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Novel application of magnetic nano-carbon composite as redox mediator in the reductive biodegradation of iopromide in anaerobic continuous systems

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Abstract

The redox-mediating capacity of magnetic reduced graphene oxide nanosacks (MNS) to promote the reductive biodegradation of the halogenated pollutant, iopromide (IOP), was tested.

Experiments were performed using glucose as electron donor in an upflow anaerobic sludge blanket (UASB) reactor under methanogenic conditions. Higher removal efficiency of IOP in the UASB reactor supplied with MNS as redox mediator was observed as compared with the control reactor lacking MNS. Results showed 82 % of IOP removal efficiency under steady state conditions in the UASB reactor enriched with MNS, while the reactor control showed IOP removal efficiency of 51 %. The precise microbial transformation pathway of IOP was elucidated by high-performance liquid chromatography coupled to mass spectroscopy (HPLC-MS) analysis. Biotransformation byproducts with lower molecular weight than IOP molecule were identified in the reactor supplied with MNS, which were not detected in the reactor control, indicating the contribution of these magnetic nano-carbon composites in the redox conversion of this halogenated pollutant. Reductive reactions of IOP favored by MNS led to complete dehalogenation of the benzene ring and partial rupture of side chains of this pollutant, which is the first step towards its complete biodegradation. Possible reductive mechanisms that took place in the biodegradation of IOP were stated. Finally, the novel and successful application of magnetic graphene composites in a continuous bioreactor to enhance the microbial transformation of IOP was demonstrated.

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Conflict of interest

The authors have declared that they have no competing interest.

Compliance with ethical standard

Ethical approval

This article does not contain studies with human participants or animal performed by any of the authors

Introduction

Removal of persistent pollutants from contaminated water is challenging due to the potential risk generated in environmental compartments. The employment of carbonaceous materials in the degradation of contaminants by redox processes has been widely reported. Especially, activated carbon (van der Zee et al. 2003; Mezohegyi et al. 2010; Pereira et al. 2010; Pereira et al. 2014), activated carbon fibers (Amezquita-Garcia et al. 2013; Emilia Rios-Del Toro et al. 2013) and carbon nanotubes (Chen et al. 2014; Fu et al. 2014) have been studied as electron shuttles in the abiotic/biotic degradation of persistent pollutants. The contribution of these carbon materials depends on the presence of quinone groups in their chemical surface, which are able to accept and donate electrons through oxidation-reduction reactions, serving as redox mediators (RM) (Leon y Leon and Radovic 1994; van der Zee and Cervantes 2009; Guin et al. 2011). Quinones are composed of two carbonyl groups in resonant form (Leon y Leon and Radovic 1994).

Graphene oxide (GO) and its partially reduced materials (RGO) have also been used as electron shuttles to promote the transformation of recalcitrant pollutants due to the redox activity of quinone groups present in their chemical surface, and to their superior catalytic activity in the zigzag edges of graphene sheets (Jiang et al. 2007), which favor the reductive transformation of these compounds (Cervantes et al. 2000; Pereira et al. 2014). For example, the redox properties of GO and RGO have been explored in the degradation of environmental pollutants like azo dyes (Lu et al. 2014; Colunga et al. 2015), nitro-compounds (Gao et al. 2011; Fu and Zhu 2013; Wang et al. 2014; Oh et al. 2014; Li et al. 2016a) and polyhalogenated organics (Fu et al. 2014). These studies indicate a feasible opportunity to apply graphene-based materials as redox catalysts in the reductive degradation of emerging contaminants. However, their implementation at full scale in biological wastewater treatment (WWT) systems is challenging due to the lack of mechanisms to maintain these catalysts inside the treatment systems, which could promote a toxicological effect on microorganisms due to their extended release into environmental compartments (Seabra et al. 2014). Accordingly, it is necessary the development of novel hybrid nano-carbon composites, with particular features that allow them to be applied in WWT systems by retaining and recovering strategies.

