Understanding the sorption and biotransformation of organic micropollutants in innovative biological wastewater treatment technologies

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2 **in innovative biological wastewater treatment technologies**

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Abstract

New technologies for wastewater treatment have been developed in the last years based on the combination of biological reactors operating under different redox conditions. Their efficiency in the removal of organic micropollutants (OMPs) has not been clearly assessed yet. This review paper is focussed on understanding the sorption and biotransformation of a selected group of 17 OMPs, including pharmaceuticals, hormones and personal care products, during biological wastewater treatment processes. Apart from considering the role of "classical" operational parameters, new factors such as biomass conformation and particle size, upward velocity applied or the addition of adsorbents have been considered.

It has been found that the OMP removal by sorption not only depends on their physico-chemical characteristics and other parameters, such as the biomass conformation and particle size, or some operational conditions also relevant. Membrane biological reactors (MBR), have shown to enhance sorption and biotransformation of some OMPs. The same applies to technologies bases on direct addition of activated carbon in bioreactors.

The OMP biotransformation degree and pathway is mainly driven by the redox potential and the primary substrate activity. The combination of different redox potentials in hybrid reactor systems can significantly enhance the overall OMP removal efficiency. Sorption and biotransformation can be synergistically promoted in biological reactors by the addition of activated carbon. The deeper knowledge of the main parameters influencing OMP removal provided by this review will allow optimizing the biological processes in the future.

Keywords: pharmaceuticals, removal mechanism, biotransformation, sorption, activated carbon

1. INTRODUCTION

The conception of wastewater treatment is moving towards a circular economy approach in the last years, in which the process economy should be balanced with the protection of natural resources and environmental sustainability (WWAP, 2017). Even though, nowadays still most wastewater treatment plants (WWTPs) are based on conventional biological treatment processes designed for the removal of organic matter

45   and nutrients through the combination of anaerobic, anoxic and aerobic bioreactors, as
46   well as complementary physico-chemical separation units. These classical approaches
47   have achieved convincing results in terms of organic matter and nutrients removal.
48   However, these installations are facing a number of challenges mostly derived from the
49   need of reducing their footprint both physically (less land area required) and, especially,
50   from an environmental point of view (less energy consumption, less sludge production,
51   fewer pollutants and green house gases emissions, etc.). The need to advance in
52   resource recovery such as nutrients or reclaimed water completes this challenging
53   scenario.

54   In this context, the problem derived from the presence of chemicals of emerging
55   concern, such as organic micropollutants (OMPs) including pharmaceuticals, hormones,
56   and personal care products, has been widely assessed in different environmental water
57   compartments (sewage, surface, ground and drinking waters) all around the world along
58   the last decades. The effluent discharges from WWTPs constitute the main source of
59   OMPs into the environment (Carmona et al., 2014; Luo et al., 2014; Clara et al., 2011;
60   Monteiro et al., 2010). It has been shown that even conventional technologies are able
61   to remove efficiently some OMPs (Belhaj et al., 2015; Nakada et al., 2006; Carballa et
62   al., 2004), although there is still a significant group of compounds with a recalcitrant
63   behaviour (Clara et al., 2011; Kim et al., 2007; Carballa et al., 2004).

64   Some innovative biological treatment technologies have demonstrated important
65   benefits in terms of operational costs and removal efficiencies for conventional
66   pollutants (organic matter, nutrients or solids). Studies about anammox-based processes,
67   hybrid systems combining different redox conditions, moving bed biofilm and packed
68   bed reactors and anaerobic treatments applied to the water line have been recently n
69   published (Bilal et al., 2017; Luo et al., 2014; Vázquez-Padín et al., 2014; Kartal et al.,
70   2013; Ettwig, 2008). However, there is still a lack of knowledge about their capacity for
71   removing OMPs.

72   There are already legal initiatives that support the importance of broadening the
73   number of pollutants when assessing the quality of water. The European Union has
74   specifically included 12 OMPs in the watch list for emerging water pollutants under the
75   2015's Water Framework Directive (WFD). These micropollutants should be monitored
76   during three years in order to consider their future inclusion in the list of priority
77   substances and to determine their respective discharge limits in function of their
78   possible toxic, estrogenic and mutagenic effects. This should be considered as an
79   indicator of the need to include the removal of OMPs as a parameter for WWTPs
80   optimization, besides other variables normally considered (e.g. efficiency of
81   macropollutant removal, reduction of the operational costs, etc.).

82   The first approach to consider the removal of micropollutants in new WWTP
83   projects is to more deeply understand their removal mechanisms, including the
84   influence of the operating conditions, as well as the role of the microbial community on

their sorption and biotransformation. The authors have a long-term experience of more than 15 years in research on the removal of OMPs in different biological processes, including from more conventional to advanced technologies. The results have shown that the combination of anoxic and aerobic conditions commonly applied in conventional denitrification processes leads to the removal of several OMPs, such as ibuprofen (IBP), naproxen (NPX), celestolide (ADBI) or roxithromycin (ROX) (Suárez et al., 2010). The application of an anaerobic pretreatment step has shown to broaden the number of OMPs that are partially biotransformed (Alvarino et al., 2014). The use of membrane bioreactors (MBR) allows operation at higher biomass concentrations and sludge retention time (SRT), which implies a higher microbial diversity. According to Reif et al. (2011), this explains the improved removal of the pharmaceuticals IBP and NPX in an MBR compared to a conventional activated sludge (CAS) unit. These results suggest that treatment strategies based on the combination of different redox potentials and on the application of high SRT (by the use of membranes, supports or granular biomass) allow reaching higher OMP removal efficiencies ==for most of the OMPs studied==.

