removal rates were higher than for dopings at lower concentration; all above 91 %. In this case, similarly to Cycle 2, the system was not able to withstand the impact of the pharmaceuticals, probably due to the low temperatures. In their study, Luo et al. (2014a) removed 25.9 ± 14.7 % of carbamazepine with an MBBR system,

when with an MBR system up to 30 % could be removed (Luo et al., 2014a). Bernhard et al. (2006) removed 13 % of carbamazepine with an MBR system and 7–10 h of HRT (Bernhard et al., 2006). Little carbamazepine was removed because it was adsorbed by the mixed liquor of the MBR system. Similarly, in the hybrid MBBR-MBR used in this research, more carbamazepine was removed at the different dopings than in the MBR system because it remained longer in the system due to the biofilm in the biological reactor reducing its adsorption and favouring its removal.

For ciprofloxacin, the removal rates in general were lower, since it is very difficult to remove biologically (Zaviska et al., 2013). The three doping mixes showed the same behaviour; the MBR system behaved more favourably than did the hybrid MBBR-MBR system, removing more ciprofloxacin. Arya et al. (2016) removed 93 % of ciprofloxacin in their laboratory-scale research using synthetic water, with an MBR system, 10 h of HRT and 3000–5000 mg/L of MLSS (Arya et al., 2016). For Doping 1, which showed statistically significant differences, the hybrid system had low removals of 31 % and 11 % for Cycles 3 and 4, respectively; and above 60 % with the MBR system. Gros et al. (2007) removed up to 63 % of ciprofloxacin

in their research with a WWTP and between 6 and 10 h of HRT (Gros et al., 2007). Doping 2 also showed statistically significant differences; the MBR system removed over 90 % in Cycle 1 and the hybrid MBBR-MBR system over 62 % in Cycle 3. Doping 3 showed high removal rates for the hybrid MBBR-MBR system, above 78 %, but lower than for the MBR system; although in this case the hybrid biomass made the system behave best in the removal of pharmaceuticals under the colder temperatures in the plant. Hybrid MBBR-MBR systems have been developed and compared with the MBR systems by Luo et al. (Luo et al., 2014a; Luo et al., 2015). The hybrid MBBR-MBR systems showed greater efficiency in removal of hydrophobic compounds than did the MBR systems under similar conditions, and furthermore it was demonstrated that with a prolonged biodegradation it overrode the adsorption of the contaminant (Cecconet et al., 2017).

In summary, the MBR system removed pharmaceutical compounds at rates ranging from 97.0 % to close to totality of ibuprofen, from 67.5 % to 94.8 % of carbamazepine and from 61.4 % to 92.1 % of ciprofloxacin. The effect of biofilm on the removal capacity was lower in relation to temperature and HRT. In the hybrid MBBR-MBR configuration, the removal

capacity ranged between 48.6 and 97.8 %, between 83.7 and 96.6 % and between 10.6 and 81.7 % for carbamazepine, ibuprofen and ciprofloxacin, respectively.

4.3. Degradation rate

The DR of each pharmaceutical compound at the different concentrations is shown in **Table 11**. In addition, regardless of the compound and the biomass, the DR increased with the initial concentration, as shown in **Figure 13**.

Table 11. *Average of degradation rate of carbamazepine, ibuprofen and ciprofloxacin for the two systems under study and for each doping.*

Cycle	$DR_{Carbamazepine}$			$DR_{Ibuprofen}$			$DR_{Ciprofloxacin}$		
	μgCarbamazepine/(h*mgMLSS)			μgIbuprofen/(h*mgMLSS)			μgCiprofloxacin/(h*mgMLSS)		
	Doping 1	Doping 2	Doping 3	Doping 1	Doping 2	Doping 3	Doping 1	Doping 2	Doping 3
1	0.86±0.16	9.31±0.70	56.04±4.87	1.01±0.10	10.44±0.76	57.28±4.43	0.09±0.02	0.93±0.03	4.93±0.00
2	0.47±0.07	6.44±1.24		0.69±0.05	6.93±1.34		0.04±0.01	0.66±0.15	
3	0.97±0.08	9.43±0.47	54.17±0.98	0.87±0.26	9.97±0.10	57.65±1.93	0.03±0.02	0.63±0.01	4.86±0.37
4	0.36±0.28	6.87±0.60	47.38±6.90	0.66±0.16	6.60±0.23	44.36±6.10	0.01±0.01	0.57±0.03	4.06±0.03

The biomass in the MBR system was inhibited in Cycle 2 during Doping 3. The system was not able to withstand the shock produced by the mix of pharmaceutical compounds at high concentration, and a foaming

phenomenon took place, removing all the biomass from the system. This total loss of biomass meant that the DR could not be determined. However, under similar conditions of HRT and temperature but including biofilm (Cycle 4) the biomass was able to withstand the shock of the pharmaceutical compounds. Biofilm processes have a greater inertia than processes based on the flocs growing (Butler and Boltz, 2013), which means that the system is not affected in the same way as a conventional MBR system when the system receives a shock, as in this case.

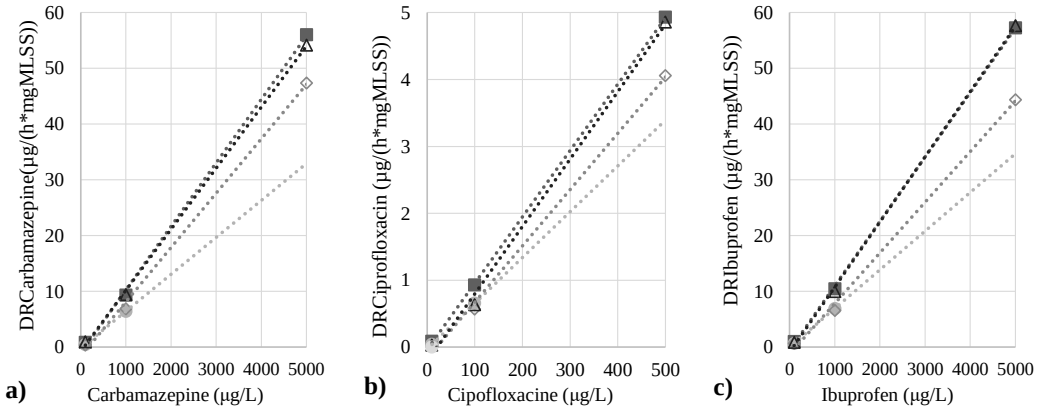

Figure 13. *Degradation rate (DR) of carbamazepine (a), ciprofloxacin (b) and ibuprofen (c) in relation to the initial concentration of each compound for the MBR system with 6 h (■) and 10 h (●) HRT and the hybrid MBR-MBBR system with 10 h (◊) and 6 h (Δ) HRT.*

A fit of the DR in relation to the initial concentration of each pharmaceutical compound can be obtained with correlation rate (R^2) higher

than 0.99. From these fittings, the relative degradation rates (rDR) from the initial concentration (C_0) (**Figure 13**) for carbamazepine were 0.0114, 0.0066, 0.0098 and 0.0110 μg/((μgC_0/L)·h·mgMLSS) for Cycles 1, 2, 3 and 4, respectively; for ibuprofen 0.0116, 0.0069, 0.0091 and 0.0117 μg/((μgC_0/L)·h·mgMLSS) for Cycles 1, 2, 3 and 4, respectively; and for ciprofloxacin 0.0099, 0.0068, 0.0084 and 0.0101 μg/((μgC_0/L)·h·mgMLSS) for Cycles 1, 2, 3 and 4, respectively.

Under the lowest HRT, the biomass activity was highest; therefore, the DR was highest too, as **Table 11** shows. For Cycles 1 and 3 (6 h of HRT), the DR was higher than for 10 h of HRT (Cycles 2 and 4), both under conventional MBR (Cycle 1) and hybrid MBBR-MBR systems (Cycle 3), independent of the pharmaceutical compound studied. Bernhard et al. (2006) found no significant correlation between the removal of pharmaceutical compounds and HRT in an MBR system but noted that their MBR system showed better removal efficiencies (even at low HRT) than a WWTP with 22 h of HRT (Bernhard et al., 2006). In the case of MBR, the rDRs in Cycle 1 were 72.7, 68.1 and 45.6 % higher than the values obtained in Cycle 3 for carbamazepine, ibuprofen and ciprofloxacin, respectively. Although in

the hybrid MBBR-MBR the same effect took place, the increase in Cycle 3 with respect to Cycle 4 was lower, with values 12.2, 28.6 and 20.2 % higher than those obtained in Cycle 4 for carbamazepine, ibuprofen and ciprofloxacin, respectively.

In relation to the type of biomass, for Cycle 4 (10 h of HRT) with the hybrid MBBR-MBR system, the rDR was somewhat higher than for Cycle 2 (10 h of HRT) with the MBR system. This may be due to the fact that the fitting in Cycle 2 was carried out with one less set of DR data, since for Doping 3 the system was inhibited by the pharmaceutical compound shock. For ibuprofen and ciprofloxacin the rDR in Cycle 3 (hybrid MBBR-MBR system) was somewhat higher than for Cycle 1 (MBR system) with 0.0117 μg/((μgC_0/L)·h·mgMLSS) and 0.0101 μg/((μgC_0/L)·h·mgMLSS) versus 0.0116 μg/((μgC_0/L)·h·mgMLSS) and 0.0099 μg/((μgC_0/L)·h·mgMLSS), respectively. With carbamazepine, the rDR did not behave same, being 0.0114 μg/((μgC_0/L)·h·mgMLSS) in Cycle 1 with the MBR system versus 0.0110 μg/((μgC_0/L)·h·mgMLSS) in Cycle 3 with the hybrid MBBR-MBR system.

Generally, the microbial activity increased with temperature (Radjenovic et al., 2008); this was observed in our research too. Temperature plays an important role in the microbial activity, solubility, reaction rate and in the physical-chemical properties of the CEC. An increase in the temperature of the effluent can reduce the concentration of dissolved oxygen and stimulate the development of specific microorganisms (Grandclément et al., 2017), thus increasing the removal rate of pharmaceutical compounds in wastewater. The DR of Cycles 1 and 3 increased with the biomass activity and so, with the temperature; i.e. if the temperature was higher, the DR was higher too for the three pharmaceutical compounds. The temperatures of Cycles 1 and 3 were above 20 °C, which resulted in the DRs being higher than for Cycles 2 and 4 when the temperatures were between 12 and 17 °C. What most affects the system is temperature, to a greater extent than the presence of a biofilm in the hybrid MBBR-MBR system, due to the typical inertial effect of the biofilm systems.

In this research, the SRT was imposed by the conditions of the system (MLSS, HRT, temperature and presence or absence of biofilm), so it is could not be modified during the operation. The SRT indicates the microbial

activity and, therefore, the degradation capacity of the system; so, in general terms, when SRT decreases, microbial activity increases and the rate of degradation of the pharmaceutical compound mix increases. Lee et al. (2003) in their research with an MBR system observed that when increasing the SRT the microbial activity decreased, affecting the removal rate of COD (Lee et al., 2003). The SRT affected the DR negatively; an increase in SRT produced a reduction in the DR for all pharmaceutical compounds. However, the presence or absence of biofilm also affected it; in the hybrid MBBR-MBR (Cycles 3 and 4) the DRs were higher than those obtained in the MBR process (Cycles 1 and 2).

In summary, the rDRs for carbamazepine, ibuprofen and ciprofloxacin were higher than 0.0066 μgcarbamazepine/(h·mgMLSS), 0.0069 μgibuprofen/(h·mgMLSS) and 0.0068 μgciprofloxacin/(h·mgMLSS). The presence of biofilm in the system (hybrid MBBR-MBR versus MBR) dampened the behaviour of the biological system, the DR being less affected than by temperature and HRT.

5. Conclusions

Considering the results obtained in this research, operating an MBR under 5,200–5,700 mg/L of MLSS, two HRTs (6 and 10 h) and three ascending concentrations of a mix of pharmaceutical compounds (ibuprofen, carbamazepine and ciprofloxacin) with conventional and hybrid MBBR-MBR configurations, the following conclusions were obtained:

- The MBR system removed pharmaceutical compounds at rates ranging from 97.0 % to close to totality of ibuprofen, from 67.5 % to 94.8 % of carbamazepine and from 61.4 % to 92.1 % of ciprofloxacin.
- The effect of biofilm on the removal capacity was lower in relation to temperature and HRT. In the hybrid MBBR-MBR configuration, the removal capacity ranged between 48.6 and 97.8 %, between 83.7 and 96.6 % and between 10.6 and 81.7 % for carbamazepine, ibuprofen and ciprofloxacin, respectively.
- The rDRs for carbamazepine, ibuprofen and ciprofloxacin were higher than 0.0066 μgcarbamazepine/(h·mgMLSS),

0.0069 μgibuprofen/(h·mgMLSS) and 0.0068 μgciprofloxacin/(h·mgMLSS).

- The presence of biofilm in the system (hybrid MBBR-MBR versus MBR) dampened the behaviour of the biological system, the DR being less affected than by temperature and HRT.
- The removal capacity and DR of pharmaceutical compounds in the system increases with the temperature and decreases with HRT. So, the MBR process could be a reliable technology for treating urban wastewater to remove ibuprofen, carbamazepine and ciprofloxacin.

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VIII - CHAPTER 5

Insight of the bacterial ecology in membrane bioreactor: operational conditions effect over dominant ecological players.

VIII - CHAPTER 5

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1. Abstract

A bench-scale bioreactor was operated under two different technologies of membrane bioreactor and hybrid moving bed bioreactor-membrane bioreactor at hydraulic retention time 6–16 h, sludge retention time 6–24 days, and mixed liquor suspended solids 3400–7800 mg/L. Analyses of their bacterial community structures showed marked differences between the two technologies for global communities but not for

dominant phylotypes, and the domination of different bacterial phylotypes for the different operational conditions. Tetrasphaera genus was ubiquitous (1.1–19.2 % relative abundance) in both bioprocesses. Fodinibacter (0.04–7.75 %) was found to positively correlate with other dominant phylotypes, highlighting the relevance of Intrasporangiaceae family in membrane-based technologies. Oligotypes distribution of dominant phylotypes showed that certain strains were favored at all operational conditions. Linkage with operational conditions determined that the presence/absence of carriers deeply impacted the relative abundance of dominant phylotypes. The results are relevant to discern the effect of operational conditions over bacterial communities in membrane-based technologies.