Lately, it has been documented the synthesis of hybrid nano-carbon composites with magnetic properties by aerosol-phase process. In this sense, the fabrication of these composites is performed by the encapsulation of magnetic nanoparticles (NP) in folded graphene sheets nanostructures with form of wrinkled RGO nanosacks (Chen et al. 2012; Chen et al. 2012; Lv et al. 2016). The application of magnetic RGO nanosacks (MNS) has been focused on radiological technologies (Chen et al. 2013) and on the removal of Cr(VI) from water (Lv et al. 2016). However, their use in reductive biotransformation processes of environmental contaminants has not been documented yet. Just a few research works have documented the synthesis and application of carbonaceous materials with magnetic properties in the redox conversion of pollutants. As an example, Pereira et al., (Pereira et al. 2017) reported the use of carbon composites with magnetic properties as redox catalysts in the reductive degradation of azo dyes in batch assays. The excellent performance of these

carbon composites can be explained by the combination of redox properties of both materials (magnetic NPs and carbon materials).

Iopromide (IOP) is a halogenated pharmaceutical compound with three iodine atoms in its aromatic ring. This compound is an X-ray contrast medium widely applied in radiological areas (Schulz et al. 2008). IOP is considered a priority contaminant because its physicochemical properties and chemical structure (Putschew et al. 2000) confer it persistency in environmental compartments (Steger-Hartmann et al. 1999; Ternes and Hirsch 2000; Onesios et al. 2009). During conventional WWT processes, IOP is resistant to microbial degradation and hence released into aquatic environments at relative high concentration ($\mu\text{g/L}$) (Ternes and Hirsch 2000; Onesios et al. 2009; Kwon et al. 2012). Consequently, it is demanded to propose novel strategies for IOP removal with the aim to be apply in the biological WWT systems. In this context, it has been studied the reductive biodegradation of IOP in an upflow anaerobic sludge blanket (UASB) reactor using anaerobic sludge enrichment with biogenic Pd^0 (Pat-Espadas et al. 2015) and metal-humic substances (Cruz-Zavala et al. 2016). Also, our previous works have employed the redox properties of GO and RGO in chemical (Toral-Sánchez et al. 2016) and the biological (Toral-Sánchez et al. 2017) reductive transformation of IOP in batch assays. Nevertheless, the application of GO-based materials in a continuous biological system for the biotransformation of IOP has not been reported yet. Accordingly, this study focused on evaluating a novel strategy for the reductive biodegradation of IOP by the use of MNS as RM in an anaerobic continuous system. The redox-mediator capacity of these nano-carbon composites was studied in a UASB reactor under methanogenic conditions. Finally, the biodegradation pathway of IOP and reductive reactions mechanisms occurring were also explained.

Material and methods

Materials and chemicals

IOP (Ultravist® 370) and Glucose (used as electron donor) were purchased from Bayer Schering Pharma and Aldrich, respectively. Basal medium solution was composed of several mineral salts (K_2HPO_4 , NaHCO_3 , MgSO_4 , NH_4Cl and CaCl_2) and trace elements dissolved in distilled water as described in previous works (Cervantes et al. 2000; Amezquita-García et al. 2013; Toral-Sánchez et al. 2016).

The fabrication of MNS was performed using an aerosol-phase methodology previously proposed (Chen et al. 2012; Chen et al. 2013; Lv et al. 2016) using a colloidal suspension of GO (0.5 mg/mL) and magnetite NP (1mg/mL). In order to demonstrate the assembly of magnetic NP in the MNS, micrographs of these nano-carbon composites were performed using a JEOL JEM-2010 High Resolution Transmission Electron Microscope (HRTEM).