This paper is a review of the behaviour of OMPs during biological wastewater treatment processes, focussing on the factors influencing sorption and biotransformation as the main OMP removal mechanisms. It is based on previous experiments on OMP removal with different technological configurations, moving from more conventional processes, such as: i) an aerobic conventional activated sludge (CAS) unit of 2 L (Alvarino et al., 2014) and ii) an upflow anaerobic sludge blanket (UASB) of 4.5 L (Alvarino et al., 2014); to more advanced integrated systems: iii) an autotrophic nitrogen removal process (ELAN®) of 200 L (Alvarino et al., 2015), iv) a hybrid anaerobic-aerobic membrane process (AnHMBR) of 176 L (Alvarino et al., 2016a) and v) a sequential batch reactor coupled to an MBR conceived for operation with direct addition of powdered activated carbon (PAC) (SeMPAC®) of 48 L (Alvarino et al., 2016b). The main characteristics of these five systems are summarized in Table 1 ==and in more detail described in supplementary information. In all the previous studies, a complete OMP mass balance was developed under steady-state conditions by considering the measured OMP concentrations in both the liquid and the solid phases. OMPs biotransformation was considered taking into account a pseudo-first order kinetic model (Alvarino et al., 2015; Ec.1).==

$$k_{biol} = \frac{C_{inf} - C_{eff} - C_{sor}}{VSS \cdot HRT}$$

(Eq.1)

==where Cinf, Ceff and Csor are the concentrations in the influent, the effluent and sorbed onto solids (μg/L); VSS is the volatile suspended solids inside the reactor (gvss/L) and HRT is the hydraulic retention time (d).==

Previous studies have focused on the removal of OMPs under a specific redox potential (Ghattas et al., 2017). Although some recent reviews have evaluated the OMP removal

during biological wastewater treatment processes in hybrid plants (Grandclément et al., 2017; Tiwari et al., 2017), they are mainly focused on the occurrence of OMPs and the evaluation of the effect of some conventional parameters, such as hydraulic or sludge retention time. In general, the information about the combination of different redox potentials in hybrid systems is still scarce. This research supposes a step forward including new parameters affecting OMP removal, especially the relationship between different factors (table S.1). Among others, the influence of biomass conformation on OMP sorption, the role of the OMP chemical structure on their cometabolic removal under different redox potentials, or the effect of adsorbents in OMP sorption and biotransformation has been analysed.

2. SORPTION OF OMPs

Sorption onto particulate matter is especially relevant when the tendency of OMPs to be retained onto sludges (primary and secondary) is high. The term sorption comprises two mechanisms: absorption (micropollutants move from the aqueous phase and enter into the lipophilic cell membrane of biomass or into the lipid fraction of the sludge due to their hydrophobicity) and adsorption (micropollutants are retained onto solids surface due to electrostatic interactions between positively charged compounds and the negatively charged surface of biomass cells) (Sipma et al., 2010).

Sorption of OMPs is a function of their physico-chemical properties, as well as of the characteristics of the sorbent agent. In the case of sludges, the octanol-water coefficient (K_{ow}) and the acid dissociation constant of OMPs (K_a) determine their sorption trend (Suárez et al., 2008; Ternes et al., 2004). Reported results indicate that those compounds with a medium or high lipophilic behaviour (high K_{ow}), such as musk fragrances or hormones, are preferentially removed by absorption onto the sludge (Suárez et al., 2008; Joss et al., 2005), independently of the biomass conformation (granular or flocculent biomass) (Alvarino et al., 2014). On the other hand, OMPs which are ionized or dissociated in the aqueous phase can be removed by electrostatic interactions with the negatively charged surfaces of the biomass, as in the case of the cationic species of the antibiotic trimethoprim (Suárez et al., 2008). The use of the solid–water distribution coefficient (K_d, in L kg^{-1}), defined as the ratio between the concentrations in the solid and liquid phases at equilibrium conditions, is commonly used to determine the fraction sorbed onto sludge (Hörsing et al., 2011).

The amount of OMP present in the solid phase can be divided into different fractions according to the strength of the binding. The first fraction relates to the amount of substance more weakly sorbed (extractable fraction), while the second is referred to that portion more strongly retained (non-extractable fraction) (Bouju et al., 2016; Barret et al., 2012; Bouju et al., 2011). The ultrasonic solvent extraction (USE) methodology is the most common technology used for measuring the OMPs extractable fraction (Ternes et al., 2004), whereas the non-extractable fraction can only be measured by using more advanced techniques such as radiolabelled isotope based methods (Bouju et al., 2011).

164 These advanced methods can be used to assess the changes over time in the distribution
165 of the radioactivity of the radiolabelled parent compound both in the liquid and solid
166 phases, as well as in the gasses produced. The ^{14}C technique was used by Alvarino et
167 al., (2016c) to determine the sorption of the antibiotic sulfamethoxazole (SMX) in
168 bioreactors using different biomass conformations (granular and flocculent biomass)
169 and applying different redox conditions (anaerobic, anoxic and aerobic). Except in the
170 case of the anaerobic granules, the contribution of the non-extractable fraction to the
171 total sorption was always above 50%, with an increasing trend along the duration of the
172 assays, and the overall removal by sorption < 6% of the total radioactivity.