2. Introduction

Wastewater management is one of the main environmental problems that urban populations face in today's world. The treatment of such waters before reuse or release to the environment is of crucial importance for the health and sustainability of the biosphere (Metcalf and Eddy, 2012). As a result, several wastewater treatment technologies have been developed

through the last century (Chen and Beck, 1997; Rodríguez-Sánchez et al., 2017). The most widely established approach in various municipal and industrial, fullscale settings is the activated sludge process (Ardern and Lockett, 1915; Ardern and Lockett, 1914; Gonzalez-Martinez et al., 2016).

In more recent years the efficiency of the activated sludge system has been surpassed by a number of novel technologies, with membrane bioreactor (MBR) based processes offering considerable promise. In the MBR, a suspended growth bioprocess is coupled with a membrane filtration system for highly efficient separation of solids and water (Stephenson et al., 2000). The membrane separation process offers several advantages over the conventional activated sludge system, including higher operational mixed liquor suspended solids (MLSS), lower infrastructural/spatial requirements of bioreactors, higher quality effluent generation, and the removal of downstream processing demands, such as sedimentation (Leyva-Díaz et al., 2015; Uribe et al., 2015). However, despite these advantages, the membrane separation process can be negatively impacted by an operational problem referred to as fouling, in which the membrane pores become clogged during filtration (He et al., 2016). Clogging of the membrane can seriously decrease

the performance of the MBR and irreversibly damage the membrane to the point of requiring replacement (Le-Clech et al., 2006; González-Martínez et al., 2014).

To mitigate this operational problem, a moving bed biofilm reactor (MBBR) was coupled to the MBR, resulting in the MBBR-MBR system. In the MBBR, a floating media is added to the bioreactor in order to promote biomass attachment and growth, which permits reactor operation at a lower MLSS while maintaining the same total biomass (Leiknes and Ødegaard, 2007). The lower MLSS benefits the membrane filtration process and makes it less prone to fouling (Leyva-Díaz et al., 2014). If a recycling flow is imposed from the membrane module to the biological reactor the system is termed hybrid MBBR-MBR, and if no such recycling exists the system is referred to as pure MBBR-MBR (Rodriguez-Sanchez et al., 2018).

It is widely accepted that biological wastewater treatment processes have an important microbiological factor defining their performance (Saunders et al., 2016). Indeed, it has been argued that the intrinsic microbial nature of wastewater treatment processes should be considered for their

design (González-Martínez et al., 2014). The recently developed Anaerobic ammonium oxidation (Anammox) process provides a clear example of the importance of understanding microbial community interactions in optimized bioreactor design and operation (Rodríguez et al., 2015). In practice, however, wastewater treatment systems are usually designed under the influence of engineering considerations only (Cydzik-Kwiatkowska and Zielińska, 2016). Thus, a comprehensive characterization of microbial ecosystems in emerging wastewater treatment technologies is essential for future optimization of these bioprocesses.

In this context, the biotechnological potential of the Earth's microbiome is still largely unknown and underutilized and initiatives have been proposed in order to fully utilize its potential for biotechnological reasons, among which wastewater treatment becomes one of the most important (Said and Or, 2017). Novel tools have been developed and are constantly evolving to provide new microbiological insights in the field of water treatment, such as the application of omics approaches in molecular biology (Gedalanga et al., 2013). Such omics approaches allow extensive interrogation of microbial community dynamics within a bioprocess, which

can influence subsequent system design iterations (Strous and Sharp, 2018). One of the most promising molecular biology techniques for the investigation of microorganisms is based on Shannon entropy clustering of genetic sequences, known as oligotypes, achieving a very sensitive characterization of the microbiome within a sample beyond the traditional operational taxonomic unit (OTU) approach (Eren et al., 2014). The characterization of oligotypes within a bioprocess could lead to a fine tuning of key operational conditions to enhance the systems performance.

Following the above considerations, in this research a bench-scale bioreactor was operated as a MBR and as a hybrid MBBR-MBR under different conditions of hydraulic retention time (HRT) (6, 10, and 16 h), solids retention time (SRT) (6–10, 11–16, and 20–24 days), and solids (from 3400 to 7800 mg/L). High throughput sequencing to determine bacterial community structure up to oligotype level was performed and examined for any linkages with operational parameters. The overall aim of the study was the analysis of important bacterial phylotypes to improve future design and operational parameters in MBR and hybrid MBBR-MBR systems.

3. Materials and methods

3.1. Bioreactor description

The research was carried out in a pilot plant located in the wastewater treatment plant (WWTP) Oeste in Granada (Spain), which was continuously fed with real urban wastewater from the primary settler. During the research two different technologies were tested based on the use of a MBR. **Figure 14** shows a diagram of the pilot plant consisting of a cylindrical tank with 272 L of volume connected to a rectangular tank of 78 L capacity where four ZENON ZW-10 membrane modules were submerged. These modules are configured as hollow fiber with external filtration with a nominal surface area of 0.93 m^2, a nominal pore size of 0.04 μm, and an absolute pore size of 0.1 μm. A blower to provide the air scouring of the membrane under a constant flow of 200 L/h was installed. To maintain a homogeneous mixture in the system, MLSS was recirculated to the biological reactor from the membrane tank with a constant flow of 90 L/h. During a first phase, the pilot plant was operated as a conventional MBR system. Upon completion the

plant was completely cleaned and carriers were incorporated into the bioreactor to create a hybrid MBBR-MBR system which was operated under the same conditions to ensure that the results were comparable. To set up the hybrid MBBR-MBR system, the biological reactor was filled with 35 % filling ratio of K1 carriers (Anoxkaldnes®). This carrier is made of high-density polyethylene and shaped into small cylinders (length 7 mm and diameter 10 mm) with a cross inside the cylinder.

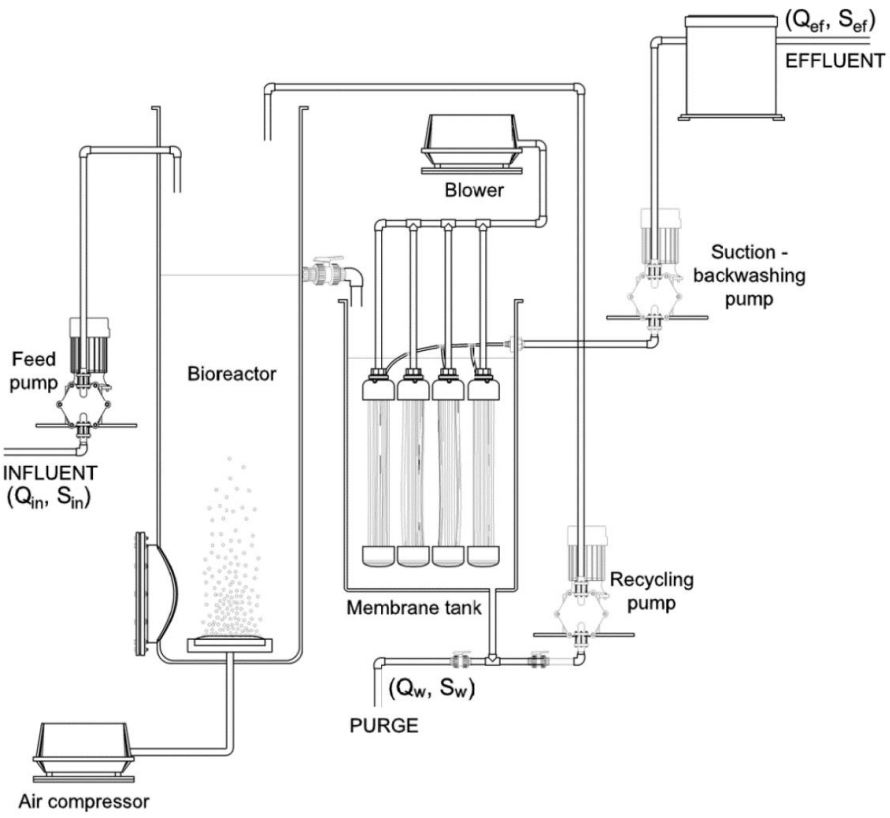

Figure 14. *Schematic of the bioreactors configuration.*

3.2. Bioreactor operation

The pilot plant was operating for 2 years under eight different operational conditions. In the different cycles the key operational variables were: HRT, with 6, 10, and 16 h being tested, respectively; and the MLSS, in the range of 3400 to 7800 mg/L, to reflect the normal range of operation in the WWTP. The SRT ranged from 6 to 24 days, depending on the temperature, the purge flow rate for each cycle, and the influent. The pilot plant was continuously fed real urban wastewater in order to simulate an authentic process with a variable influent caused by the seasonal fluctuation. The same HRT and MLSS was maintained with the MBR and hybrid MBBR-MBR, however, the presence of biofilm in the bioreactor and the typical variation of temperature and influent characteristics caused a slight variation in the SRT during the research.

The operational parameters (HRT, SRT, MLSS, and temperature) and the effluent concentrations of nutrients, for each of the cycle samples collected for the bacterial study are presented in **Table 12**. During the

research, the temperature for each cycle was different depending on the season, varying from 8 to 30 °C.

Table 12. *Operational parameters of the bioreactors analyzed in the study.*

Sample	Technology	HRT (h)	SRT (d)	MLSS (mg/L)	COD (mg/L)	BOD (mg/L)	NH_4^+ (mg/L)	Temperature (°C)
1	MBR	6	9.81	4891.52	38.51	17	43.33	16.9
2	MBR	6	9.81	4596.97	32.12	16	53.39	15.8
3	MBR	10	16.87	4000.61	56.00	11	54.77	28.6
4	MBR	10	16.87	3389.09	26.86	18	48.19	29.5
5	MBR	6	11.23	6140.00	60.15	4	44.36	20.5
6	MBR	6	11.23	5904.24	63.42	21	33.32	19.6
7	MBR	6	11.23	4816.97	47.10	8	36.63	21.2
8	MBR	10	21.67	5710.91	69.94	50	45.86	10.5
9	MBR	10	21.67	4903.03	60.15	24	52.41	11.2
10	MBR	16	13.56	4807.27	102.57	21	38.09	9.4
11	MBR	16	13.56	4800.00	99.30	15	56.47	8.1
12	Hybrid MBBR-MBR	10	23.89	6520.00	63.34	8	52.77	20.4
13	Hybrid MBBR-MBR	10	23.89	5310.91	46.85	11	43.94	20.1
14	Hybrid MBBR-MBR	10	23.89	5109.09	40.25	3	54.89	20.3
15	Hybrid MBBR-MBR	6	6.02	4954.05	106.21	7	42.75	27.5
16	Hybrid MBBR-MBR	6	6.02	5476.36	40.25	3	56.97	29.8
17	Hybrid MBBR-MBR	6	6.02	5654.55	56.74	5	65.04	29.4
18	Hybrid MBBR-MBR	6	8.40	7261.21	81.35	6	52.29	21.6
19	Hybrid MBBR-MBR	6	8.40	7806.67	81.57	5	62.65	20.3

3.3. Physicochemical analyses

Physicochemical analyses were performed on samples from the feed tank, biological reactor, and membrane tank, respectively. The temperature was measured continuously in the biological tank of the pilot WWTP with a thermometer (LANGE LDOTM/SC 100). The COD, BOD_5, and MLSS were determined according to the method of the American Public Health Association, the American Water Works Association and the Water Environment Federation. pH and conductivity were determined using a pH meter (Crison pH 25®) and a conductivity meter (Crison CM 35®), respectively. The ammonia was determined by ion chromatography, using a conductivity meter (Methrom®). For the separation of cations, Metrosep C4-150 column was used with nitric acid (1.7 mmol/L) and dipicolinic acid (0.7 mmol/L) as eluent.

Statistical significance of differential values among the influent and effluent concentrations of COD, BOD_5, and NH_4^+ were analyzed by the means of PERMANOVA calculation through 9999 bootstrap simulations using Euclidean distances computed in PASTv3 software.

3.4. Collection of biological samples and high throughput sequencing

The biomass samples were extracted from the membrane tank during the stationary phase under each of the operational conditions. This biomass contained the suspended biomass for both the MBR and the hybrid MBBR-MBR systems and biomass detached from the carriers in the case of the hybrid MBBR-MBR system. This sampling provided a proper comparison of the effect of the operational conditions over the biomass in the MBR and the hybrid MBBR-MBR systems. The biomass samples were taken immediately to the laboratory, where they were subjected to centrifugation at 821.73 g for 10 min at room temperature. The resulting supernatant was discarded, and the pelleted biomass was stored at −20 °C for subsequent DNA extraction. The DNA was extracted from each of the pelleted biomass samples using a FastDNA SPIN Kit for Soil (MP Biomedicals) according to the manufacturer's instructions. The extracted DNA pools were kept at −20 °C and sent to RTLGenomics laboratory (Lubbock, Texas) for high throughput sequencing using Illumina MiSeq equipment with the Illumina MiSeq Reagents Kit v3. The primer pair 27F-519R was selected for the amplification of the hypervariable regions V1-V2-V3 of the 16S rRNA gene

of the domain Bacteria. The PCR amplification was developed following that utilized by Rodriguez-Sanchez et al. (Rodriguez-Sanchez et al., 2018).

3.5. Bioinformatics pipeline and ecological analysis of samples

The raw data obtained from the high throughput sequencing were processed for ecological analysis using mothur v1.39.5 and VSEARCH (Schloss et al., 2009; Rognes et al., 2016).

First, paired-end reads were merged into contigs through Needleman alignment conditions and assuming a consensus of ambiguous base for overlapping bases with Phred score difference lower than 6. The generated contigs were screened to remove those with any ambiguous base or with more than eight homopolymers. The remaining contigs were then aligned against the SiLVA SEED v132 database using the k-nearest neighbor with a k-mer size of 8 bp and under Needleman conditions and contigs that failed to start and end at the selected primers positions were discarded for the analysis. After alignment screening, contigs were subjected to chimera detection and deletion by VSEARCH implemented in mothur in a selfreference fashion. Nonchimeric sequences were then taxonomically

classified against the RDP Trainset16 using the Wang algorithm and under a cutoff of 80 %. The sequences that could not be classified within the Bacteria domain were removed from the analysis. Later, sequencing errors were reduced by the means of preclustering taking 1 bp difference for each 100 bp of contig (Huse et al., 2010). Then, following Unno (Unno, 2015), singleton sequences were removed from the analysis. Thereafter, a Phylip distance matrix was calculated for all contigs, and the distance data were then taken for the clusterization of contigs into OTUs, which was done using the OptiClust algorithm under 100 iterations in a 3 % difference threshold and using the Matthew's correlation coefficient as metric for the method (Westcott and Schloss, 2015). After clustering, singleton clusters were deemed as failures and removed from the analysis.