Source of anaerobic sludge and activation

Methanogenic granular sludge from a full-scale UASB reactor treating brewery wastewater (Sonora, Mexico) was used as inoculum to evaluate the reductive biotransformation of IOP. Volatile suspended solids (VSS) of the methanogenic sludge were previously estimated at

6.15 % based on wet weight (Pat-Espadas et al. 2015). The methanogenic sludge was acclimated for one month in a lab-scale UASB reactor with volume of 1.5 L at room temperature ($25 \pm 2^\circ\text{C}$). The hydraulic residence time (HRT) was 24 h. During this operational period, the bioreactor was constantly fed with 1 g/L of chemical oxygen demand (COD) provided as glucose and mineral medium, reaching a constant COD removal $> 90\%$ under steady state conditions.

Microbial degradation of iopromide in UASB reactors

Two lab-scale UASB reactors (330 mL of working volume) were simultaneously operated and inoculated with 10 g VSS/L of previously acclimated methanogenic granular sludge. The working reactor (R-MN) was enriched with MNS and tailored with a magnetic trap made with 30 H neodymium magnets and in stainless steel to the upper part of the reactor in order to retain and recover the MNS dispersed in the reactor. The control reactor (R-ctrl) was inoculated without MNS. Reactors were operated for 28 days with a constant HRT of 12 h at room temperature ($25 \pm 2^\circ\text{C}$) and sheltered from light to inhibit the photodegradation of IOP and biotransformation byproducts. The reactors were fed during the operational period with the mineral medium described above.

Before reactors operation, 150 mL of MNS solution with a concentration of 85 mg/L was mixed with the stabilized sludge in the R-MN and then pre-incubated for 48 h. After this period, R-MN and R-ctrl were continually supplied with 1 g/L of COD provided as glucose and an initial concentration of 200 $\mu\text{g/L}$ of IOP. During the operational period of R-MN, three pulses of 100 mL of the aforementioned MNS solution were applied on days 7, 15 and 22. The performance of both bioreactors was evaluated by monitoring the effluent pH, ORP of the system and COD removal efficiency. Samples were taken from the effluent of the anaerobic bioreactors at selected time periods to determine the IOP removal and generation of its biodegradation byproducts as described below.

Analytical procedures

The IOP concentration in the influent and effluent of both bioreactors was determined by high-performance liquid chromatography (HPLC) using an Agilent Technology 1260 series chromatograph as previously described (Toral-Sánchez et al. 2016; Toral-Sánchez et al. 2017). Identification of biotransformation byproducts of IOP was carried out by HPLC coupled to mass spectroscopy (HPLC-MS) in a Varian® 500-MS ion trap mass spectrometer as reported in previous work (Toral-Sánchez et al. 2017). VSS and COD was estimated according to standard methods (Eaton and Franson 2005). The ORP and pH of the biological system was monitored using a Thermo Scientific STARA2110 Orion Star A211 benchtop pH meter with electrode stand.

Results

Microbial transformation of IOP in anaerobic continuous systems

The reductive biodegradation of IOP was assessed in continuous UASB reactors (R-ctrl and R-MNS). COD removal efficiency, as well as ORP and pH values monitored during the operational period of the bioreactors are shown in Fig. 1. COD removal efficiency (Fig. 1A)

of R-MN remained constant with an average of 93.7 ± 2.22 %. Similarly, ORP of the system (Fig. 1B) and effluent pH values (Fig. 1C) of R-MN remained constant during the same operational period with values of -190.1 ± 19.36 mV and 7.27 ± 0.17 , respectively. No significant variations of these parameters were detected in R-ctrl (see Fig. 1) with respect to R-MN, which indicates that there was no negative impact in the performance of the reactor supplemented with MNS. In addition, Table 1 shows the performance of R-MN and R-ctrl during the operational period. The average ORP for R-MN and R-ctrl was -190.1 ± 19.36 mV and -179.97 ± 10.76 mV, respectively. Also, the effluent pH for R-MN and R-ctrl was 7.27 ± 0.17 and 7.26 ± 0.06 , respectively. Under methanogenic conditions, the optimal ORP of the system is around -200 mV and the range of pH is between 6.5 and 7.5 (Fetzer and Conrad 1993). These results suggest that the methanogenic conditions remained optimal during the operational period in both reactors. Also, it can be observed that COD removal efficiency, ORP and pH values of R-MN remained constant when the pulses of MNS were applied (see stages in Table 1), which confirms the absence of negative impact on this bioreactor when enriched with this carbon composites as electron shuttles.