173 Among the different factors which exert a significant role on sorption of OMPs
174 onto sludges, the following have been especially highlighted in WWTPs:

175 *Biomass conformation*

176 The structure of biomass (type and particle size distribution) is expected to have an
177 impact on the removal of micropollutants, as it affects mass transfer between the target
178 compound and the microorganisms. Among suspended biomass, granular conformation
179 is typical of anaerobic reactors (upflow anaerobic sludge blanket (UASB), expanded
180 granular sludge bed (EGSB)), whereas flocculent agglomerations are usually present in
181 activated sludge based processes. Many innovative hybrid configurations consider the
182 use of supports for biofilm development. When flocculent and granular biomasses are
183 compared in terms of the relative amount of OMPs sorbed onto sludge (i.e. referred to
184 the concentration in the liquid phase, as determined by the K_d coefficient, table S.2),
185 higher values are obtained for flocculent biomass, which might be attributed to the
186 higher specific surface area of the floccules compared to the granules (Alvarino et al.,
187 2016a; Alvarino et al., 2014). The same effect is observed when comparing granules
188 with different particle sizes, being sorption enhanced as the granular size decreases
189 (Alvarino et al., 2015). In spite of the lower relative amount of OMPs sorbed onto
190 granular sludge compared to flocculent biomass, the contribution of sorption to the
191 OMPs removal in reactors working with this type of biomass (such as UASB units) is
192 higher since usual biomass concentrations are very high compared to CAS units (7-30
193 vs. 1-2 g_{VSS} L^{-1}, respectively) (Alvarino et al., 2016a; Alvarino et al., 2014).

194 In the case of the granular biomass, sorption behaviour has been described by an
195 intraparticle diffusion model comprising a two-stage diffusion process: starting with a
196 quick sorption of OMPs onto the surface of the granules followed by a slower
197 intramolecular diffusion step towards deeper layers (Alvarino et al., 2015; Shi et al.,
198 2011). Consequently, the sorbed concentration of the lipophilic OMPs (e.g. musk
199 fragrances) on granular biomass can increase with time, as reported by Alvarino et al.
200 (2016b; 2014). In such cases, the mass balances in granular systems cannot be based on
201 one unique sorption coefficient (K_d) for each OMP, whereas with flocculent biomass the
202 equilibrium is normally reached quickly being the K_d approach appropriate. In order to
203 predict sorption onto biofilms, the two-stage diffusion model was successfully used as

in the case of granules (Wicke et al., 2007; Headley et al., 1998). A biofilm is usually considered as a matrix constituted by extracellular polymeric substances (EPS) in which biomass cells are embedded (Wunder et al., 2011). These EPS may determine different behavior towards OMP removal mechanisms, since their structure shows lower interaction with lipophilic OMPs than the biomass, reducing significantly their sorption onto the biofilm (Wang et al., 2002). EPS can also influence the sorption of hydrophilic OMPs, since cationic and anionic functional groups are available in EPS chemical structure, making possible the establishment of electrostatic interactions (Wunder et al., 2011; Flemming et al., 1996). On the other hand, the apolar functional groups of EPS proteins may sorb apolar organic compounds (Flemming et al., 1996).

Hydrodynamic parameters

Hydraulic retention time (HRT) is directly related to the contact time between the wastewater and the sludge inside the reactor, which is usually in the range of hours for biological reactors. Thisis enough to reach the sorption equilibrium among both phases in flocculent biomass processes (Alvarino et al., 2014; Fernandez-Fontaina et al., 2012). However, as stated in the previous section, the sorption equilibrium is not achieved under these conditions when operating with granular biomass (e.g. in UASB or EGSB reactors). Experiments carried out in UASB reactors at different HRT showed that higher HRT applied in those granular systems led to an enhanced removal of musk fragrances by sorption (the most lipophilic OMPs considered), with negligible effect on the other OMPs (Alvarino et al., 2016a; Alvarino et al., 2014), due to the increase in the contact time. In upflow reactors (such as the UASB concept), the upward velocity applied affects the hydrodynamics and degree of turbulence existing in the sludge bed. It was shown that the increase of the upward velocity enhances the removal of the lipophilic OMPs, due to the direct impact on the liquid-solid OMP mass transfer (Alvarino et al., 2014). Therefore, the application of an internal recirculation to decouple the HRT and the upward velocity would be an alternative to enhance the removal by sorption in granules (Alvarino et al., 2014).

pH and temperature

Sorption is also affected by wastewater characteristics such as its temperature or pH, although the narrow variation range of these variables during a conventional WWTP operation makes these parameters not very relevant. Sorption has been correlated inversely with temperature in the case of the hormone 17α-ethinylestradiol (EE2), with a reduction of K_d values of 20-25% when the temperature was increased from 10 to 30ºC (Zeng et al., 2009). On the other hand, the role of pH on sorption has been related with the dissociation of certain OMPs (through the acid dissociation constant pKa), which can result in the generation of positively charged compounds (prone to interact with the negatively charged surface of sludges) or anions (low interaction). Thus, the cationic species would be adsorbed by Van der Waals-type interactions (Suárez et al., 2008; Golet et al., 2003).