For taxonomic classification of OTUs, the contig with lowest distance to all other contigs within each of the OTUs was taken as representative of that OTU. Then, representative sequences were classified against the MiDAS 2.0. (McIlroy et al., 2017). The taxonomic classification was done through the Wang method with a cutoff of 80 %.

3.6. Analyses of similarity of samples and correlation between OTUs

The similarity of samples was analyzed using a phylogenetic and an OTU approach. For the phylogenetic similarity, the Phylip distance matrix was taken for the calculation of a phylogenetic tree through the relaxed neighbor joining algorithm using Clearcut software (Evans et al., 2006). The phylogenetic tree was then used for the calculation of weighted UniFrac through 1000 iterations and subsampling to the smallest sample size among all samples (Lozupone et al., 2011). For the OTU approach similarity, the OUT table generated was taken for the calculation of singular value decomposition, prior to correction of zero values through Bayesian multiplicative replacement by the count zero multiplicative method using zComponents package implemented in R statistical software and a transformation to the centered logratio using robCompositions package implemented in R statistical software (Bian et al., 2017).

The correlation between OTUs was done by proportionality measure using the ρ function (Erb and Notredame, 2016). This was calculated among the dominant OTUs (OTUs with > 5 % relative abundance in at least one

sample). The calculation was developed through generation of Dirichlet distributions under 128 Monte-Carlo simulations using ALDEx2 package implemented in R, and the results were then computed using the package propr implemented in R.

3.7. Influence of technology and environmental parameters

The influence of environmental parameters over the OUT distribution in the samples was calculated by expected effect size, multivariate redundancy analysis and PERMANOVA analysis. For expected effect size method, calculation was done through generation of Dirichlet distributions under 128 Monte-Carlo simulations using ALDEx2 package implemented in R software. The data generated were then used to calculate the sensitivity of OTUs with respect to technology. For multivariate redundancy analysis, the data from environmental parameters (HRT, SRT, MLSS, effluent BOD_5, effluent COD, effluent NH_4^+, and temperature) were normalized to the LOG(X+1) transformation except the presence of carriers, which was treated as a binary variable for presence-absence scenario; and then the OTU distribution and the normalized environmental parameters data were used

to calculate a multivariate redundancy analysis by 499 unconstrained Monte-Carlo simulations under a full permutation model through CANOCO 4.5 for Windows software. The PERMANOVA analyses were calculated using PASTv3 software. To observe the influence of technology, HRT, SRT, MLSS, temperature, and the influent and effluent concentrations of COD, BOD_5, and NH_4^+ over the whole bacterial communities in the MBR and hybrid MBBR-MBR systems, computations based on Bray–Curtis distance and obtained by 9999 bootstrap permutations were used.

3.8. Oligotyping analysis of OTUs of interest

Several OTUs of interest were taken in order to discern their oligotypes distribution. For this purpose, all contigs assigned to these OTUs were used for calculation. First, a Shannon entropy calculation was computed over the alignment of all contigs within the OTU of interest. The results of the Shannon entropy analysis were subsequently taken to configure the oligotype distribution attending to a number of high-entropy nodes, starting with the highest entropy node and adding the following in order one-by-one until the purity score of the oligotypes distribution was equal or higher

than 0.90. (Gonzalez-Martinez et al., 2018). For construction of the oligotype distribution and removal of noise, the minimum substantive abundance was taken as 200. The oligotype distributions were subjected to differential presence analysis to determine their variability with respect to technology through expected effect size and with operational variables through multivariate redundancy analysis, which were conducted in the same fashion as described above.

3.9. Prediction of metagenome in the biological samples

The whole metagenome in the biological samples was predicted using PICRUSt software (Langille et al., 2013). Following the guidelines offered by the developers, the representative sequences of OTUs were classified against the GreenGenes database 13_5 release through the k-nearest neighbor method using a kmersearch method with kmer size of 8 bp and a phylogenetic cutoff of 80 %. The taxonomy table obtained was normalized, and from the normalized table the metagenome of all taxonomies was predicted using PICRUSt.

4. Results and discussion

4.1. Performance of the bioreactors

During the research, the influent of the pilot plant presented a normal value of organic matter in a real urban wastewater with an average COD of 572 mgO_2/L ± 116 mgO_2/L and average BOD_5 of 352 ± 73 mgO_2/L. In relation to the suspended solids, total suspended solids in the influent ranged between 87 and 172 mg/L. The pH and conductivity changed during the research between 7.00 and 8.10 for pH and between 858 and 1471 μS/cm for conductivity. Influent ammonium was 59 ± 15 mgN/L. These fluctuations are caused by the use of real urban wastewater from a standard sanitation system. The PERMANOVA analysis showed that the effluent values of BOD_5 were influenced by technology, and the effluent COD values were influenced by the SRT and HRT ($p < 0.05$) (**Table 13**). The NH_4^+ influent and effluent values were affected by the technology and SRT, and therefore no statistical significance of better performance could be given based on this data.

Table 13. *PERMANOVA results from the analysis of differences of the effluent concentrations in the MBR and hybrid MBBR-MBR at the operational conditions tested.*

	COD		BOD_5		NH_4^+	
	F	**p-value**	**F**	**p-value**	**F**	**p-value**
Technology	0.1937	0.6571	8.4800	0.0027*	4.6230	0.0482*
Temperature	0.6276	0.7699	65.3000	0.1748	2.5410	0.3949
SRT	4.8470	0.0271*	1.5350	0.2244	4.4470	0.0036*
HRT	4.8470	0.0296*	1.5350	0.2215	0.1042	0.8943
MLSS	0.7175	0.6967	3.8710	0.3078	0.7315	0.7233

In both MBR and hybrid MBBR-MBR systems, a high removal of organic matter was obtained. The organic matter removal increased with MLSS, HRT, and temperature. The highest removal rate was obtained for the cycles at higher temperature in both systems. Within the MBR system, an average organic matter removal measured as COD of 88.40 ± 4.0 % was achieved, being higher than 84 % for the HRT of 16 h. For organic matter in the form of BOD_5, the performance was 93.69 ± 4.6 %, reaching higher than 95 % with HRT of 10 and 16 h.

However, the hybrid MBBR-MBR system did not achieve higher average COD removal than obtained in the MBR system, being 88.31 ± 6.8 % (mean value for all conditions tested for the MBBR-MBR).

In contrast to the observations regarding organic matter, ammonium removal efficiency was found to differ between the test systems. Although HRT, temperature, and MLSS were observed to effect the ammonia removal, the presence of biofilm was identified as the most influential variable. Within the MBR system, the ammonium removal was lower, with an average value of 11.7 ± 9.5 %, and the highest value of 31 ± 0.0 % corresponding to an HRT of 10 h. The hybrid system removed more ammonium than the MBR, with an average of 20 ± 7 %. The highest value for ammonium removal, 30 %, corresponded to a 6 h HRT and a temperature of 22 °C.

4.2. Analysis of similarity of biological samples

The singular value decomposition of the biological samples showed that they were primarily differentiated by the SRT and the operational technology, whereas no clustering of samples was observed for the HRT or MLSS at operation (**Figure 15**).

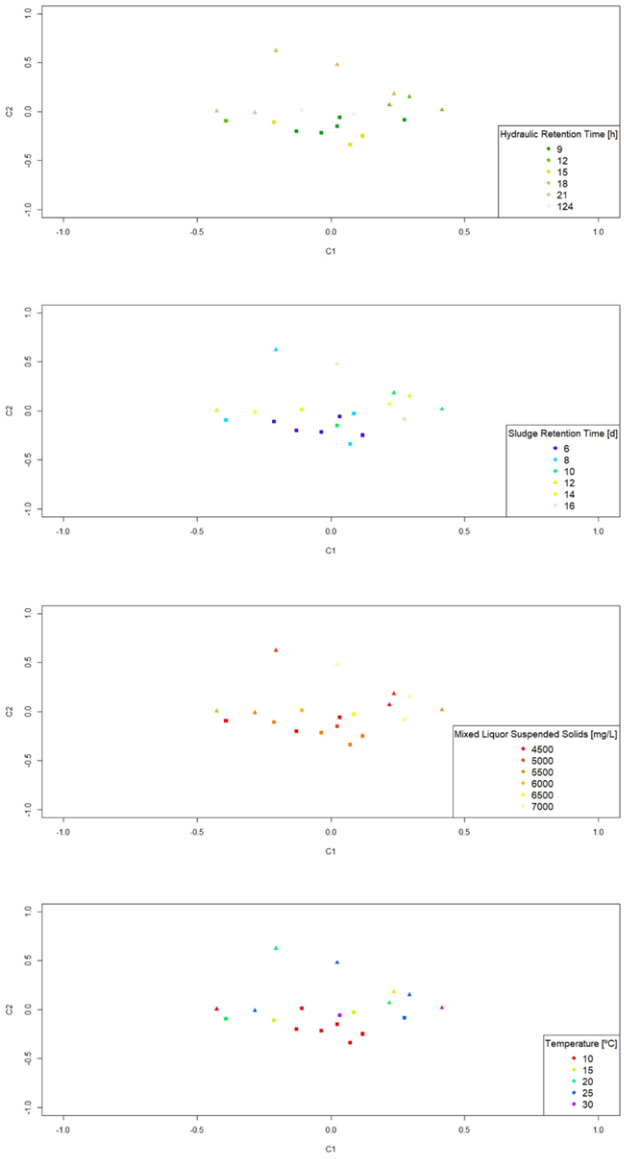

Figure 15. *Singular value decomposition of the zerocorrected centered log-ratio transformed bacterial community structure data (squares represent the MBR samples and triangles represent the hybrid MBBR-MBR samples).*

Differences between the two technologies were also demonstrated by the weighted UniFrac analysis, in which all biological samples from the hybrid MBBR-MBR system but two were clustered together at around the 57–58 % similarity (**Figure 16**).

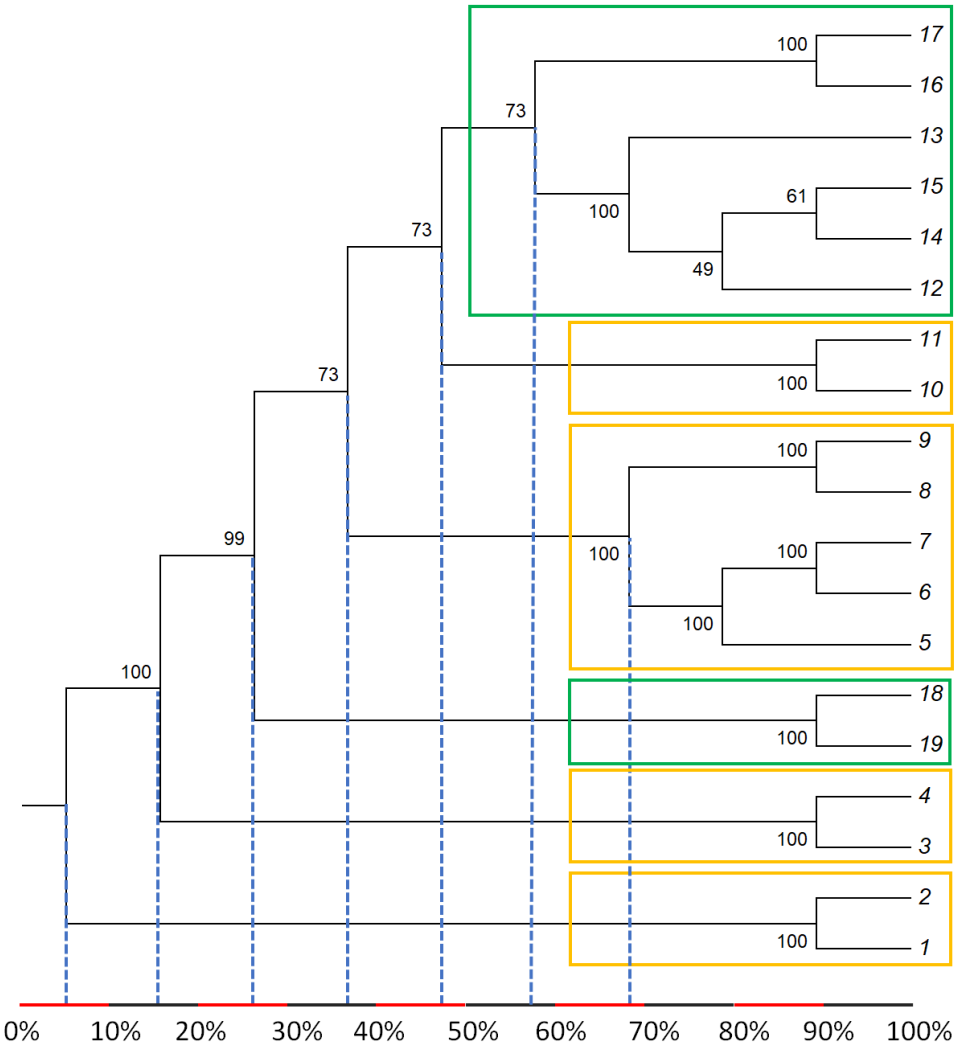

Figure 16. *Weighted UniFrac analysis of the biological samples.*

In this sense, the samples from the MBR and the hybrid MBBR-MBR separated at around the 47–48 % similarity. SRT and HRT defined the groups for the MBR, separating those that shared both operational values, regardless of the MLSS in operation. The two samples from the hybrid MBBR-MBR that were separated from the others had higher MLSS at operation (7261.21 and 7806.67 mg/L, respectively) than all other samples. It is possible that the different operational MLSS concentrations affected the bacterial community composition of the hybrid MBBR-MBR, as observed in hybrid MBBR-MBR systems under variable salinity conditions (Rodriguez-Sanchez et al., 2018). This trend was also shared by almost all samples from the hybrid MBBR-MBR. In contrast, the samples from the MBR were more separated than those from the hybrid MBBR-MBR, suggesting that bacterial communities in the hybrid MBBR-MBR had more stability with respect to operational conditions than those in the MBR. Therefore, it is possible that the introduction of carriers could protect the bacterial community thriving in the hybrid MBBR-MBR against the operational conditions, which would enhance the stability of the system.

4.3. Bacterial community structure of the MBR and hybrid MBBR-MBR

The most important OTUs (> 5 % relative abundance in at least one sample) are shown in **Figure 17**. There was only one OTU that was found at > 1 % relative abundance in all samples, and it was related to Tetrasphaera genus. This suggested that Tetrasphaera was a core microorganism in the MBR and hybrid MBBR-MBR. It has been previously reported that Tetrasphaera is a dominant phosphate-accumulating organism in enhanced biological phosphorous removal systems (Nguyen et al., 2011; Marques et al., 2017; Stokholm-Bjerregaard et al., 2017). Tetrasphaera was very prevalent (> 10 % relative abundance) in the hybrid MBBR-MBR samples operated at high HRT and SRT.