On the other hand, biodegradation of IOP was also assessed in both UASB reactors at a constant concentration of $200 \mu\text{g/L}$ for 28 days, with glucose provided as an electron donor and carbon source. The removal efficiency of IOP during the operational period for both reactors is shown in Fig. 2 and Table 1. It can be observed in R-MN and R-ctrl a low and similar IOP removal (~ 40 to 55 %) in the first six days of operation, suggesting an acclimation period of both inocula to the experimental conditions. However, when the first pulse of MNS was applied in R-MN (day 7), an abrupt increase in the IOP removal was observed from 54.4 ± 1.6 to 81.05 ± 1.1 %, which indicates a clear contribution of MNS in the biotic reduction of this emerging pollutant. Subsequently, R-MN achieved an average IOP removal of 82.01 ± 3.83 %. In contrast, R-ctrl achieved only 51.18 ± 6.7 % in average of IOP removal after the sixth day of operation. These results indicate a better performance in terms of IOP removal in R-MN as compared to that observed in R-ctrl, which suggest that the catalytic activity of MNS plays a fundamental role during the reductive biodegradation of IOP under methanogenic conditions.

The morphology of MNS was observed by high resolution micrographs (Fig. S1 of supporting information). It can be seen that magnetic NP are immobilized inside of nanosacks with a few NP appearing on the outside of this nano-carbon composite (Chen et al. 2013). The presence of magnetic NP in MNS could contribute to IOP removal as it was observed in R-MN (see Fig.2). These observations can be explained by the excellent reducing power of magnetic NPs (Allen et al. 1974; Scott et al. 2005; Gorski et al. 2010).

Biotransformation pathway of IOP in UASB reactor

With the aim to propose a precise microbial transformation pathway of IOP, byproducts generated from its biodegradation from both reactors (R-MN and R-ctrl) were identified by HPLC-MS at specific time intervals during the operational period. The molecular weights (m/z) of 28 biotransformation products (TP) were recognized in R-MN. Proposed chemical structure and elemental composition of the TPs are shown in Fig. 3. The chemical structure

modification of IOP along the operational period of R-MN will be concisely explained below.

TP with m/z of 788.89, appearing in the first sampling day, indicates a dehydration reaction in side chain B. In sampling day 5, six additional byproducts from TP 788.89 were observed. TPs with a m/z of 714.5, 774.4, 758.5, 744.5, 662.5 and 634.7 imply partial rupture of side chain A and B, including dehydration, demethylation and decarboxylation reactions, and cleavage of the C-N bond. Specially, while TP 634.7 implies the removal of one iodine atom (dehalogenation) from the aromatic ring, and TP 662.5 involves the rupture of side chain B. After 10 days of operational period, six new byproducts were generated with different chemical structures. TPs identified with a m/z of 742.6, 690.6, 674.6 and 728.3 come from either TP 774.4, 758.5 or 744.5. Structures of TP 742.6 and 728.3 involve demethylation and dehydration reactions in side chain A and B, respectively. TPs 690.6 and 674.6 involve demethylation and decarboxylation reactions, cleavage of amide bond and subsequent rupture of side chain B ramification. The proposed structure of TP 322.8 exhibits several reductive reactions in side chain A, B and C of either TP 662.5 or 634.7, which included the rupture of C-N bond, demethylation reactions and the double dehalogenation of the benzene ring. TP 390.8 results from dehydration, demethylation and amide bond rupture in side chain A, cleavage of C-N bond in side chain B with a subsequent removal of two iodine molecules of the benzene ring.