Membrane-based reactors

245   Membrane biological reactors are currently widely used for urban and industrial
246   wastewaters. These systems are based on the combination of a suspended biomass
247   biological reactor and a membrane filtration step in the microfiltration or ultrafiltration
248   range (0.1-1 and 0.01-0.1 μm, respectively). In spite of these low pore sizes, OMPs are
249   not retained by means of size exclusion. Only nanofiltration or reverse osmosis
250   membranes are able to remove OMPs by physical means (Schäfer et al, 2011).
251   Membranes used in MBRs can be classified according to their hydrophilic or
252   hydrophobic nature depending on the properties of the construction material. In the case
253   of the hydrophobic membranes (e.g. polyethersulfone membranes), lipophilic OMPs
254   such as musk fragrances could be retained by sorption onto the membrane (Jermann et
255   al, 2009). Sorption of OMPs by membranes may be influenced by the establishment of
256   electrostatic interactions among the pollutant and the membrane surface (Ghaemi et al.,
257   2011). Nghiem et al. (2002) reported that OMPs can be retained by adsorption onto
258   membranes by the formation of hydrogen bonds, dropping the retention when the
259   membrane is saturated. No significant retention of OMPs within the membrane material
260   is expected for hydrophilic and neutrally charged membranes, such as the well-known
261   Kubota or Zenon modules (flat sheet and hollow fibre, respectively). The interaction
262   among OMPs and the cake layer formed on the membrane surface is another issue
263   which deserves attention, as retention by this layer through sorption and/or size
264   exclusion is possible (Wu et al., 2013; Terzic et al., 2005). This effect was reported for
265   musk fragrances in an MBR using a hydrophilic non-charged microfiltration membrane,
266   where an increase in the solid phase concentrations of these compounds was observed
267   with time due to their retention in the cake layer (Alvarino et al., 2016a). Higher
268   removal for diclofenac (DCF) and ROX was observed in an ultrafiltration MBR
269   compared to a microfiltration MBR, and these differences were also attributed to the
270   retention by the cake layer (Alvarino et al., 2017).

271   *Use of adsorbents*

272   The use of commercially available activated carbon (AC) in different
273   conformations (granular, powdered, pellets, etc.) is a well-established alternative for the
274   removal of a wide number of xenobiotics such as pesticides, taste and odor compounds,
275   and OMPs. This alternative is especially interesting for OMPs which exhibit a low
276   affinity to be retained onto sludge but, on the contrary, with a high affinity to interact
277   with other sorbent agents such as activated carbons (Table 2) (Paredes et al., 2016;
278   Kovalova et al., 2013). The sorption efficiency reached with activated carbon is a
279   function of the characteristics of the OMP considered, such as its electrical charge, and
280   its distribution coefficient (log D). This coefficient depends on the octanol-water
281   distribution coefficient (K_{ow}) and the acid dissociation constant (pK_a) (Kovalova et al.,
282   2013; Nguyen et al., 2012). Compounds with high log D values, such as carbamazepine
283   (CBZ) or DCF, are readily sorbed onto PAC. Consequently, removal efficiencies above

99% were achieved in an MBR operated with a direct AC addition of 1 g/L (Alvarino et al., 2017). No influence of the use of PAC was observed in this reactor in the case of IBP, NPX or SMX, that are compounds with log D < 1. A gradual saturation of the PAC with time was observed in this system (Alvarino et al., 2017, 2016b). The propensity to breakthrough is related to the charge of the compounds that were retained. The saturation was first observed for negatively charged compounds (such as DCF), afterwards for the neutral substances (such as CBZ), and finally for the positively charged OMPs such as trimethoprim (TMP) (Nguyen et al., 2012, Alvarino et al., 2017, 2016b). The efficiency of sorption onto the AC is very much influenced by the quality of the wastewater treated due to the competition of the matrix with OMPs, as reported by Paredes et al. (2016).

Table 2. OMPs sorption on the biomass and the activated carbon

3. BIOTRANSFORMATION OF OMPs DURING BIOLOGICAL WASTEWATER TREATMENT

Biotransformation of OMPs in WWTPs is related to the chemical reactions induced by the presence of microorganisms in water, such as bacteria, which assimilate the pollutants as growth or maintenance substrates leading to their elimination (Tran et al., 2013; Yang et al., 2013). Although in biological processes complete biomineralization of some OMPs is possible, for most of them the removal is only partial, being some compounds completely recalcitrant (Collado et al., 2014; Clara et al., 2004). Apart from the operational conditions imposed in a given WWTP (HRT, SRT, temperature, process technology, etc.), the biotransformation of OMPs is also a function of the chemical structure of each compound and the concentrations at which they are present. The OMP concentrations determine the type of metabolism (primary or cometabolism), while the type and activity of biomass (which depends on the redox conditions imposed) determine the degree of biotransformation.

Influence of biomass activity: metabolism and cometabolism

The OMPs are present at much lower concentrations than the macropollutants (μg L^{-1} to ng L^{-1} vs. mg L^{-1}, respectively). Thus, it is expected that the energy generated by the OMP biotransformation is not enough to promote the growth of biomass, which implies that the presence of a primary substrate is necessary for inducing the enzymes for the OMP biotransformation by means of cometabolism. During this process, the persistent compounds are converted into more biotransformable intermediate products within the global metabolic pathways (Yi and Harper, 2007).