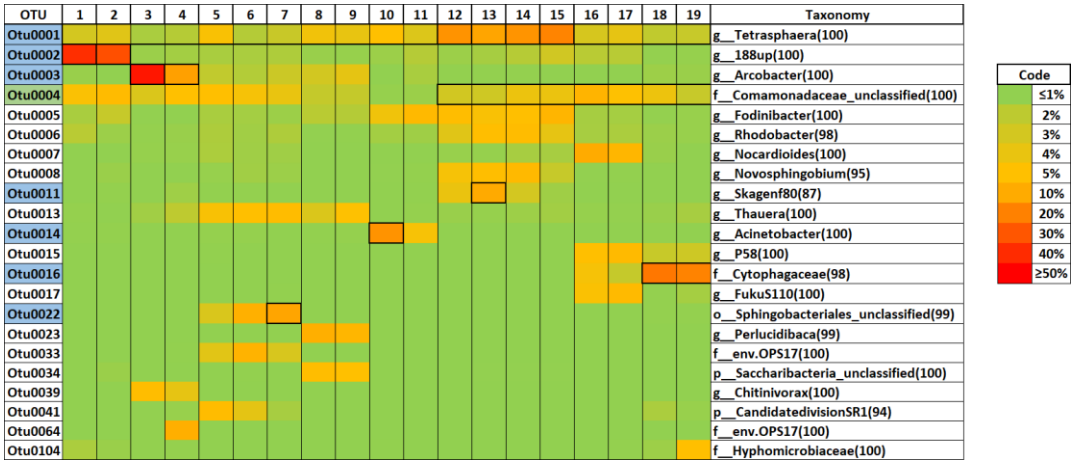

Figure 17. *Heat map of important OTUs.*

Another OTU which was found in all hybrid MBBR-MBR samples was related to a member of the Comamonadaceae family. This OTU was also represented in all samples from the MBR except for those operated at a 16 h HRT. It is possible therefore that this unclassified Comamonadaceae clone may also be of importance in the two membrane-based systems. A subsequent BLAST search revealed 99 % similarity between this Otu0004 and Simplicispira clones KY031326.1 and KY031325.1 (99 % cover and 0 e-value).

Several OTUs were strongly associated with certain operational conditions in the MBR and hybrid MBBR-MBR. Otu0002, affiliated to 188up clone, was dominant for the MBR system operated at low HRT and SRT. The

Otu0003, classified as Arcobacter, was dominant in the MBR at medium HRT and SRT. Arcobacter genus comprises pathogenic strains that have been identified in wastewater treatment for their fast-heterotrophic kinetics (they can form dominant populations in short HRT activated sludge processes) as well as their ability for denitrification (Gonzalez-Martinez et al., 2016).

The unclassified Sphingobacteriales was found to be prevalent at short HRT and medium SRT. Sphingobacteriales-related OTUs have been reported as dominant phylotypes in MBBR and MBR systems and have been linked to degradation of recalcitrant organic compounds (Xue et al., 2016; Torresi et al., 2018). Also, Otu0014, affiliated as Acinetobacter, was dominant at high HRT and medium SRT. Acinetobacter genus, which contains some pathogenic strains, has been widely reported in wastewater treatment systems such as MBR and Acinetobacter heterotrophic-nitrifying strains have previously been isolated from these systems (Zhao et al., 2010; Harb and Hong, 2017). The clone Skagenf80 was very abundant in the hybrid MBBR-MBR at medium HRT and high SRT. Finally, the uncultured Cytophagaceae clone was dominant at short HRT and SRT. Cytophagaceae

members identified in MBR systems have previously been reported to be associated with the removal of recalcitrant total organic carbon (TOC) (Phan et al., 2016).

The proportionality analysis showed that several OTUs were positively correlated (**Figure 18**), that is, their relative abundance increased together in the biological samples with statistical significance ($\rho > 0.65$). In this sense, Otu0001 was correlated with Otu0005 and Otu0006, Otu0008 with Otu0006, Otu0011 with Otu0008, Otu0005 with Otu0006, and Otu0016 with Otu0017, respectively. Conversely, Otu0005 was negatively correlated ($\rho < -0.65$) with Otu0041. Therefore, Otu0005 and Otu0006 were significantly linked with the relative abundance of other dominant OTUs in the system. These OTUs were taxonomically classified as Fodinibacter and Rhodobacter. The only isolated strain of Fodinibacter, F. luteus, was found in a salt mine environment and showed capabilities for aerobic degradation of organic matter and reduction of nitrate to nitrite (Wang et al., 2009). Fodinibacter has not been previously identified as a dominant phylotype in wastewater treatment system. In this sense, the presence of Fodinibacterrelated bacteria in the MBR and hybrid MBBR-MBR opens new

insights into the ecological roles of this genus in membrane-based technologies. Rhodobacter OTUs were reported to be favored at short HRT conditions in MBR systems (Silva et al., 2016). Moreover, Fodinibacter and Rhodobacter were correlated with the ubiquitous OTU in the MBR and hybrid MBBR-MBR under all conditions, that is, Tetrasphaera, which suggests the potential importance these two OTUs for the functioning of the bioreactors. Conversely, the Skagenf80 clone showed correlation with Novosphingobium, the Cytophagaceae clone showed correlation with the FukuS110 clone, and there was a negative correlation between Fodinibacter and the Candidate Division SR1 clone. It has been shown that Intrasporangiaceae members, among which Fodinibacter and Tetrasphaera are included, have the capacity to accumulate phosphate (Kong et al., 2005). The proportionality analysis further suggested the high ecological relevance of Fodinibacter and Rhodobacter in the MBR and hybrid MBBR-MBR under the operational conditions tested.

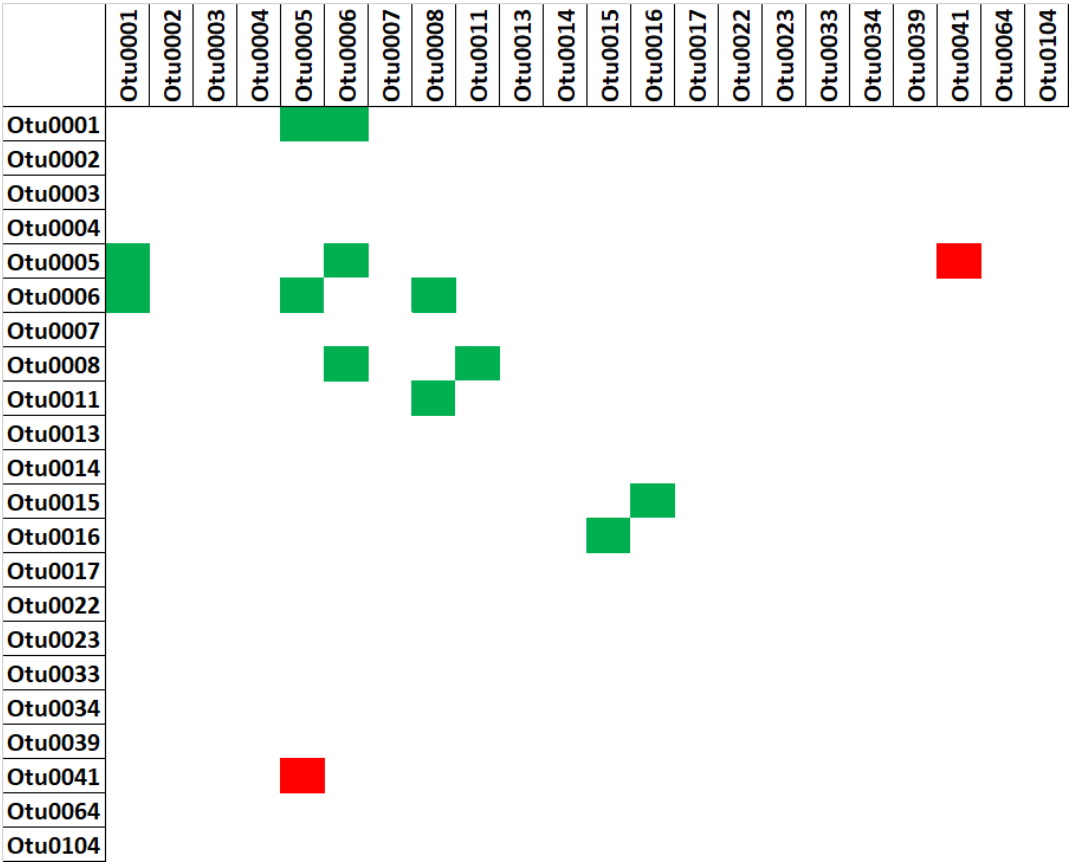

Figure 18. *Results of the proportionality analysis among important OTUs (green cell indicates a ρ coefficient value > 0.6).*

4.4. Linkage of bacterial community structure and operational parameters

The PERMANOVA analysis regarding the bacterial community structure showed that the technology, the HRT, and the SRT had significant statistical influence ($p < 0.05$) over the bacterial community structure present

in the bioreactors (**Table 14**). Conversely, other parameters such as temperature, MLSS concentration, or influent or effluent concentrations of COD, BOD_5, and NH_4^+ did not show significant effect ($p > 0.05$) over the bacterial community composition. In this sense, only the operational variables were of importance for the composition of the bacterial community structure. These findings are supported by previous, similar reports showing that HRT and SRT are variables that greatly influence the bacterial populations' dynamics in MBR and MBBR-MBR systems (Rodriguez-Sanchez et al., 2018; Reboleiro-Rivas et al., 2016). It was interesting to notice that the bacterial communities were not influenced by influent concentrations of organic matter and ammonium

Table 14. *PERMANOVA analysis showing the influence of the operational parameters and the effluent concentrations of COD, BOD_5 and NH_4^+ over the bacterial community structure of the MBR and hybrid MBBR-MBR during all the experimentation period.*

	F	**p-value**
Technology	3.151	0.0008*
Temperature	0.6984	0.9655
SRT	2.125	0.0049*
HRT	7.248	0.0001*
MLSS	1.054	0.3316
COD	1.391	0.1374
BOD_5	0.8804	0.7471
NH_4^+	0.415	0.9926

The expected effect size comparing the distribution at the MBR and hybrid MBBR-MBR showed that only Otu0003, Otu0036, Otu0037, Otu0040 and Otu0076 and Otu0091 had significantly different relative abundances between the two technologies (**Figure 19**). All of these except Otu0003, classified as Arcobacter (6.56 % mean in the MBR vs. 0.16 % mean in the hybrid MBBR-MBR), were of relatively low prevalence in both reactors (0.16 % vs. 0.61 %, 0.06 % vs. 0.59 %, 0.15 % vs. 0.52 %, 0.01 % vs. 0.44 % and 0.08 % vs. 0.27 %) and were classified as PeM15 clone, PeM15 clone, Roseomonas, GKS98 freshwater group clone, and Reyranella, respectively. This result suggested that the relative abundance of dominant OTUs except Arcobacter was not different between the MBR and the hybrid MBBR-MBR regardless of the operational conditions. Thus, operational parameters were important for the selection of dominant OTUs instead of technological configuration of the bioreactor, which highlights the adaptation of the dominant OTUs to certain operational scenarios as shown in **Figure 17**.

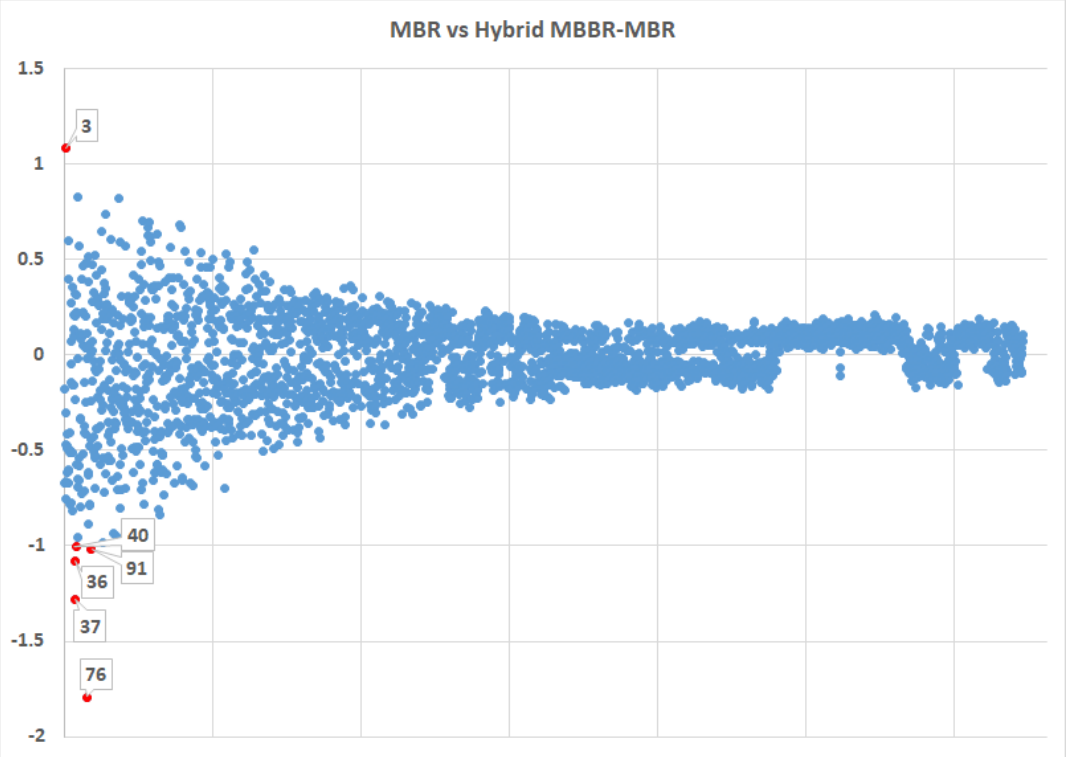

Figure 19. *Expected effect size discriminating the effect of technological configuration over the OTUs in the biological samples.*

The dominant OTUs showed correlation with operational variables during the operation period (**Figure 20**a). The SRT and HRT were positively correlated, while these were inversely correlated with the MLSS and temperature. The effluent BOD_5 was correlated with HRT and SRT. Conversely, these parameters were mainly independent of the effluent COD and NH_4^+.