Samples collected after 14 days of operation contained three additional byproducts derived from IOP degradation. TPs 762.5, 576.7 and 336.8 come from TP 774.4, 728.3 and 390.8, respectively. The chemical modification of TP 762.5 involves demethylation of side chain A. TP 576.7 suggests demethylation in side chain A, cleavage of C-N bond in side chain B and deiodination of the aromatic ring. The structure of TP 336.8 implies the rupture of C-N bond in side chain A, rupture of side chain B ramification and dehalogenation of two iodine atoms (HI) of the aromatic ring. Furthermore, after 18 days of operation, generation of four new byproducts was observed. The rupture of C-N bond in side chain B, the loss of H₂O molecules (dehydration) and demethylation reactions in side chain A of TP 762.5 or 714.5 resulted in the evolution of TPs 670.7 and 684.7. The structure proposed for TP 538.7 comes from either TP 762.5, 690.6 or 674.7, which implies the rupture of side chain B ramification and elimination of one iodine atom. Also, dehydration, demethylation and decarboxylation reactions of side chain C of TPs 336.8 and 322.8, as well as the rupture of C-N bond of side chain A, rupture of side chain B ramification and subsequent deionidation of the aromatic ring of TP 576.7 result in the generation of TP 316.9.

Structures of TPs with a m/z of 730.3, 616.5, 588.7, 548.7 and 276.9 appeared in day 28 of the operational period. TPs 616.5 and 588.7 result from several reactions, such as demethylation and dehydration of side chain A and B, rupture of C-N and amide bonds in side chain A and rupture of side chain B ramification of either TPs 670.7 or 684.7. TP 548.7 suggests deionidation of the aromatic ring (HI) of TP 674.6. The rupture of side chain B ramification and the completely dehalogenation of the aromatic ring (removal of three iodine atoms) of TP 576.7 results in the generation of TP 276.9. Finally, two new byproducts were detected on day 31 (end of the operational period). Structure of TP 306.8 shows a partial rupture in side chain C of TP 336.8 and 316.9, including demethylation and removal of one

molecule of H₂O. TP 254.8 involves demethylation and dehydration of side chain A of TP 276.9. Similar fragmentation pathway of IOP and *m/z* of biodegradation byproducts identified in this research are suggested in our previous works (Toral-Sánchez et al. 2016; Toral-Sánchez et al. 2017) as well as in others studies using different chemical or biological degradation strategies (Pérez et al. 2006; Schulz et al. 2008; Lütke Eversloh et al. 2014; Gros et al. 2014; Pat-Espadas et al. 2015; Toral-Sánchez et al. 2016; Cruz-Zavala et al. 2016; Toral-Sánchez et al. 2017).

Moreover, TPs with lower *m/z* (648.6 and 588.7) in R-ctrl were also identified. Therefore, it can be suggested that the enrichment of MNS as electron shuttles in the R-MN promoted a greater degree of IOP biotransformation, which was manifested by the generation of biological byproducts with lower *m/z* values as compared with the byproducts formed in R-ctrl. Furthermore, it can be observed the complete dehalogenation of the aromatic ring of IOP molecule (TPs 276.9 and 254.8) during the operational period of R-MN.

Discussion

The present study reports the novel application of hybrid graphene materials, in the form of MNS, as RM in the biotransformation of a halogenated pharmaceutical pollutant (IOP) in an UASB reactor. The use of GO and RGO has previously been demonstrated as effective electron shuttles to improve the degradation of this pharmaceutical in chemical and biological batch incubations (Toral-Sánchez et al. 2016; Toral-Sánchez et al. 2017). However, a mechanism to retain and recover these catalysts in a biological continuous system is necessary in order to employ GO-based materials for the reductive transformation of persistent pollutants presents in WWT facilities. In this sense, the present work reports a strategy to retain novel nano-carbon composites in an UASB bioreactor by magnetic field.