Cometabolism has been widely studied in the case of nitrification. The simultaneous elimination of ammonium and different OMPs has been reported for EE2,

IBP, NPX, trichloroethylene or isoproturon, as a consequence of the cometabolic biotransformations induced by autotrophic aerobic bacteria (Figure 1) (Fernandez-Fontaina et al., 2012, Helbling et al., 2012; Yi and Harper et al., 2007; Yi et al., 2006). The enzyme ammonium monooxygenase (AMO) is suggested to be the catalyst responsible for the OMP/NH_3 cometabolism (Yi et al., 2006). Park et al., (2017) studied the cometabolic removal of OMP by AMO and classified the OMPs in three categories in function of the cometabolic biotransformation rates: i) OMPs with a biological kinetic constant (k_{biol}) > 1 L g_{VSS}^{-1} d^{-1}, such as atenolol, thiamphenicol, and fenoprofen; ii) OMPs with a k_{biol} between 0.1- 1 L g_{VSS}^{-1} d^{-1}, such as ketoprofen, propranolol, metoprolol, diltiazem, or bezafibrate and iii) OMPs with a k_{biol} < 0.1 L g_{VSS}^{-1} d^{-1}, such as TMP and indomethacin. A correlation between the removal rate of three OMPs (IBP, bisphenol-A, and triclosan TCS) and the nitritation activity in a nitritation-anammox process was previously observed, evidencing the role of the AMO bacteria in the biotransformation of these particular compounds (Alvarino et al., 2015). In fact, the metabolites 1-hydroxyibuprofen and 2-hydroxyibuprofen were detected in biological processes under aerobic conditions (CAS, aerobic MBRs, and nitrifying activated sludge) as intermediates of IBP biotransformation (Fernandez-Fontaina et al., 2016; Alvarino et al., 2014; Zwiener et al., 2002). The formation of both metabolites might be related to the enzymatic reactions catalyzed by AMO bacteria that utilize elemental oxygen to incorporate the hydroxyl function into the chemical structure of the persistent compounds (Figure 1a), increasing their biodegradability (Chang et al., 1997). The removal of OMPs has been correlated to the specific nitrification rate in enriched nitrifying cultures (Fernandez-Fontaina et al., 2012; Helbling et al., 2012; Yi and Harper et al., 2007). Tran et al. (2009) suggested that the OMP removals depend on the concentration of both substrates: OMPs and ammonium.

Although less studied than the autotrophic nitrifying metabolism, the effect of the heterotrophic activity on the removal of OMPs under aerobic conditions was also reported (Alvarino et al., 2016a; Fernandez-Fontaina et al., 2016; Tran et al., 2013; Tran et al., 2009). An aerobic activated sludge reactor used as a post-treatment of a previous anaerobic step, operated at a low heterotrophic activity, was spiked with pulses of methanol (primary organic substrate) and a mixture of OMPs (Alvarino et al., 2016a). During this experiment, a correlation between the reactor heterotrophic activity and the removal rates of most OMPs was observed. The exceptions were those OMPs whose cometabolisms had previously shown to depend on the nitrifying activity. The removal of the antibiotic SMX by aerobic autotrophic and heterotrophic cometabolism has also been studied (Alvarino et al., 2016c; Fernandez-Fontaina et al., 2016; Müller et al., 2013). A positive influence of the presence of organic matter as primary substrate was observed in the removal rate of this antibiotic, whereas no influence was found related to the ammonium content in the feeding. This confirmed that SMX removal is only influenced by the activity of aerobic heterotrophs, while the role of nitrifying bacteria are not relevant (Alvarino et al., 2016c; Muller et al., 2013). Although the removal of

IBP by cometabolism in the presence of nitrifiers is well documented, the use of nitrification inhibitors showed that heterotrophs also contribute positively to its biotransformation in CAS systems (Fernandez-Fontaina et al., 2016; Tran et al., 2009). Therefore, the effect of both types of microorganisms has to be taken into account when studying the removal of IBP by cometabolism.

Less information is available in the case of biomass grown under anoxic or anaerobic conditions. Pomiès et al., (2015) assessed the removal of atenolol under anoxic and aerobic conditions and determined that cometabolism using organic carbon as primary substrate was the main removal mechanism under both redox conditions. The same behavior was observed for the antibiotic SMX by Alvarino et al. (2016c). Recent studies reported a correlation between the methanogenic primary metabolism and OMPs removal in the case of the pharmaceuticals NPX, SMX, and TMP, during urban wastewater treatment in UASB reactors (Alvarino et al., 2016a; Alvarino et al., 2014).

Influence of the redox potential

The redox conditions applied to the biological process (aerobic, anoxic, anaerobic) are crucial to determine the microbial populations developed in the reactor and, therefore, the biotransformation pathway of a given pollutant. The degree of biotransformation is very much influenced by the OMP chemical structure. Table 3 shows a summary of removal efficiencies measured for a selection of OMPs under the most common redox potentials applied in WWTPs: aerobic (CAS unit, Table 1), anaerobic (UASB reactor, Table 1) and anoxic conditions (during denitrification in the SeMPAC® process, Table 1) (Alvarino et al., 2016b, 2014). Among the micropollutants considered in Table 3, most of them were readily biotransformed under aerobic conditions, such as the pharmaceuticals IBP, NPX and ROX, hormones, musk fragrances, fluoxetine (FLX) and TCS. In general, it can be seen that aerobic systems are more efficient for the widest group of compounds, although anaerobic units can improve the biotransformation of some substances (e.g. SMX, TMP). Nitrification usually enhances biotransformation, whereas the anoxic conditions imposed in denitrification units are not especially relevant for the biotransformation of OMPs. The use of synthetic supports enhances the removal of OMPs due to the improvement of the nitrification (Alvarino et al., 2016a; di Trapani et al., 2014). Finally, there is a group of pharmaceutical recalcitrant compounds (CBZ, diazepam (DZP), DCF) which are not affected by the redox conditions applied. Under positive redox conditions, the dissolved oxygen concentration influences the removal of several OMPs (Stadler et al., 2015): atenolol is readily removed under pure aerobic conditions, whereas the removal of SMX increases in microaerobic conditions.