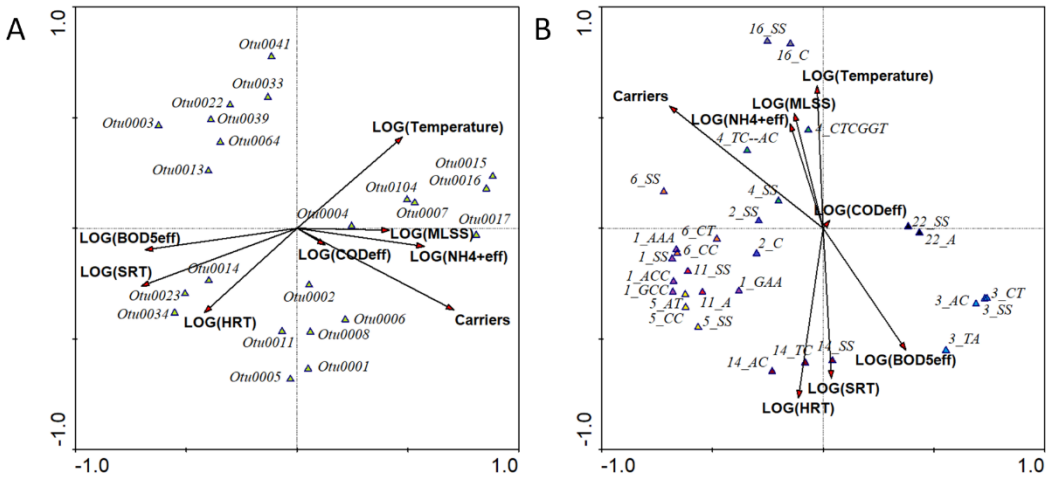

Figure 20. *Biplots linking important OTUs with environmental parameters (A) and oligotypes distribution of dominant OTUs with environmental parameters (B).*

Three clusters of dominant OTUs could be distinguished. One was positively correlated with effluent concentrations of COD, NH_4^+, and MLSS, and contained the Hyphomicrobiaceae clone, the P58 clone, the Cytophagaceae clone, and the FukuS110 clone. The other group was correlated with effluent BOD_5, HRT, and SRT, and accounted for Acinetobacter, the Saccharibacteria clone, and the env.OPS17 clone. The third group was related with SRT and temperature and contained all other dominant OTUs.

The dominant OTUs more related to effluent BOD_5 were Acinetobacter and the Saccharibacteria clone, while significant negative correlations were

associated with Nocardioides and Rhodobacter. The Hyphomicrobiaceae and Cytophagaceae clones were the most positively correlated OTUs with NH_4^+ concentrations in the effluent, while for Arcobacter, Thauera, and the clone Skagenf80 the opposite trend was observed. In this way, the multivariate redundancy analysis highlighted the ecological importance of Arcobacter, Thauera, Nocardioides, Rhodobacter, the Saccharibacteria clone, the Hyphomicrobiaceae clone, and the Cytophagaceae within the bioprocesses studied.

The presence of carriers in the bioreactor was shown to select for dominant OTUs related to Tetrasphaera, Fodinibacter, and Rhodobacter, which seemed to be the most important OTUs in the bioreactors in terms of correlation with other species or ubiquitousness. However, the dominant OTU related to Arcobacter was strongly and negatively correlated with the presence of carriers in the bioreactor. Thus, the presence or absence of carriers within the system was found to influence the appearance of certain dominant OTUs. This result has previously been observed among the nitrifying communities in MBR and hybrid MBBR-MBR treating urban

wastewater (Leyva-Díaz et al., 2015), but such studies did not investigate the impact of different operational conditions in terms of HRT, SRT, and MLSS.

The SRT and HRT, which showed a significant statistical effect over the shaping of the bacterial communities in the bioreactors as by PERMANOVA analysis, were pivotal for the clustering of certain clusters of OTUs. In this sense, OTUs positively correlated with the HRT and SRT were identified with Otu0034 (classified as a Saccharibacteria member), Otu0023 (Perlucidibaca), Otu0014 (Acinetobacter), Otu0001 (Tetrasphaera), Otu0003 (Arcobacter) and Otu0005 (Fodinibacter), among others. OTUs prevalent at low HRT and SRT were Otu0015 (P58 clone), Otu0016 (a Cytophagaceae clone), Otu0017 (a FukuS1110 clone), Otu0007 (Nocardioides) and Otu0004 (a Comamonadaceae clone), among others. As observed, different SRT (6–24 d) and HRT (6–16 h) selected for very different phylogenies in the MBR and hybrid MBBR-MBR.

4.5. Oligotyping analysis of dominant OTUs

The distribution of oligotypes in the dominant OTUs showed the predominance of a certain oligotype over all other competitors and the sensu stricto type (**Figure 21Figure 21.**).

Figure 21. *Distribution of oligotypes of dominant OTUs.*

Thus, the bioprocesses selected for a certain oligotype that outcompeted all others at all operational conditions in both technologies. BLAST searches against the NCBI nt database showed that the oligotypes were related at genus level, highlighting the importance of Tetrasphaera elongata, the uncultured bacterium clone 188up, Arcobacter butzleri, Acidovorax sp. strain W1-6, the uncultured candidate division TM7 bacterium clone Skagenf80, Acinetobacter johnsonii strain ATCC 17909, the uncultured Flexibacteraceae bacterium clone LiUU-3-229, and the uncultured Sphingobacteria bacterium clone SeqSEEZ81 (**Table 15**).

Table 15. *BLAST results against the NCBI nt database of the representative sequences of oligotypes found for the dominant OTUs*

OTU	0001	0002	0003	0004	0005	0006	0011	0014	0016	0022
Oligotype	ACC	C	AC	TC--AC	CC	CC	A	TC	C	A
Accession number	NR_024735	AY212640	L14626	MF370622	AY710281	AB077986	DQ640696	NR_117624	AY509281	JN367106
evalue	0	0	0	0	0	0	0	0	0	3.00E-177
Coverage	99	98	99	99	99	99	96	99	97	99
Identity	99	99	97	99	99	99	97	98	92	90
Taxonomy	*Tetrasphaeraelongata*	Uncultured bacterium clone 188up	*Arcobacter butzlerii*	*Acidovorax sp.* strain W1-6	Uncultured *Intrasporangiaceae* bacterium clone ska19	*Rhodobacterglu conicum*	Uncultured candidate division *TM7* bacterium clone Skagenf80	*Acinetobacter johnsonii*strain ATCC 17909	Uncultured *Flexibacteraceae* bacterium clone LiUU-3-229	Uncultured *Sphingobacteria* bacterium clone *SeqSEEZ81*
Competing oligotype 1	GCC		TA	CTCGGC	AT	CT		AC		
Accession number	Y14596		NR_025905	KF826883	LN869375	KC295211		NR_119114		
evalue	0		0	0	0	0		0		
Coverage	99		99	98	96	96		98		
Identity	98		99	98	100	99		99		
Taxonomy	*Tetrasphaeravaronensis*		*Arcobacter cryaerophilus*	*Acidovorax sp.* B2	Uncultured *Intrasporangiaceae* bacterium isolate OTU 23	*Rhodobacter sp.* BA31		*Acinetobacter johnsonii*strain DSM 6963		

Table 16. BLAST results against the NCBI nt database of the representative sequences of oligotypes found for the dominant OTUs (continue)

OTU	0001	0002	0003	0004	0005	0006	0011	0014	0016	0022
Competing oligotype 2	GAA		CT							
Accession number	NR_043460		NR_117024							
evalue	0		0							
Coverage	97		100							
Identity	98		97							
Taxonomy	*Tetrasphaeravanveenii*		*Arcobacterthrophiarum*							
Competing oligotype 3	AAA									
Accession number	NR_024735									
evalue	0									
Coverage	99									
Identity	99									
Taxonomy	*Tetrasphaeraelongata*strain Lp2									

The multivariate redundancy analysis showing the linkage of oligotypes with the environmental variables revealed that the oligotypes from the same OTU clustered together except for Rhodobacter (**Figure 20**b). Ordination of oligotypes with respect to environmental variables was similar as the ordination of the OTU they belong to under the same environmental variables. The highest oligotype variability was found for Otu0004 and Otu0006, in which the sensu stricto distribution were less positively correlated with temperature, MLSS and effluent NH_4^+, respectively. These results indicated that different oligotypes had very similar ecological niches and suggested that the dominant oligotypes were still dominant at all operational conditions tested. The expected effect size suggested that there were no differences for the relative abundance of oligotypes with respect to technology. The presence of carriers in the bioreactor did not show any influence in the ordination of oligotypes within an OTU. Thus, the different environmental parameters and technological configurations tested promoted the presence of the same dominant oligotypes, suggesting a strong capacity for adaptation to varying

conditions. These dominant oligotypes could be of relevance for the ecology of MBR and MBBR-MBR systems.

4.6. Prediction of metagenome in the biological samples and insight into potential function of dominant phylotype: Focus on nitrification, denitrification, and phosphorus removal

The contributions of the dominant OTUs to the activity involved in the nitrogen and phosphate cycles in wastewater treatment systems, as defined by Gonzalez-Martinez et al. and Gil-Pulido et al. (Gonzalez-Martinez et al., 2018b; Gil-Pulido et al., 2018), are shown in **Figure 22**. With respect to nitrification genes, the autotrophic ammonia oxidation genes amo and hao were not found in any of the samples. This result highlighted the importance of heterotrophic nitrification in the MBR and hybrid MBBR-MBR, which should be further analyzed in the future. The metagenome prediction results suggested that the potential ammonium oxidation was carried out by heterotrophic nitrification metabolisms, among which the cytochrome c-552 was the only one found to be active for the high-relative-abundance phylotypes. Only Otu0010 had a contribution to the activity of this

cytochrome (in a range of 0.01–26.43 % in all samples) among the top-abundant phylotypes. Otu0010 was affiliated to Tessaracoccus genus as determined from the MiDAS 2.0 database.

Figure 22. *Heat map showing the contribution to the selected genes involved in the nitrogen cycle and phosphate cycle in wastewater treatment systems for the dominant OTUs found in the MBR and hybrid MBBR-MBR at all conditions tested.*

Interestingly, there was a high potential contribution of the dominant phylotypes in the MBR and the MBBR-MBR systems with respect to the

nitrate and nitrite reduction. The Otu0022 was the top potential contributor to the nitrate reductase (NADH) activity (25.00–100.00 % in the samples where it was present). Other OTUs with high contributions to nitrate reduction were Otu0006, Otu0007, Otu0008, Otu0009, Otu0011, Otu0013, Otu0014, and Otu0016. These results are in accordance with the multivariate redundancy analysis (RDA), in which OTUs Otu0011 and Otu0013 (the Skagenf80 clone and Thauera) were negatively correlated with effluent NH_4^+. This may suggest that they could thrive in the bioreactors at lower NH_4^+ concentration which, linked to their potential contribution to nitrate reduction, may imply their ecological role in denitrification in the MBR and hybrid MBBR-MBR.

Many dominant phylotypes could potentially develop nitrite reduction. In addition to all those with the capability to reduce nitrate, OTUs Otu0002, Otu0003, Otu0004, Otu0005, and Otu0011 were identified for potential nitrite reduction activity. The negative correlation of Otu0003, classified as Arcobacter, with effluent NH_4^+ could suggest its potential role as a nitrite reducer in the bioprocesses studied, similarly to the relationships between NH_4^+ and OTUs related to Skagenf80 andThauera.

Many dominant OTUs appear to have the potential to contribute polyphosphate kinase capacities, which has been closely related to the phosphate biological removal (Gil-Pulido et al., 2018). These were Otu0002, Otu0003, Otu0004, Otu0005, Otu0006, Otu0007, Otu0008, Otu0009, Otu0013, Otu0014, Otu0016, and Otu0022. Thus, these OTUs could be potentially involved in the removal of phosphate in the MBR and MBBR-MBR systems.

The authors also note the metabolic versatility of several of the dominant OTUs, among which those related to Rhodobacter, Nocardioides, Novosphingobium, Thauera, Acinetobacter, the Cytophagaceae clone, and the Sphingobacteriales clone were potentially identified for complete denitrification and phosphorous removal. The metagenome prediction results therefore suggested that the dominant phylotypes identified in the MBR and hybrid MBBR-MBR systems could potentially develop ecological roles of nutrient removal in addition to organic matter removal.

The lack of dominant phylotypes with potential nitrification metabolism and the high abundance of denitrifiers suggested that it is possible that nitrification is the most sensitive step in the nitrogen removal

process in MBR and hybrid MBBRMBR systems. Future design and operation of MBR and hybrid MBBR-MBR treating urban wastewater should take into account that potential nitrification activity would be linked to minor phylotypes. Greater emphasis should be put on this pathway to ensure optimization of the bioprocess to achieve efficient nitrogen elimination.

However, no activity in the nutrient cycling was predicted for the dominant Otu0001. In spite of this, the metagenome prediction of this OTU showed that it has a very high contribution for a number of genes, some of which are shown in **Table 17**.

Table 17. *Genes for which the Otu0001 was the top contributor among all samples.*

Gene	Mean contribution across all samples (%)
UDP-apiose/xylose synthase	92.42
licheninase [EC:3.2.1.73]	92.40
alpha-1,3-rhamnosyltransferase [EC:2.4.1.-]	92.35
protein MpaA	92.33
CDP-glycerol glycerophosphotransferase [EC:2.7.8.12]	92.14
chorismate mutase / prephenate dehydrogenase [EC:5.4.99.5 1.3.1.12]	92.03
phosphonopyruvate decarboxylase [EC:4.1.1.82]	91.90
deoxyribonuclease I [EC:3.1.21.1]	91.77
phosphoenolpyruvate phosphomutase [EC:5.4.2.9]	91.74
anthranilate synthase/phosphoribosyltransferase [EC:4.1.3.27 2.4.2.18]	91.62
cysteine/O-acetylserine efflux protein	91.43

Among them, the high contribution (mean value of 92.14 % in all samples) of mpaA protein suggested the potential high activity of this OTU in the hydrolysis of murein peptides. The hydrolysis of murein components could be related to the destruction of cell walls, which could suggest the potential role of Tetrasphaera in the scavenging of biomass in the MBR and hybrid MBBR-MBR under the conditions tested. The scavenging role of Tetrasphaera would also be related to its high activity of licheninase (92.40 % mean value), which has been linked to degradation of complex organic compounds found in lichens. The high potential activity related to the phosphotransferase system (phosphonopyruvate decarboxylase, phosphoenolpyruvate phosphomutase, and cysteine/O-acetylserine efflux protein) may suggest that Tetrasphaera thrived in the bioprocesses studied through this particular metabolism.