Results from biological experiments conducted in R-MN showed high removal efficiency of IOP. Also, it was demonstrated that the redox catalytic activity of MNS promoted a further biotransformation degree of this priority pollutant. This pharmaceutical is a halogenated compound with three iodine atoms bonded to the benzene ring, and hydroxyl and carboxyl groups in their side chains, which makes it a persistent environmental contaminant (Putschew et al. 2000; Pat-Espadas et al. 2015). It has been reported that the degradation of halogenated organics is performed by reductive dehalogenation reactions that take place under reducing environments (−400 mV) and generally requires an external electron donor (Knackmuss 1996; Pat-Espadas et al. 2015). As discussed in the results section, the average value of ORP during the operational period of R-MN was -190.1 ± 19.36 mV, which suggests that the system conditions drove the reductive dehalogenation (deionidation) of IOP as observed in the biotransformation pathway of this pollutant (see Fig. 3). On the other hand, it has been reported that the resistance to microbial degradation of halogenated compounds is related to the bond strength between carbon atoms and halogenated groups. Accordingly, it is expected that iodinated organics are more susceptible to biodegradation than other halogenated compounds since C-I bonds are weaker (209 kJ/mol) than others halogen-carbon bonds (C-Br (280 kJ/mol), C-Cl (397 kJ/mol) and C-F (536 kJ/mol)) (Rittmann and McCarty 1999).

As documented by several authors (Knitt et al. 2008; Schulz et al. 2008; Onesios et al. 2009), microbial degradation of IOP is challenging due to its high biochemical stability and resistance to reductive transformation. Also, IOP molecule presents a low removal during the application of biological conventional treatments (activated sludge) due to their high polarity ($\log K_{OW} = -2.33$) and high water solubility (0.97 mol/L), thus sorption onto sludge or sediments is negligible (Steger-Hartmann et al. 1999; Petrovic and Barceló 2007). Accordingly, several chemical or biological strategies have been studied in order to facilitate the removal of this halogenated contaminant. As examples, it has been explored the removal of IOP by electron beam irradiation technology (Kwon et al. 2012), advanced oxidation (Jeong et al. 2010; Azerrad et al. 2014), reductive hydrodehalogenation with porous palladium-nickel catalyst (Knitt et al. 2008), oxidation by electrochemical treatment (Lütke Eversloh et al. 2014), ozone oxidation (Snyder et al. 2006), as well as aerobic degradation strategies by activated sludge (Kalsch 1999), *Pseudomonas* (Xu et al. 2014) and fungus *Trametes versicolor* (Gros et al. 2014). Anaerobic strategies using methanogenic consortia in UASB reactors have also been employed in order to evaluate their potential application in the reductive transformation of IOP. Pat-Espadas et al., reported an IOP removal efficiency of 81 % using immobilized biogenic Pd(0) as catalyst (Pat-Espadas et al. 2015). Also, Cruz-Zavala et al., achieved a IOP removal efficiency of 80 % applying iron-humic complexes as RM (Cruz-Zavala et al. 2016). In addition, these works proposed the biotransformation pathway of IOP, where reductive reactions of IOP molecule generated byproducts with simpler chemical structure. Accordingly, this research work also proposed the biotransformation pathway of IOP using carbon-based composites (MNS) as RM in UASB reactor. Proposed biotransformation pathway (see Fig. 3) implies that MNS promoted deiodination of aromatic ring and rupture of hydroxyl, carboxyl and methyl bonds from side chains of IOP molecule. The reductive reactions that took place in R-MN could be explained by complex mechanisms that are related to the redox properties and catalytic activity of MNS as electron shuttle.