Table 3. OMPs biotransformation in function of the redox potential

NPX, SMX, and TMP are readily biotransformed compounds under anaerobic conditions (Table 3), which is associated with their chemical structure. The presence of an amide group in the structure of SMX makes its biotransformation under aerobic conditions difficult, but it can be biotransformed by reductive reactions due to the presence of a strong electron-withdrawing group such as the sulfonyl (Field, 2002). Alvarino et al. (2016a) studied the intermediate products produced during the cometabolic biotransformation of SMX under anaerobic conditions. One of the metabolites of SMX detected under anaerobic conditions was more polar than the parent compound, which suggests a pathway through the breakage of SMX at the sulfonyl group (Alvarino et al., 2016c) (Figure 2). Besides, a second metabolite observed during the biological biotransformation of SMX under anaerobic conditions was proposed to be formed by the attack at the N-O bond of the 3-amino-5-methyl-isoxazole ring (Alvarino et al., 2016c; Hayase et al., 1982). In the case of the antibiotic TMP, the substituted pyrimidine is a functional group readily biotransformed under anaerobic conditions (Figure 2) (Adrian and Suflita, 1994), whereas the carboxylic group of NPX promotes its biotransformation by hydroxylation (Musson et al., 2010). Other evidences of biotransformation under anaerobic conditions are those reported by Falås et al., (2016), who observed the O-demethylation of the aromatic methoxy group of the antidepressant venlafaxine; Veetil et al. (2012) showed the anaerobic biotransformation of TCS under methanogenic conditions; or the works of Ghattas et al. (2017), who determined that the antimicrobial agent TCS is transformed into chlorocatechol and 2,4-dichlorophenol by a cleavage of the diphenyl ether.

The pharmaceutical NPX is readily biotransformed under both, anaerobic and aerobic redox potentials (Figure 1b). Nitrification has been demonstrated to affect positively its biotransformation (Fernandez-Fontaina et al., 2012), which suggests that the pathway for NPX removal is based on the cometabolic removal of the aromatic ether through O-dealkylation induced by AMO bacteria (Quintana et al., 2005). In this way, the hormones estrone (E1), β-estradiol (E2) and EE2 are easily biotransformed under aerobic conditions due to the presence of aromatic rings substituted by hydroxyl groups that can be cometabolised by AMO bacteria (Figure 1c, Chang et al., 1997).

In general, lower OMP removals were obtained under anoxic conditions when compared with similar systems in the presence of oxygen. For example, Dorival-Garcia et al. (2013) reported a medium biotransformation of quinolone (14-40%) under aerobic conditions, whereas under anoxic conditions was observed a recalcitrant behaviour. Alvarino et al. (2016b) has followed the removal of some OMPs during one complete cycle in the sequential batch reactor (SBR) used in the SeMPAC® process prior to the addition of PAC. During the anoxic stage, a recalcitrant behavior was observed for most of the micropollutants except in the case of musk fragrances (ADBI, tonalide (AHTN), galaxolide (HHCB)), sulfamethoxazole and fluoxetine. The minor biotransformations during denitrification might be related to the lower oxidation potential of the nitrate compared to oxygen. In the case of the lipophilic compounds, the removal occurred

mainly during the denitrification stage in the SeMPAC® process, but it was principally attributed to sorption onto the sludge since this was the first stage of the SBR cycle (Alvarino et al., 2016b).

One of the trends in the water industry the development of innovative WWTP configurations is the combination of different redox potentials (spatially distributed in different tanks or using a time distribution in one single tank) to enhance the microbial diversity able to biotransform a wider range of complex pollutants, while maintaining a high degree in the organic matter and nutrient removal processes (Luo et al., 2014). Table 4 shows the results obtained in terms of OMPs biotransformation efficiencies for several wastewater treatments which combine different redox potentials: 1) Anaerobic-aerobic MBR (AnHMBR), comprising an UASB reactor followed by an aerobic MBR operated with carriers (to promote nitrification); 2) One-stage granular autotrophic nitrogen removal sidestream system (the ELAN® process based on Anammox) in which anoxic and aerobic conditions occur (Vázquez-Padín et al., 2014); 3) Sequential batch reactor, which combines nitrifying and denitrifying conditions, coupled to a MBR (SeMPAC® system before the addition of PAC to evaluate only the biological process). As expected, CBZ, DZP, and DCF were not biotransformed in any of these processes, according to their recalcitrant behavior. The removal of IBP, NPX, and hormones was clearly higher in the autotrophic N removal process than in the conventional nitrification-denitrification system, very likely as a result of the higher nitritation activity developed in autotrophic N removal processes (98 and 85% of removal for IBP and NPX and 80 and 65% for NPX, respectively). The opposite behavior was found for the antibiotic SMX, which was readily biotransformed in the conventional nitrification-denitrification process, whereas a removal below 60% was found in the autotrophic N removal reactor (Table 4). This difference can be explained based on the evidence that SMX is cometabolically degraded during primary heterotrophic processes (Müller et al., 2013; Alvarino et al., 2016c). The same applies to FLX that was also preferentially removed during a heterotrophic conventional nitrification-denitrification process compared to the autotrophic N removal process (Table 4), independently from the nitrification activity. In general, the removal efficiencies obtained for OMPs in the autotrophic N removal reactor were similar to those obtained in enriched nitrifying processes (Fernandez-Fontaina et al., 2012). Since both processes have in common the nitritation step, the cometabolism by the ammonia monooxygenase (AMO) bacteria appears to be the key factor in their removal, as evidenced by a wide number of OMPs, such as IBP or TCS.