5. Conclusions

A bench-scale conventional MBR and a hybrid MBBRMBR were operated for the treatment of real urban wastewater under HRT 6–16 h, SRT 6–24 days, and MLSS 3400–7800 mg/L to study their bacterial ecology. The

bacterial community structure was affected mainly by technology choice, with SRT and HRT also impacting to a certain degree. Tetrasphaera was found in all biological samples, highlighting its important role in membrane-based technologies, which could be linked to scavenging of other organisms such as bacteria and lichens, as suggested by metagenome prediction. The presence of carriers greatly affected the relative abundance of dominant OTUs. Dominant OTUs had a low evenness oligotype distribution under all conditions with all oligotypes from the same OTU sharing an ecological niche. Many dominant phylotypes had potential activity for denitrification and phosphorous removal, while nitrification activity was not found among these phylotypes. The results obtained enhance our knowledge about the microbial communities in membrane-based technologies for the treatment of urban wastewater and highlight the relevance of commonly disregarded phylotypes from the Intrasporangiaceae family.

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IX - RESULTS AND DISCUSSION

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Given that throughout the chapters 1, 2, 3, 4 and 5 have already been presented and discussed, in an extremely detailed manner, each of the partial results obtained throughout these years of research, our objective in this section is nothing more than trying to evaluate them together, highlighting exclusively from each of them those findings that we consider especially relevant when responding to both the main objective of this research and the secondary ones.

We intend, in this way, that this analysis serves mainly to convey an overview of the behavior and influence on the elimination of different types of drugs and contaminants of emerging concern, by MBR and MBBR-MBR systems working at low hydraulic retention times.

All these results will be discussed based on the scientific literature consulted, to finally determine in the conclusions of this study, whether this type of systems can be an alternative to conventional treatments in cases of expansion of active sludge plants that have remained underdeveloped and

to what extent can contribute to eliminate certain compounds of emerging concern, such as those studied in the present investigation.

About the kinetic study, respect to the influence of temperature on the start-up of the MBR, it has been shown that the values of Y_H and b_H increased with temperature. In this regard, temperature of mixed liquor had an effect on the growth and decay of biomass. Regarding Y_H, this trend was in accordance with the necessity of biomass for satisfying its maintenance energy requirements previous to use oxygen for biomass growth. In relation to b_H, the decay increased with the temperature since the microbial activity also rose (Pollice et al., 2007). Moreover, the b_H values obtained in this study were lower than those reported by Ekama et al., (1986) for conventional activated sludge systems (0.24 day^{-1}), so the fraction of biomass oxidized per day was lower for the MBR.

Otherwise, napierian logarithm of the heterotrophic kinetic parameters was correlated with the inverse of temperature, except for the values of phase 4, characterized by the lowest temperature (11.5 °C).

This deviation of the values corresponding to phase 4 in relation to the general trend is probably due to the more favorable operation conditions of HRT and MLSS that characterized this phase (HRT = 10 h and MLSS = 6,000 mg/L), cancelling out the effect of temperature. This is supported by the fact that $\mu_{m,H}$, $K_{M,H}$, b_H are more positively correlated with the variables HRT and MLVSS than temperature. This effect was also observed when the $r_{su,H}$ and COD removal were analysed.

Heterotrophic biomasses from phase 2 and phase 3, which were characterized by the highest temperatures, 30.1 °C and 22.9 °C, respectively, showed the highest values of $r_{su,H}$. However, heterotrophic biomass corresponding to phase 4 had higher values of $r_{su,H}$ than heterotrophic bacteria from phase 1 in spite of its lower value of temperature (11.5 °C). This is explained as a consequence of the higher influence of HRT and MLSS compared with temperature.

Finally, the lowest values for $r_{su,H}$ were in phase 1, with the most unfavourable operational conditions regarding HRT and MLSS, with values

of 6 h and 4,000 mg/L, respectively, as well as a low value of temperature (14.2 °C).

Furthermore, it should be hightlighted that heterotrophic biomass required less time for organic matter oxidation during the start-up of phase 2 due to its higher $r_{su,H}$. Moreover, less time would be required to accomplish a steady state in the operational conditions of the start-up of phase 2.

This was in accordance with the COD removal efficiencies obtained in the four phases. Heterotrophic biomass from phase 2 showed the highest COD removal, followed by the biomass from phases 3 and 4. Results obtained concerning COD removal for phase 4 also highlighted the higher influence of HRT and MLSS in relation to temperature. During the phase 1, the lowest values of COD removal performance were obtained due to their less advantageous operation conditions. In light of this, Arévalo et al., (2014) got COD removal rates varying between 98.0 % and 98.9 % at HRT of 35 h and temperature fluctuations from 9 °C to 33 °C, which were higher than those obtained in this research (Figure 8b). Poyatos et al. worked with a MBR

at HRT values of 8.05 h and 11.71 h and temperature variations from 8.3 °C to 23.9 °C, obtaining COD removal efficiencies similar to those obtained in this study (84-94 %) (Poyatos et al., 2008).

About the kinetic study of the effect of bisphenol A on the rates of organic matter removal, decay and biomass generation in a MBR, as we could see detailed in chapter 2, the main results obtained were:

Regarding dynamic and static oxygen uptake rates: R_S and OUR, in phases 1 and 4 the respirometric assays in absence of BPA had a higher duration (5,938 s and 4,350 s) than those obtained in presence of this compound (4,325 s and 1,544 s). Nevertheless, in phases 2 and 3, the presence of BPA increased the duration of the respirometric test, 4,214 s and 2,772 s, respectively, in relation to the experiments without BPA, 3,650 s and 1,743 s, respectively. Therefore, the presence of BPA at lower temperatures, 18.6 °C for phase 1 and 12.1 °C for phase 4, decreased the duration of the respirometric test.

In general, the BPA reduced the three maximum values of R_S of each respirometric test. This occurred in all operation phases with the exception

of phase 3, which could be due to the fact that working at high biomass concentration (7,000 mg/L) compensated the effect of BPA for this phase.

Independently of the presence of BPA, the duration of the respirometric assays decreased from phase 1 to phase 3, probably due to the fact that phase 2 worked at more favorable operation conditions (HRT = 10 h, T = 31.1 °C and SRT = 16.87 day) than phase 1 (HRT = 6 h, T = 18.6 °C and SRT = 9.81 day). The reduction of duration from phase 2 to phase 3 could be based on the higher biomass concentration corresponding to phase 3 (7,000 mg/L) compared with phase 2 (4,000 mg/L). However, the lowest operation temperature (12.1 °C) of phase 4 cancelled out the most favorable operation conditions regarding HRT (10 h), MLSS (7,000 mg/L) and SRT (21.67 day).

The presence of BPA reduced the maximum value of OUR, i.e. the OUR_{end}, in all operation phases. OUR_{end} was reduced from 6.099 to 5.147 $mgO_2/(L \cdot h)$ for phase 1, from 6.670 to 6.222 $mgO_2/(L \cdot h)$ for phase 2, from 6.501 to 5.764 $mgO_2/(L \cdot h)$ for phase 3, and from 6.453 to 5.364 $mgO_2/(L \cdot h)$ for phase 4.

Regarding heterotrophic kinetic modeling, the amount of hetrotrophic biomass produced per substrate oxidized was higher in presence of BPA ($Y_{H,BPA}$) than in absence of BPA ($Y_{H,n/BPA}$), with the exception of phase 3. Specifically, the increase of $Y_{H,n/BPA}$ values was 2.61-7.37 %, for phase 2 and phase 4, respectively, higher in presence of BPA.

Regarding the maximum specific growth rate, the values of $\mu_{m,H,BPA}$, in presence of BPA, were higher than those obtained in absence of BPA ($\mu_{m,H,n/BPA}$). The values of $\mu_{m,H,BPA}$ exceeded those obtained without BPA in 51.85 % for phase 1, 46.66 % in phase 2, 20.07 % in phase 3 and 23.83 % in phase 4. This implied that the heterotrophic biomass required less time to oxidize organic matter in presence of BPA for all operation conditions. The same trend could be observed for $K_{M,BPA}$ and $K_{M,n/BPA}$. In this regard, the values of $K_{M,BPA}$ were higher than $K_{M,n/BPA}$, with the exception of phase 4, probably due to the operation at the lowest temperature (12.1 °C). The increase percentages of $K_{M,BPA}$ in relation to $K_{M,n/BPA}$ were 53.25 % for phase 1, 38.57 % for phase 2 and 58.61 % for phase 3.

The effect of these variations in kinetic parameters that characterize the heterotrophic biomass in the MBR system are included in the values of $r_{su,H,n/BPA}$ and $r_{su,H,BPA}$. In this regard, it must be highlighted that the substrate degradation rate was increased in presence of BPA, with increases of 46.51 % for phase 1, 24.39 % for phase 2, 12.68 % for phase 3 and 21.95 % for phase 4. That is the reason why organic matter was degraded faster in presence of BPA than in absence of this compound. Heterotrophic biomass corresponding to phase 2 had the highest $r_{su,H}$ regardless of the presence of BPA, which could be due to the highest operation temperature (31.1 °C) and high HRT (10 h), according to **Table 2**. The values of $r_{su,H}$ for phase 3 were lower than those obtained in phase 2 since the temperature, HRT and SRT were also lower, i.e. 20.8 °C, 6 h and 11.23 day, respectively, in spite of its higher value of MLSS. Heterotrophic biomass subjected to the operation conditions of phase 4 showed lower values of $r_{su,H}$ than those corresponding to phase 3, probably due to its lower temperature (12.1 °C). However, these values were higher than those obtained in phase 1 despite the fact that the temperature of phase 1 was higher (18.6 °C). This could be due to the more favorable operation conditions of phase 4 regarding HRT (10 h), MLSS (7,000

mg/L) and SRT (21.67 day) in relation to those characterizing phase 1 (HRT = 6 h, MLSS = 4,000 mg/L, SRT = 9.81 day).

The decay rate for heterotrophic biomass was higher in phase 2 due to its higher temperature (31.1 °C), followed by phase 3 (20.8 °C), phase 1 (18.6 °C) and phase 4 (12.1 °C). It must be pointed out that the presence of BPA reduced the biomass decay rate for heterotrophic biomass as $b_{H,BPA}$ values were lower than $b_{H,n/BPA}$, with reduction percentages of 4.84 % for phase 1, 3.91 % for phase 2, 5.67 % for phase 3 and 9.17 % for phase 4. This implied lower quantity of biomass oxidized per day in presence of BPA. This was supported by the lowest values for OUR_{end}.

The values of $r'_{x,H}$ in absence and presence of BPA had a similar trend to that observed for $r_{su,H}$. The highest biomass growth rate occurred in phase 2, which showed the highest values of HRT and T, followed by phase 3, phase 4 and, finally, phase 1. Thus, at highest temperature (phase 2), $r'_{x,H}$ had the highest value and more organic matter was oxidized to CO_2. The net cell growth rate for heterotrophic biomass increased in presence of BPA, i.e.

72.48 % for phase 1, 28.75 % for phase 2, 13.11 % for phase 3 and 31.25 % for phase 4, which corroborated the highest values for Y_H in presence of BPA.

Regarding the multivariate statistical analysis using Canoco software, the temperature presented a positive correlation with the substrate degradation rate for organic matter removal and the decay coefficient for heterotrophic biomass in absence and presence of BPA ($r_{su,H,n/BPA}$, $r_{su,H,BPA}$, $b_{H,n/BPA}$ and $b_{H,BPA}$). Moreover, temperature showed a higher influence on the species of the system than the rest of variables due to the higher length of its vector.

The HRT and SRT had also a positive correlation with the $r_{su,H,n/BPA}$, $r_{su,H,BPA}$, $b_{H,n/BPA}$ and $b_{H,BPA}$ although their effect on the species was lower than in the case of temperature due to the lower length of their vectors. This supported that the values of $r_{su,H}$ and $r'_{x,H}$ were higher for phase 4 than those obtained for phase 1 in spite of the lower value of T (12.1 °C), whose effect was cancelled out by the HRT and SRT.

Finally, the MLVSS concentration had almost no influence on the $r_{su,H}$ and b_H since the angles between these vectors were of approximately 90º.

This explained that the values of $r_{su,H}$, b_H and $r'_{x,H}$ for phase 2 (MLSS = 4,000 mg/L) were higher than those corresponding to phases 3 and 4 (MLSS = 7,000 mg/L), prevailing the effect of T, HRT and SRT.

About the impact of ciprofloxacin, carbamazepine and ibuprofen on a membrane bioreactor system, as we could see in chapter 3, the main results obtained were:

In the influent characterization, the differences observed were typical from real urban wastewater in relation to the daily fluctuations. During the doping cycles not differences were observed in the pH and conductivity of the biomass getting 7.63±0.38, 7.48±0.60, 7.90±0.16 and 1.91±0.11 of pH and 1,191±142, 1,478±41, 1349±112 and 1.406±15 μS/cm of conductivity both to the cycles 0, 1, 2 and 3 respectively.

The concentration of biomass showed an important change once the doping was done, i.e. cycles 1, 2 and 3 showed statistically significant differences with cycle 0 but not itself. The MLSS decreased from 4,552±535 mg/L in cycle 0 to around 3 g/L in the rest of the cycles (3,003±217, 3,216±163 and 3,381±252 mg/L to cycle 1, 2 and 3, respectively); however, the fixed

solids remained relatively constant, around 600 mg/L. With respect to the organic matter consumption, in spite of the biomass reduction, the organic matter removal remained relatively constant.

About the pharmaceutical degradation, the three compounds presented a high removal, especially at the lowest concentrations. The efficiency of the system to remove pharmaceutical was slightly lower in doping 2 as a consequence of the lower temperature in relation to the other dopings.

Both ibuprofen and ciprofloxacin were not detected in the effluent (removal efficiency above 100 %) and the highest efficiencies were reached for ibuprofen (higher than 94.7 %), however, the yield obtained with carbamazepine was slightly lower, with an average efficiency ranging from 71.9 to 92.9 %. The relative degradation rates from the initial concentration of each pharmaceutical were 0.0154 μg/((μgC$_0$/L)·h·mgMLSS) for carbamazepine, 0.0152 μg/((μgC$_0$/L)·h·mgMLSS) for ciprofloxacin and 0.0160 μg/((μgC$_0$/L)·h·mgMLSS) for ibuprofen.

About the kinetic modeling, the values of Y_H were similar in the different cycles whereas the biomass had higher values of $\mu_{m,H}$ and K_M under the addition of different pharmaceuticals than in the control phase. When the mix of carbamazepine, ciprofloxacin and ibuprofen was added to the MBR system in growing concentrations, the heterotrophic biomass showed higher substrate degradation rates for organic matter, which varied between 183.97 $mgO_2/(L \cdot h)$ and 192.88 $mgO_2/(L \cdot h)$. These values almost doubled the $r_{su,H}$ of the control cycle in absence of pharmaceuticals.