It has been documented that quinone groups present as oxygenated functionalities of GO sheets improve the redox conversion of environmental contaminants (Larsen et al. 2000; van der Zee et al. 2003), including IOP (Toral-Sánchez et al. 2016; Toral-Sánchez et al. 2017), due to their superior redox-mediating capacity (Montes-Morán et al. 2004). In addition, it has been stated that the redox activity of quinone groups is fundamental in the electron transfer process towards electron acceptor pollutants, improving their reductive transformation reactions (van der Zee and Cervantes 2009; Fu and Zhu 2013; Toral-Sánchez et al. 2016). In this sense, previous works have documented a great stability of carbonyl groups of GO sheets after thermal reduction processes (Gao et al. 2010; Huh 2011; Pei and Cheng 2012). As reported by Chen et al., hybrid MNS are conformed of RGO sheets produced by thermal reduction process during synthesis of filled nanosacks (Chen et al. 2013). Also, our previous work showed a high distribution and stability of carbonyl groups in RGO sheets after the reduction process (Toral-Sánchez et al. 2016). As a consequence, it can be proposed that the redox-mediating capacity of quinone groups present in rGO sheets of MNS contributed to the reductive reactions of IOP performed in R-MN as observed in previous section (see Fig. 1 and 3). Accordingly, it is expected that the catalytic activity of

quinone moieties is involved in the biotransformation mechanisms of this halogenated pollutant.

Moreover, it has been documented that the catalytic activity of graphene sheets improves the conversion rate of chemical compounds by reductive reactions (Enoki et al. 2007; Gao et al. 2011). The π -electrons from zigzag edges of RGO have the ability to react with the functionalities bonded of aromatic ring of IOP molecule because of their great chemical reactivity (Jiang et al. 2007). As aforementioned, MNS are conformed by RGO sheets, which could interact with iodine atoms and functional groups of side chains of IOP due to the π -electrons reactivity, promoting its molecular activation (Toral-Sánchez et al. 2016). These facts suggest another reductive biotransformation mechanism of IOP, which is well founded by the formation of biological TPs as a result of reductive reactions that underwent this halogenated contaminant (see transformation pathway, Fig. 3).

Furthermore, it has been reported that magnetic NP have an excellent reducing power due to their ability to transfer electrons towards electron-accepting reactants and to their great catalytic activity (Allen et al. 1974; Scott et al. 2005; Gorski et al. 2010). These unique properties are due to magnetite, which contains both iron species (Fe(II) and Fe(III)) in its chemical structure, which exhibit reduction-oxidation reactions (Scott et al. 2005; White and Peterson 1996). Accordingly, it can be proposed that the reductive biotransformation mechanism of IOP probably implied the transfer of electrons from this couple towards quinone groups on RGO sheets of MNS, which can accept electrons and then transfer them through the graphene sheets (Leon y Leon and Radovic 1994). Finally, the electrons can be accepted by the final electron acceptor, IOP (Pereira et al. 2017).

Until now, chemical mechanisms involved in the microbial transformation of IOP have been proposed. However, the biological processes involved in the reductive reactions of IOP are key drivers in its biotransformation as reported in our previous work (Toral-Sánchez et al. 2017). It has been documented that the biological activity of microorganisms by extracellular electron transfer (EET) process is fundamental for improving the reductive biodegradation of environmental contaminants (Summers et al. 2010; Wang et al. 2014). EET process mediated by redox shuttles is an important microbial electron transfer pathway due to the presence of extracellular polymeric substances (EPS), which contain electron transport species in the gap between cells and electron acceptor/donor substances (Marsili et al. 2008; Xiao et al. 2017). In this sense, it has been reported that EPS from electroactive microorganisms exhibit redox activity due to the presence of proteins, humic substances, flavins and c-type cytochrome enzymes, serving as electron transfer mediators in EPS (Cervantes et al. 2007; Flemming et al. 2007; Marsili et al. 2008; Li et al. 2016b; Xiao et al. 2017). The redox properties of EPS play an important role in the redox conversion of pollutants, such as U(VI) (Canstein et al. 2008; Cao et al. 2011), nitrobenzene (Wang et al. 2013) and azo dyes (Kudlich et al. 1997).