Table 4. *OMPs removal in the systems with combination of redox potential*

The design of innovative systems is frequently driven by the valorisation of the organic matter present in sewage through its conversion into methane, which is possible in anaerobic processes. So, low temperature anaerobic pre-treatment processes are being

installed before the aerobic biological units, allowing the exposure of OMPs to different redox conditions. In general, the contribution of the anaerobic treatment to the overall OMP removal has shown to be low (Falås et al., 2016; Xue et al., 2010). Even though, this combination has demonstrated to enhance the removal of some aerobically persistent OMPs such as venlafaxine, diatrizoate, and tramadol (Falås et al., 2016). In order to further elucidate the OMPs removal mechanisms occurring in the anaerobic-aerobic integrated systems, a separate analysis of the individual processes was carried out by comparing the performances of individual UASB and CAS reactors with the integrated AnHMBR concept (Table 1). The removal efficiencies of the OMPs readily biotransformed under anaerobic conditions were similar in the first stage of the AnHMBR and in the UASB reactor (Alvarino et al., 2016a; Alvarino et al., 2014). On the contrary, in the case of the OMPs readily biotransformed under aerobic conditions, the removal was much lower in the AnHMBR compared to the CAS unit. This was attributed to the low COD level entering the aerobic stage of the AnHMR (in spite of operating at an ambient temperature of around 20ºC), which confirms the cometabolism role in the removal of OMPs. Also, lower nitrification was achieved in the aerobic stage of the AnHMBR (below 60%) compared to the CAS unit (around 90%) due to the washout of these slow-growing bacteria during reactor purges. This hampered the removal of several OMPs, whose removal had been previously shown to depend on the nitrification activity. The further improvement of the nitrifying activity in the AnHMBR system, as a consequence of the introduction of Kaldnes rings as a support for the growth of the slowly growing nitrifying bacteria, led to the restoration of the removal efficiencies of OMPs such as IBP or hormones (Alvarino et al., 2016a).

Parameters influencing k_{biol} values

Biological kinetic constants (k_{biol}, in L g_{VSS}^{-1} d^{-1}) are usually obtained in kinetic batch tests considering pseudo-first order kinetics (Joss et al., 2006). These k_{biol} values correspond to the maximum biological activity and are not affected by other operational conditions (Alvarino et al., 2014). Even though, biotransformation of OMPs depends on their chemical structure and the redox conditions applied, which should be taken into account when using previously published data. These experiments are performed without limitation of the primary substrate to follow the OMP biotransformation by cometabolism.

If the k_{biol} values are obtained under the steady-state operation of a biological reactor, they are influenced by the operational conditions, such as biomass concentration or hydraulic retention time, and therefore are specific to the process studied. These specific k_{biol} values are determined by a mass balance applied to each OMP, as shown in equation 1 in the case of a continuous stirred tank reactor (CSTR) model and considering pseudo-first order kinetics for OMP biotransformation.

Figure 3 shows a summary of k_{biol} values obtained for a representative group of OMPs by Alvarino et al. (2016a; 2014) (Table S1 gives the detailed data for all OMPs)

520 under different selected operational reactor conditions (Table 1). Accordingly, OMP
521 biotransformation degree could be classified as low, medium and high for k_{biol} <0,1 0.1-
522 1 and >1 L g_{VSS}^{-1} d^{-1}, respectively, in these particular studies. Figure 3 shows that, in
523 general, the highest k_{biol} were achieved under aerobic conditions with full nitrification
524 (CAS unit). The influence of the nitrification rate on the removal of some OMPs was
525 evidenced by the lower k_{biol} obtained in the aerobic post-treatment step of the AnHMBR
526 compared to the CAS unit (Figure 3, Table S.3). The decrease in k_{biol} when nitrification
527 was lower was especially important in the case of NPX, whereas no influence on the
528 behaviour of the hormone E2 was evidenced.

529 Under anaerobic conditions, only three OMPs achieve the medium-high ranges:
530 SMX and NPX, whose k_{biol} values are close to the limit between the medium and high
531 ranges, and TMP, with the highest value located in the high range. It is worth to
532 remember that the k_{biol} calculations are strongly affected by biomass concentration (Eq.
533 1). Thus, the one order of magnitude higher sludge concentrations used in the UASB
534 compared to the CAS unit (30-40 vs. 1-2 g_{VSS} L^{-1}, respectively) implied that even in the
535 case of the readily biotransformable OMPs, such as TMP, SMX or NPX, moderate k_{biol}
536 values were obtained (Alvarino et al., 2016a, 2014).