Furthermore, the endogenous or decay coefficients for the biomass under the pharmaceuticals effect were higher than that obtained in the control cycle without these chemicals. Thus, percentages in the range of 9.11 % and 11.16 % of the total quantity of heterotrophic biomass were oxidized per day in the doping cycles, while the percentage of biomass oxidized was 7.41 % in the control cycle.

The cell growth rate and the cell decay rate increased under doping 1, doping 2 and doping 3, so the substrate consumption was compensated, which supports the similar removal efficiencies of organic matter as COD

and BOD_5 both in the control and doping phases. In light of this, in spite of the synergy derived from the use of three pharmaceuticals, the effect of the addition of growing concentrations from doping 1 cycle to doping 3 cycle, and the extended period of time during the doping cycles (9 days), the biomass became stabilized and the organic matter degradation rate and cell decay rate for heterotrophic bacteria remained constant.

Regarding the **effect of the biomass on the biodegradation capacity of a mix of pharmaceuticals (carbamazepine, ibuprofen and ciprofloxacin) in a membrane bioreactor**, as we could see in chapter 4, the concentrations in the influent were did not vary statistically and for the four cycles they were similar. Both systems tested, MBR and hybrid MBBR-MBR, removed pharmaceuticals at high rates, higher than 60 %, although ciprofloxacin in doping 1 for cycle 4 had a very low removal rate: about 11 %.

In general, ibuprofen reached the objective of high removal rates in both systems used in this research. At the lowest doping level, which did not show statistically significant differences between the different cycles under study, it was almost completely removed by the MBR system.

However, for the hybrid MBBR-MBR system the removal rates were lower than with the MBR system: above 83 % and 88 % for cycles 3 and 4, respectively. In the case of doping 2, there were statistically significant differences between cycles 1 and 4. In cycle 1, no ibuprofen was detected in the effluent and in cycle 4 the removal rate was 89 %, being the highest removal rate with the MBR system, which worked at an HRT of 6 h and a temperature above 20 °C.

This research has shown that the moving bed and membrane bioreactors for the treatment of wastewater working at low hydraulic retention time, guarantee a high rate of elimination of conventional and emerging pollutants, showing a considerable ability to adapt and stabilize the sludge in stress situations generated by incremental doping with different types and concentrations of drugs.

About the **insight on the bacterial ecology in membrane bioreactor**, as we could see in chapter 5, the main results obtained were:

In both MBR and hybrid MBBR-MBR systems, a high removal of organic matter was obtained. The organic matter removal increased with

MLSS, HRT and temperature. The hybrid MBBR-MBR system did not achieve higher average COD removal than the obtained in the MBR system, being 88.31 ± 6.8 % (mean value for all conditions tested for the MBBR-MBR).

The singular value decomposition of the biological samples showed that they were primarily differentiated by the SRT and the operational technology, whereas no clustering of samples was observed for the HRT or MLSS at operation.

The samples from the MBR and the hybrid MBBR-MBR separated at around the 47-48 % similarity. SRT and HRT defined the groups for the MBR, separating those that shared both operational values, regardless of the MLSS in operation. The two samples from the hybrid MBBR-MBR that were separated from the others had higher MLSS at operation (7,261.21 and 7,806.67 mg/L, respectively) than all other samples. It is possible that the different operational MLSS concentrations affected the bacterial community composition of the hybrid MBBR-MBR, as observed in hybrid MBBR-MBR systems under variable salinity conditions (Rodriguez-Sanchez et al., 2018). In contrast, the samples from the MBR were more separated than those from

the hybrid MBBR-MBR, suggesting that bacterial communities in the hybrid MBBR-MBR had more stability with respect to operational conditions than those in the MBR. Therefore, it is possible that the introduction of carriers could protect the bacterial community thriving in the hybrid MBBR-MBR against the operational conditions, which would enhance the stability of the system.

There was only one OTU that was found at > 1 % relative abundance in all samples, and it was related to Tetrasphaera genus. This suggested that Tetrasphaera was a core microorganism in the MBR and hybrid MBBR-MBR showing a high prevalence (> 10 % relative abundance) in the hybrid MBBR-MBR samples operated at high HRT and SRT.

The unclassified Sphingobacteriales was found to be prevalent at short HRT and medium SRT. Sphingobacteriales-related OTUs have been reported as dominant phylotypes in MBBR and MBR systems and have been linked to degradation of recalcitrant organic compounds (Xue et al., 2016; Torresi et al., 2018).

The proportionality analysis showed that several OTUs were positively correlated, i.e. their relative abundance increased together in the biological samples with statistical significance ($\rho > 0.65$). In this sense, Otu0001 was correlated with Otu0005 and Otu0006, Otu0008 with Otu0006, Otu0011 with Otu0008, Otu0005 with Otu0006, and Otu0016 with Otu0017, respectively. On the other hand, Otu0005 was negatively correlated ($\rho < -0.65$) with Otu0041.

The PERMANOVA analysis regarding the bacterial community structure showed that the technology, the HRT and the SRT had significant statistical influence ($p < 0.05$) over the bacterial community structure present in the bioreactors. On the other hand, other parameters such as temperature, MLSS concentration or influent or effluent concentrations of COD, BOD_5 and NH_4^+ did not show significant effect ($p > 0.05$) over the bacterial community composition.

The dominant OTUs showed correlation with operational variables during the operation period. The SRT and HRT were positively correlated, while these were inversely correlated with the MLSS and temperature. The

effluent BOD_5 was correlated with HRT and SRT. On the other hand, these parameters were mainly independent of the effluent COD and NH_4^+.

The dominant OTUs more related to effluent BOD_5 were Acinetobacter and the Saccharibacteria clone, while significant negative correlations were associated with Nocardioides and Rhodobacter.

The presence of carriers in the bioreactor was shown to select for dominant OTUs related to Tetrasphaera, Fodinibacter and Rhodobacter, which seemed to be the most important OTUs in the bioreactors in terms of correlation with other species or ubiquitousness. The presence or absence of carriers within the system was found to influence the appearance of certain dominant OTUs. This result has previously been observed among the nitrifying communities in MBR and hybrid MBBR-MBR treating urban wastewater (Leyva-Díaz et al., 2015), but such studies did not investigate the impact of different operational conditions in terms of HRT, SRT and MLSS.

The multivariate redundancy analysis showing the linkage of oligotypes with the environmental variables revealed that the oligotypes from the same OTU clustered together except for Rhodobacter.

The expected effect size suggested that there were no differences for the relative abundance of oligotypes with respect to technology. The presence of carriers in the bioreactor did not show any influence in the ordination of oligotypes within an OTU. Thus, the different environmental parameters and technological configurations tested promoted the presence of the same dominant oligotypes, suggesting a strong capacity for adaptation to varying conditions. These dominant oligotypes could be of relevance for the ecology of MBR and MBBR-MBR systems.

The metagenome prediction results suggested that the potential ammonium oxidation was carried out by heterotrophic nitrification metabolisms, among which the cytochrome c-552 was the only one found to be active for the high-relative-abundance phylotypes. Interestingly, there was a high potential contribution of the dominant phylotypes in the MBR and the MBBR-MBR systems with respect to the nitrate and nitrite reduction. The metagenome prediction results suggested that the dominant phylotypes identified in the MBR and hybrid MBBR-MBR systems could potentially develop ecological roles of nutrient removal in addition to organic matter removal.

The lack of dominant phylotypes with potential nitrification metabolism and the high abundance of denitrifiers suggested that it is possible that nitrification is the most sensitive step in the nitrogen removal process in MBR and hybrid MBBR-MBR systems.

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X - CONCLUSIONS

X - CONCLUSIONS

General Conclusions

Given that the detail of the partial conclusions to each of the objectives initially proposed has been presented throughout this thesis in each of their respective chapters (1-5), we will present in a synthetic way the summary of the main conclusions obtained after 2 years of scientific research.

1. On the effect of temperature on the heterotrophic biomass of a MBR, which was modeled considering the Arrhenius equation and the Monod model in four different start-up phases analyzed at HRT values of 6 h and 10 h, with concentrations of MLSS in stationary states of 4,000 mg/L and 6,000 mg/L, and temperatures of 11.5 °C, 14.2° C, 22.9° C and 30.1 °C, we conclude that:

- In phase 4, the most favorable of all the studied, with 10 h of HRT and 6,000 mg/L of MLSS, there was a breach of the general trend for the adjustment of Arrhenius, probably as a result of

the compensation of the effect of the temperature (11.5 °C) under such conditions. The confirmation of this circumstance is found when analyzing the highest elimination values of $r_{su,H}$, and COD for phase 4 compared, for example, with those of phase 1 (HRT = 6 h, MLSS = 4,000 mg/L and T = 14.2 °C).

- On the other hand, the heterotrophic biomass showed the highest $r_{su,H}$, during phase 2, under the operating conditions of HRT = 10 h, MLSS = 4,000 mg/L and T = 30.1 °C, which meant less time to oxidize the organic matter during the start-up phase and less time to reach the steady state.

2. Regarding the kinetic results, using a respirometer, for the heterotrophic biomass contained in MBR simulating an intrusion of BPA in a MBR system in four different phases of operation, with HRT between 6 and 10 h, MLSS oscillating between 4,000 and 7,000 mg/L, temperatures between (12.1 and 31.1 °C) and SRT between 9.81 and 21.67 days, we conclude that:

- In the presence of BPA, the rate of degradation of the substrate for the elimination of organic matter, $r_{su,H}$, increased with increasing percentages of 46.51 % for phase 1, 24.39 % for phase 2, 12.68 % for phase 3 and 21.95 % for phase 4, which led to a faster consumption of organic matter than in the absence of BPA. The heterotrophic biomass belonging to operation phase 2 (HRT = 10 h, T = 31.1 °C) showed the highest $r_{su,H}$ in the absence of BPA (143.84 $mgO_2/(L \cdot h)$) and in the presence of BPA (190.22 $mgO_2/(L \cdot h)$). A similar trend was observed for the growth rate of the net heterotrophic biomass, $r'_{su,H}$.

- The rate of decomposition of the heterotrophic biomass was reduced as a consequence of the presence of BPA, which implies lower amounts of oxidized biomass per day, with values of 0.0715 day^{-1} for phase 1, 0.1304 day^{-1} for phase 2, 0.0871 day^{-1} for phase 3 and 0.0354 day^{-1} for phase 4. In summary, the presence of BPA increased the rate of degradation of the substrate for the elimination of organic matter and the net growth rate of the heterotrophic biomass, and the rate of decomposition of

heterotrophic biomass decreased. Therefore, the heterotrophic biomass of the MBR was not inhibited by the presence of BPA, showing an adaptation capacity to improve the rates of elimination of organic matter and the generation of biomass, and to reduce the rate of decomposition. Among all the analyzed variables, the temperature was the one that showed a greater influence in the rates of elimination of organic matter, decomposition and generation of biomass.

3. Given the results obtained in our MBR pilot plant, treating real urban wastewater with 6 h of HRT, 7.5 days of SRT and 4,552 ± 535 mg/L of MLSS under three different shocks of increasing concentrations of pharmaceutical products at 5, 0.5 and 5 mg/L of carbamazepine, ciprofloxacin and ibuprofen, respectively, we can conclude that the MBR process can be used as a reliable technology to eliminate most of these pharmaceutical products, without reducing the elimination capacity of organic matter, as:

- The addition of compounds of pharmaceutical origin did not affect the organic matter elimination capacity, that is, the rates of elimination of TOC, COD and BOD_5 did not show statistically significant differences (value of $p < 0.05$). The elimination rates of TOC, COD and BOD_5 were higher than 83.78 ± 1.72 %, 89.70 ± 1.37 % and 96.02 ± 0.26%, respectively.
- The elimination rates of ibuprofen, ciprofloxacin and carbamazepine were higher than 94.7 %, 88.7 % and 71.9 %, respectively, under the three concentrations analyzed.
- The degradation rates were 0.0154 μgC/((μgC_0/L)·h·mgMLSS) for carbamazepine, 0.0152 μgC/((μgC_0/L)·h·mgMLSS) for ciprofloxacin and 0.0160 μgC/((μgC_0/L)·h·mgMLSS) for ibuprofen.
- Statistically significant differences were observed in MLVSS, which indicated that there was a decrease in the biomass present in the MBR system. However, these differences were not observed in the elimination of organic matter, since the higher cell growth rate annulled the higher rate of cellular

decomposition due to the chemical stress caused by the addition of pharmaceutical products in the MBR system. This showed that the elimination of COD and BOD_5 was similar in the control phase and in the doping cycles. Therefore, despite the synergistic effect caused by the use of three pharmaceutical products, the influence of adding increasing concentrations of them and the effect of the long period of time during the doping cycles, contributed to the stabilization of the biomass, such and as shown by the degradation rate of organic matter (183.97-192.88 $mgO_2/(L \cdot h)$ and the decay coefficient (0.0911-0.1116 day^{-1}) after the increase from the control cycle.

4. Taking into account the results obtained in this investigation, operating a MBR under 5,200-5,700 mg/L of MLSS, two different HRT (6 and 10 h) and three ascending concentrations of a mixture of pharmaceutical compounds (ibuprofen, carbamazepine and ciprofloxacin) with conventional and hybrid MBBR-MBR configurations, we can conclude that the MBR process could be a reliable technology to treat urban wastewater to eliminate these emerging contaminants, since:

- The MBR system eliminated pharmaceutical compounds at rates that were 97.0 % close to the totality of ibuprofen, from 67.5 % to 94.8 % in the case of carbamazepine and from 61.4 % to 92.1 % in the case of ciprofloxacin.
- The effect of the biofilm on the elimination capacity was lower in relation to temperature and HRT. In the hybrid configuration of MBBR-MBR, the elimination capacity ranged between 48.6 and 97.8 %, between 83.7 and 96.6 % and between 10.6 and 81.7 % for carbamazepine, ibuprofen and ciprofloxacin, respectively.
- The degradation rates for the evaluated pharmaceuticals were greater than 0.0066 μgcarbamazepine/(h·mgMLSS), 0.0069 μgibuprofen/(h·mgSSLM) and 0.0068 μgciprofloxacin/(h·mgSSLM).
- The presence of biofilm in the hybrid MBBR-MBR system against the MBR damped the behavior of the biological system, seeing the degradation rate less affected by temperature and HRT.

- The elimination capacity and the degradation rate of the pharmaceutical compounds in the system increase with temperature and decrease with the HRT.