On the other hand, it has been reported the presence of electroactive microorganisms in methanogenic consortia, which promote interspecies electron transfer processes for the production of methane (Lee et al. 2016; Li et al. 2018; Feng et al. 2018). In addition, several works have documented that the presence of GO-based materials improve the transfer of

electrons by EET mechanisms, favoring the reductive biological reactions of organic contaminants (Reguera et al. 2005; Logan and Rabaey 2012; Patil et al. 2012). Accordingly, it is possible to suggest that microbial EET processes are involved in the biotransformation of IOP due to the contribution of redox EPS secreted by methanogenic microorganisms in the presence of MNS.

Finally, another biological mechanism implies the biological oxidation of substrate (glucose) by the methanogenic consortium, which generates electrons that can be donated directly to the final electron acceptor (IOP). Also, the electron transfer process may take place from the oxidation of glucose to quinone groups of RGO sheets of MNS, which can accept electrons. After that, electrons can be donated by quinone groups (forming hydroquinone groups) and then, transfer them through graphene sheets (Leon y Leon and Radovic 1994). Finally, the electrons can be accepted by IOP molecule. Similar mechanisms are proposed in previous works (Pereira et al. 2017).

According to these mechanisms, it can be concluded that the redox behavior of MNS contributed in the redox conversion of IOP since R-MN showed an average IOP removal of 82 % while R-ctrl only achieved 51 % of IOP removal.

The biotransformation mechanisms proposed above may be significant drivers on the reductive reactions of IOP. The anaerobic continuous experiments conducted with MNS as RM showed the generation of byproducts with simpler structure than IOP molecule, which are more susceptible to complete biodegradation, mainly due to the reductive deiodination of aromatic ring of this pollutant. The present study exhibits, for the first time, the reductive biodegradation of IOP using MNS as electron shuttle. The novel application of MNS as RM in UASB reactor was demonstrated for the removal of IOP. The great catalytic activity of MNS was evident due to the high removal efficiency and further biotransformation degree of IOP. The main advantage of this reductive transformation strategy depends on the simultaneous use of the redox activity of MNS and their magnetic properties to be captured inside the biological continuous process. Finally, the removal strategy proposed in this study shows a feasible option to apply hybrid graphene-based materials in biological WWT systems.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Performance of both reactors (R-MN and R-ctrl) regarding to (A) COD removal efficiency, (B) ORP of the system and (C) effluents pH values, as a function of operational period.

Figure 2.
Performance of R-MN and R-ctrl regarding IOP removal efficiency during the whole operational period.

Figure 3.

Reductive biotransformation pathway of IOP in the UASB reactor supplied with MNS as RM.

Table 1

Performance of R-MN and R-ctrl after application of MNS pulses along the operational period

	Start-up		Stage 1		Stage 2		Stage 3	
			1st pulse		2nd pulse		3rd pulse	
	Day 0–6		Day 7–15		Day 16–22		Day 23–31	
	R-ctrl	R-MN	R-ctrl	R-MN	R-ctrl	R-MN	R-ctrl	R-MN
IOP removal (%)	41.89 ± 0.85	51.20 ± 1.31	51.55 ± 1.63	78.32 ± 1.36	51.39 ± 4.19	83.92 ± 1.33	56.35 ± 1.71	81.41 ± 1.73
COD removal (%)	94.06 ± 1.47	93.30 ± 1.25	95.10 ± 0.67	93.07 ± 1.22	94.16 ± 1.48	95.50 ± 0.61	93.72 ± 0.71	93.32 ± 0.91
ORP (mV)	166.56 ± 3.59	180.78 ± 7.83	174.82 ± 8.37	191.72 ± 12.45	195.77 ± 5.24	188.13 ± 4.14	182.76 ± 4.82	189.55 ± 3.99
pH	7.29 ± 0.06	7.41 ± 0.08	7.44 ± 0.04	7.29 ± 0.06	7.08 ± 0.01	7.12 ± 0.07	7.29 ± 0.09	7.27 ± 0.07