537 Although HRT is a factor included in k_{biol} calculations (Eq. 1), its influence under
538 normal operation conditions was not so pronounced (Figure 3). A decrease in HRT in
539 the CAS unit from 24 to 12 h, implied a general increase in the k_{biol} values, except for
540 the persistent compounds (Table S.3). This was due to the fact that the OMPs removal
541 efficiencies achieved were not influenced by the applied HRT, indicating that the
542 capacity of the reactor for OMP removal was oversized.

543 ***Figure 3.*** *OMPs k_{biol}l values constants determined in the reactors operated*
544 *under aerobic (at different nitrifying activities) and anaerobic conditions*
545 *(Alvarino et al., 2016a, 2014)*

546 *Influence of the simultaneous addition of sorbent agents*

547 The direct addition of activated carbon in bioreactors has been reported as positive to
548 improve the removal of carbon and nitrogen, as well as to reduce potential toxic effects
549 caused by inhibitors (Cecen and Atkas, 2011; Widjaja et al., 2004). In this kind of
550 systems both biotransformation and sorption are enhanced: the first through the
551 potential growth of a biofilm onto the AC and the latter due to the high sorption
552 capacity of this agent. This explains that, although the main removal mechanism related
553 to the AC addition into biological treatment processes is related to its high sorption
554 capacity, other factors are influencing the biological process (Alvarino et al., 2016b;
555 Rattier et al., 2012; Cecen and Aktas, 2011; Sun et al., 2008; Ying and Ping, 2006):

- The AC fosters longer retention of the sorbed OMP inside the biological reactors that might promote an enhancement in their biotransformation, especially for those compounds with medium biodegradability;
- The presence of AC promotes the growth of a biofilm, which can provide additional redox environments (e.g. presence of anoxic/anaerobic inner biofilm layers even in aerated tanks) and thus, a wider microbial diversity.
- The addition of AC promotes the nitrification that is related to the removal of several OMPs, such as hormones or the pharmaceutical IBP;
- AC bioregeneration can improve the OMP removal by sorption onto the PAC and is based on:
    a. Once the OMP solid-liquid equilibrium is achieved, adsorbed OMPs can desorb out and be removed by biotransformation.
    b. Adsorbed OMPs can be removed by a extracellular biotransformation carried out by the microbial enzymes secreted by the attached biomass inside the carbon pores;
- The sorption capacity of the solid matrix is enhanced (e.g. CBZ is highly retained by AC whereas HHCB is sorbed preferentially by the sludge);
- Wastewaters with inhibitory substances can be biologically treated since their concentration in the liquid phase can be reduced below their IC_{50} due to sorption onto the AC. The AC addition in the biological treatment reduce the effluente toxicity;
- The presence of AC in bioreactors has shown benefits in their operation (e.g. better biomass settling properties), especially in MBRs (less fouling, better management of trans membrane pressure, etc.).

One of the innovative configurations testing this concept is the SeMPAC® process (Table 1), a technology patented by the authors (patent ES 2 362 298 B2), which is now in process of extension at international level (European Patent application EP12777603.7). This process is based on the direct addition of PAC (1 g L^{-1}) in a SBR which is coupled with an external ultrafiltration membrane chamber. It has been shown that OMP removal in the SeMPAC® process occurs synergistically through biotransformation, sorption onto the sludge (5-8 g_{VSS} L^{-1}) and sorption onto the PAC (Alvarino et al., 2016b). For example, an increase in the removal by biotransformation and sorption for moderate biotransformable compounds, such as the antibiotics TMP, SMX, erythromycin (ERY) and ROX and the antidepressant FLX, has been reported in the presence of PAC. The k_{biol} values obtained for SMX and FLX increased from 1.3-1.5 L g_{VSS}^{-1} d^{-1} in the absence of PAC, to values higher than 4 L g_{VSS}^{-1} d^{-1} with PAC (Alvarino et al., 2016b), which can be considered as an evidence of an enhanced biotransformation.

CONCLUSIONS

A comparative analysis of different technologies used for biological wastewater treatment was carried out to further understand which operational parameters influence the two main OMP removal mechanisms, namely sorption and biotransformation. Several parameters that were usually not considered in previous works were included in the analysis, leading to the following main conclusions:

- Sorption onto the sludge is affected by the particle size. Only in the case of the flocculent biomass, the sorption equilibrium is quickly achieved, while sorption on the granules is affected by several parameters, such as the hydraulic and the sludge retention time.

- When using MBR systems, the physico-chemical characteristic of the membrane determines its sorption capacity. Hydrophobic membranes are able to retain lipophilic OMPs, whereas charged membranes can retain OMPs by electrostatic interactions.

- The simultaneous addition of activated carbon in bioreactors enhances the removal of recalcitrant OMPs by sorption. The tendency for each OMP to be retained by sorption is a function of the distribution coefficient and its electrical charge.

- The degree of OMP biotransformation strongly depends on the applied redox potential. The chemical structure of the OMPs determines if the compound is prone to go through oxidative or reductive pathways. The integration of different redox potentials in hybrid advanced biological configurations constitutes a good alternative to enhance the removal efficiencies of OMPs. The cometabolic removal of OMPs depends on several parameters, such as the biomass primary metabolic activity, hydraulic retention time or biomass concentration.

- The direct addition of PAC in biological reactors enhances the removal of OMPs not only by sorption but also synergistically by biotransformation, in the case of OMPs with a medium biodegradability.

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