5. In a MBR and a hybrid MBBR-MBR operated on a pilot scale for the treatment of real urban wastewater, under HRT 6-16 h, SRT 6-24 days and MLSS 3,400-7,800 mg/L to study its bacterial ecology, it was found that the structure of the bacterial community was affected mainly by the chosen technology, also affecting SRT and HRT. It was also concluded that:

- In all biological samples, with a relative abundance of 1.1-19.2 %, Tetrasphaera appeared, demonstrating its important role in membrane-based technologies, which could be related to the elimination of other organisms such as bacteria and lichens, as suggests the prediction of the metagenome.
- The presence of carriers affected to a large extent the relative abundance of the dominant OTUs. The dominant OTUs had a distribution of low uniformity oligotypes in all conditions, with

all the oligonucleotides of the same OTU sharing an ecological niche. Many dominant phylotypes had potential activity for denitrification and phosphorus removal, while nitrification activity was not found among these phylotypes.

- The results obtained have served to improve our knowledge about the microbial communities associated with membrane-based technologies for the treatment of urban wastewater, highlighting the relevance of the phylotypes that are not commonly considered in the Intrasporangiaceae family.

Application Conclusions

As general conclusions of application we can say that throughout this investigation it has been demonstrated that in the start-up of this type of MBR and MBBR-MBR systems working at low HRT, the influence of environmental conditions is greater than in the case of working with higher HRT.

It has been proven that the technologies of mobile bed and membrane bioreactors for the treatment of wastewater, working with a low HRT of over

6 h, guarantee a high rate of elimination of conventional and emerging pollutants, showing a considerable capacity to adapt and stabilize their sludge in situations of stress generated by incremental doping with different types and concentrations of pharmaceuticals.

Therefore, this type of technology can be a technically viable alternative to the conventional treatment of activated sludge at half load, when considering the possible extension of the biological treatment of a sewage treatment plant, which today is a generalized need that it affects many plants around the world, constituting an interesting alternative to consider in the face of the growing regulatory restrictions that are going to be extended around the increasingly demanding requirements for the elimination of pharmaceuticals and contaminants of emerging concern in wastewater discharges.

XI - CONCLUSIONES

XI - CONCLUSIONES

Conclusiones Generales

Dado que los detalles de las conclusiones parciales de cada uno de los objetivos se han ido presentado paulatinamente a lo largo de esta tesis en cada uno de sus respectivos capítulos (1-5), plantearemos de una manera sintética el resumen de las principales conclusiones obtenidas tras 2 años de investigaciones científicas.

1. Sobre el efecto de la temperatura en la biomasa heterótrofa de un BRM, que fue modelizado considerando la ecuación de Arrhenius y el modelo de Monod en cuatro fases de puesta en marcha diferentes analizadas a valores de TRH de 6 h y 10 h, con concentraciones de SSLM en estados estacionarios de 4,000 mg/L y 6,000 mg/L, y temperaturas de 11.5 °C, 14.2 °C, 22.9 °C y 30.1 °C, concluimos que:

- En la fase 4, la más favorable de todas las estudiadas, con 10 h de TRH y 6000 mg/L de MLSS, se observó un incumplimiento de la tendencia general para el ajuste de Arrhenius,

seguramente como consecuencia de la compensación del efecto de la temperatura (11.5 °C) en dichas condiciones. La confirmación de esta circunsancia la encontramos al analizar los mayores valores de eliminación de $r_{su,H}$ y DQO para la fase 4 en comparación, por ejemplo, con los de la fase 1 (TRH = 6 h, SSLM = 4,000 mg/L y T = 14.2 °C).

- Por otro lado, la biomasa heterótrofa mostró el $r_{su,H}$ más alto durante la fase 2, bajo las condiciones de operación de TRH = 10 h, SSLM = 4,000 mg/L y T = 30.1 °C, lo que implicó menos tiempo para oxidar la materia orgánica durante la fase de puesta en marcha y menos tiempo para alcanzar el estado estacionario.

2. Respecto a los resultados cinéticos, empleando un respirómetro, para la biomasa heterotrófica contenida en BRM simulando una intrusión de BPA en un sistema BRM en cuatro fases diferentes de operación, con TRH comprendidos entre 6 y 10 h, MLSS oscilando entre 4,000 y 7,000 mg/L, temperaturas entre (12.1 y 31.1 °C) y SRT de entre 9.81 y 21.67 días, concluimos que:

- En presencia de BPA, la tasa de degradación del sustrato para la eliminación de materia orgánica, $r_{su,H}$, aumentó con porcentajes crecientes de 46.51 % para la fase 1, 24.39 % para la fase 2, 12.68 % para la fase 3 y 21.95 % para la fase 4, lo que acarreó un consumo de materia orgánica más rápido que en ausencia de BPA. La biomasa heterotrófica perteneciente a la fase de operación 2 (TRH = 10 h, T = 31.1 °C) mostró el $r_{su,H}$ más alto en ausencia de BPA (143.84 $mgO_2/(L \cdot h)$) y en presencia de BPA (190.22 $mgO_2/(L \cdot h)$). Se observó una tendencia similar para la tasa de crecimiento de la biomasa heterótrofa neta, $r'_{su,H}$.
- La tasa de descomposición de la biomasa heterotrófica se vió disminuida como consecuencia de la presencia de BPA, lo que implica menores cantidades de biomasa oxidada por día, con valores de 0.0715 $día^{-1}$ para la fase 1, 0.1304 $día^{-1}$ para la fase 2, 0.0871 $día^{-1}$ para la fase 3 y 0.0354 $día^{-1}$ para la fase 4. En resumen, la presencia de BPA aumentó la tasa de degradación del sustrato para la eliminación de materia orgánica y la tasa neta de crecimiento de la biomasa heterótrofa, y disminuyó la

tasa de descomposición de la biomasa heterotrófica. Por lo tanto, la biomasa heterótrofa del BRM no fue inhibida por la presencia de BPA, mostrando una capacidad de adaptación para mejorar las tasas de eliminación de materia orgánica y la generación de biomasa, y para reducir la tasa de descomposición. De entre todas las variables analizadas, la temperatura fue la que mostró una mayor influencia en las tasas de eliminación de materia orgánica, descomposición y generación de biomasa.

3. Dados los resultados obtenidos en nuestra planta piloto BRM, tratando aguas residuales urbanas reales con 6 h de TRH, 7.5 días de TRC y 4,552 ± 535 mg/L de SSLM bajo tres choques diferentes de concentraciones crecientes de productos farmacéuticos a 5, 0.5 y 5 mg/L de carbamazepina, ciprofloxacina e ibuprofeno, respectivamente, podemos concluir que el proceso BRM puede utilizarse como una tecnología fiable para eliminar la mayor parte de estos productos farmacéuticos, sin reducir la capacidad de eliminación de materia orgánica, ya que:

- La adición de compuestos de origen farmacéutico no afectó a la capacidad de eliminación de materia orgánica, es decir, las tasas de eliminación de COT, DQO y DBO_5 no presentaron diferencias estadísticamente significativas (valor de $p < 0.05$). Las tasas de eliminación de COT, DQO y DBO_5 fueron superiores a 83.78 ± 1.72, 89.70 ± 1.37 y 96.02 ± 0.26 %, respectivamente.
- Las tasas de eliminación de ibuprofeno, ciprofloxacina y carbamazepina fueron superiores a 94.7, 88.7 y 71.9 %, respectivamente, bajo las tres concentraciones analizadas.
- Las tasas de degradación fueron 0.0154 $\mu gC/((\mu gC_0/L)\cdot h\cdot mgMLSS)$ para carbamazepina, 0.0152 $\mu gC/((\mu gC_0/L)\cdot h\cdot mgMLSS)$ para ciprofloxacina y 0.0160 $\mu gC/((\mu gC_0/L)\cdot h\cdot mgMLSS)$ para ibuprofeno.
- Se observaron diferencias estadísticamente significativas en SSLMV, lo que indicó que hubo una disminución en la biomasa presente en el sistema MBR. Sin embargo, estas diferencias no se observaron en la eliminación de materia orgánica, ya que la

mayor tasa de crecimiento celular anuló la mayor tasa de descomposición celular debido al estrés químico causado por la adición de productos farmacéuticos en el sistema BRM. Esto mostró que la eliminación de DQO y DBO_5 fue similar en la fase de control y en los ciclos de dopaje. Por lo tanto, a pesar del efecto sinérgico causado por el uso de tres productos farmacéuticos, la influencia de agregar concentraciones crecientes de ellos y el efecto del largo período de tiempo durante los ciclos de dopaje, contribuyó a la estabilización de la biomasa, tal y como demuestra la tasa de degradación de materia orgánica (183.97-192.88 $mgO_2/(L \cdot h)$ y el coeficiente de decaimiento (0.0911-0.1116 $día^{-1}$) después del aumento desde el ciclo de control.

4. Teniendo en cuenta los resultados obtenidos en esta investigación, operando un BRM por debajo de 5,200–5,700 mg/L de SSLM, dos TRH distintos (6 y 10 h) y tres concentraciones ascendentes de una mezcla de compuestos farmacéuticos (ibuprofeno, carbamazepina y ciprofloxacina) con configuraciones RBLM-BRM convencionales e híbridas, podemos

concluir que el proceso BRM podría ser una tecnología confiable para tratar aguas residuales urbanas para eliminar dichos contaminantes emergentes, ya que:

- El sistema BRM eliminó los compuestos farmacéuticos a tasas que fueron del 97.0 % a cerca de la totalidad del ibuprofeno, del 67.5 % al 94.8 % en el caso de la carbamazepina y del 61.4 % al 92.1 % en el de la ciprofloxacina.
- El efecto de la biopelícula en la capacidad de eliminación fue menor en relación con la temperatura y el TRH. En la configuración híbrida de RBLM-BRM, la capacidad de eliminación osciló entre 48.6 y 97.8 %, entre 83.7 y 96.6 % y entre 10.6 y 81.7 % para carbamazepina, ibuprofeno y ciprofloxacina, respectivamente.
- Las tasas de degradación para los medicamentos evaluados fueron superiores a 0.0066 μgcarbamazepina/(h·mgSSLM), 0.0069 μgibuprofeno/(h·mgSSLM) y 0.0068 μgciprofloxacina/(h·mgSSLM).

- La presencia de biopelícula en el sistema RBLM-BRM híbrido frente al BRM amortiguó el comportamiento del sistema biológico, viéndose la tasa de degradación menos afectada por la temperatura y el TRH.
- La capacidad de eliminación y la tasa de degradación de los compuestos farmacéuticos en el sistema aumentan con la temperatura y disminuyen con el TRH

5. En un BRM y un RBLM-BRM híbrido operados a escala piloto para el tratamiento de aguas residuales urbanas reales, bajo TRH 6-16 h, TRC 6-24 días y SSLM 3,400-7,800 mg/L para estudiar su ecología bacteriana, se comprobó que la estructura de la comunidad bacteriana quedaba afectada principalmente por la tecnología elegida, afectando también el TRC y el TRH. Además se concluyó que:

- En todas las muestras biológicas, con una abundancia relativa del 1.1-19.2 %, aparecía Tetrasphaera demostrando así su importante papel en las tecnologías basadas en membranas, que podrían estar relacionadas con la eliminación de otros

organismos como bacterias y líquenes, como lo sugiere la predicción del metagenoma.

- La presencia de carriers afectó en gran medida la abundancia relativa de las OTUs dominantes. Las OTUs dominantes tenían una distribución de oligotipos de baja uniformidad en todas las condiciones, con todos los oligotipos de la misma OTU compartiendo un nicho ecológico. Muchos filotipos dominantes tenían actividad potencial para la desnitrificación y la eliminación de fósforo, mientras que la actividad de nitrificación no se encontró entre estos filotipos.
- Los resultados obtenidos han servido para mejoran nuestro conocimiento sobre las comunidades microbianas asociadas a tecnologías basadas en membranas para el tratamiento de aguas residuales urbanas, destacnado la relevancia de los filotipos que comúnmente no se tienen en cuenta en la familia Intrasporangiaceae.

Conclusiones de Aplicación

Como conclusiones generales de aplicación podemos decir que a lo largo de esta investigación se ha demostrado que en la puesta en marcha de este tipo de sistemas BRM y BRLM-BRM trabajando a bajos TRH, la influencia de las condiciones ambientales es mayor que en el caso de trabajar con TRH superiores.

Se ha comprobado que las tecnologías de biorreactores de lecho móvil y de membrana para el tratamiento de aguas residuales, trabajando a bajo TRH por encima de 6 h, garantizan una alta tasa de eliminación de contaminantes convencionales y emergentes, mostrando una capacidad considerable para adaptarse y estabilizar sus fangos en situaciones de estrés generadas por el dopaje incremental con diferentes tipos y concentraciones de fármacos.

Por lo tanto, este tipo de tecnologías pueden ser una alternativa técnicamente viable al tratamiento convencional de lodos activados a media carga, a la hora de considerar la posible ampliación del tratamiento biológico de una estación depuradora de aguas residuales, que hoy es una necesidad

generalizada que afecta a muchas plantas en todo el mundo, constituyendo igualmente una interesante alternativa a considerar frente a las crecientes restricciones normativas que se van a ir extendiendo en torno a los cada vez más exigentes requerimientos de eliminación de fármacos y contaminantes de preocupación emergente en los vertidos de aguas residuales.

XII - FUTURE RESEARCH LINES

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As a complement and/or continuity to the studies carried out and presented throughout this document, a series of research lines that could be carried out in the future are proposed:

- Scaling of the MBR, MBBR-MBR systems for treatment of wastewater with pharmaceutical compounds.
- Study of other compounds of emergent concern, with advanced membrane systems.
- Study of coupled treatments of membrane processes with advanced oxidation processes.
- Analysis of operating costs and implantation of MBR reactors, and comparative with other systems.
- Study of the influence of sludge digestion on bioreactors with membranes for their correct stabilization.

XIII – FUTURAS LÍNEAS DE INVESTIGACIÓN

XIII - FUTURAS LÍNEAS DE INVESTIGACIÓN

Como complemento y/o continuidad a los estudios llevados a cabo y presentados en este documento, se propone una serie de líneas de investigación que podrían llevarse a cabo en el futuro:

- Escalado de sistemas BRM y RBLM-BRM para el tratamiento de agua residual con compuestos farmacéuticos.
- Estudio de otros contaminantes de preocupación emergente con sistemas avanzados de membrana.
- Estudio de tratamientos combinados consistentes en procesos de membrana con procesos de oxidación avanzada.
- Análisis de los costes operativos y de implantación de los BRM y comparativa con otros sistemas.
- Estudio de la influencia de la digestión del fango en biorreactores con membranas para su correcta estabilización.