

This is the Post-print version of the following article: *Eduardo Toral-Sánchez, J. Rene Rangel-Mendez, Juan A. Ascacio Valdés, Cristóbal N. Aguilar, Francisco J. Cervantes, Tailoring partially reduced graphene oxide as redox mediator for enhanced biotransformation of iopromide under methanogenic and sulfate-reducing conditions, Bioresource Technology, Volume 223, 2017, Pages 269-276*, which has been published in final form at: <https://doi.org/10.1016/j.biortech.2016.10.062>

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Accepted Manuscript

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PII: S0960-8524(16)31476-6
DOI: <http://dx.doi.org/10.1016/j.biortech.2016.10.062>
Reference: BITE 17217

To appear in: *Bioresource Technology*

Received Date: 10 September 2016
Revised Date: 19 October 2016
Accepted Date: 20 October 2016

Please cite this article as: Toral-Sánchez, E., Rene Rangel-Mendez, J., Ascacio Valdés, J.A., Aguilar, C.N., Cervantes, F.J., Tailoring partially reduced graphene oxide as redox mediator for enhanced biotransformation of iopromide under methanogenic and sulfate-reducing conditions, *Bioresource Technology* (2016), doi: <http://dx.doi.org/10.1016/j.biortech.2016.10.062>

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Tailoring partially reduced graphene oxide as redox mediator for enhanced biotransformation of iopromide under methanogenic and sulfate-reducing conditions

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Abstract

This work reports the first successful application of graphene oxide (GO) and partially reduced GO (rGO) as redox mediator (RM) to increase the biotransformation of the recalcitrant iodinated contrast medium, iopromide (IOP). Results showed that GO-based materials promoted up to 5.5 and 2.8-fold faster biotransformation of IOP by anaerobic sludge under methanogenic and sulfate-reducing conditions, respectively. Correlation between the extended of reduction of GO and its redox-mediating capacity was demonstrated, which was reflected in faster removal and greater extent of biotransformation of IOP. Further analysis indicated that the biotransformation pathway of IOP involved multiple reactions including deiodination, decarboxylation, demethylation, dehydration and N-dealkylation. GO-based materials could be strategically tailored and integrated in biological treatment systems to effectively enhance the redox conversion of recalcitrant pollutants commonly found in wastewater treatment systems and industrial effluents.

Keywords: Biotransformation, graphene oxide, iopromide, redox mediator, wastewater.

1. Introduction

In recent years, a substantial number of emerging pollutants are released into the environment around the world. Iodinated X-ray contrast media (ICM), such as iopromide (IOP), are pharmaceuticals widely used in intravascular administration with global consumption of approximately 3.5×10^6 kg/yr (Hennebel et al., 2010), which are used for brain and body imaging, and mostly excreted in urine or feces due to remarkable stability to human metabolism (Knitt et al., 2008). Because of its chemical properties and low biodegradability, IOP is a recalcitrant pollutant, which is poorly removed in conventional wastewater treatment facilities, and thus it is usually released unaltered into receiving water bodies (Onesios et al., 2009; Ternes and Hirsch, 2000). Accordingly, IOP has been detected in effluents from sewage treatment plants, surface water systems, groundwater, and even in drinking water (Busetti et al., 2010; Putschew et al., 2000). Moreover, it has been reported that exposure to this pollutant could cause human health impacts, such as vomiting, nausea, hives, headache, etc. (Bottinor et al., 2013). In consequence, it is necessary to develop novel treatment techniques to efficiently remove it from contaminated waters.

In this context, biological treatment systems have been explored as feasible options to remove ICM, such as IOP, from water. Recently, few studies have reported the biotransformation of IOP under anaerobic conditions, which includes the use of biogenic palladium(0) (Pat-Espadas et al., 2015) and metal-humic acid complexes (Cruz-Zavala et al., 2016) immobilized in granular sludge as bio-catalyst. Also, the IOP reduction in an abiotic system has been studied by the application of hydrogen gas in combination with supported palladium and porous nickel catalysts (Knitt et al., 2008). On the other hand, it has also been reported the use of redox mediators (RM) as good option to accelerate the reductive biotransformation of electron-accepting pollutants in contaminated waters (Van der Zee and Cervantes, 2009). In this sense, carbon based materials like granular activated carbon (GAC) (Pereira et al., 2010), activated carbon fibers (ACF) (Amezquita-Garcia et al., 2013; Emilia Rios-Del Toro et al., 2013), carbon nanotubes (Chen et al., 2014), black carbon (Gong et al., 2014) and carbon xerogels (Pereira et al., 2014), have been applied as RM to increase the reductive transformation of different recalcitrant pollutants, since they have a diversity of surface oxygenated groups like quinone groups, which can mediate the reduction of these contaminants (Cervantes et al., 2000; Pereira et al., 2014).

Nowadays, the application of graphene-based nanomaterials has a great interest in many research areas because it possesses unique properties such as mechanical strength, high surface area, rapid electron transfer capacity (Geim, 2009) and extraordinary catalytic activity (Enoki et al., 2007). Also, graphene-based materials, such as graphene oxide (GO), present a wide array of oxygenated groups in their chemical structure, like carbonyl groups, which can mediate the transformation of pollutants by enhancing electron transfer processes (Cervantes et al., 2000; Pereira et al., 2014). In addition, it was reported that the graphene basal planes of GO-based materials have very high electrical conductivity (Dreyer et al., 2009), which is another mechanism to mediate the redox conversion of contaminants by electron transfer on the graphitic carbon surface (Fu and Zhu, 2013).

Lately, it has been reported that GO and partially reduced graphene oxide (rGO), can facilitate the biotic and abiotic reduction of recalcitrant pollutants, such as azo dyes (Colunga et al., 2015; Fu and Zhu, 2013), nitroaromatic compounds (Fu and Zhu, 2013; Gao et al., 2011; Wang et al., 2014; Oh et al., 2014) and halogenated pollutants (Fu et al., 2014). Moreover, the chemical transformation of IOP using GO and rGO as RM and the importance of the physical and chemical properties of GO-based materials on the reductive process have been reported recently (Toral-Sánchez et al., 2016). Accordingly, it can be inferred that these graphene-based materials could mediate the redox conversion of emerging recalcitrant pollutants in biological treatment systems. However, the use of these carbon nanomaterials on the biological transformation of pharmaceuticals, like IOP, has not been studied yet.

The aim of the present study was to evaluate, for the first time, the use of GO and rGO materials as RM to achieve the biological transformation of IOP under two relevant environmental conditions: methanogenic and sulfate-reducing. Furthermore, the importance of intrinsic properties of GO-based materials in the ability to act as electron shuttle is studied. Mechanisms taking place in the biotransformation of IOP are also elucidated.

2. Materials and methods

2.1. Materials and chemicals

All chemicals with 99% purity were used as received. IOP (CAS No. 73334-07-3) was obtained from Bayer Schering Pharma with commercial name Ultravist® 370. The basal

medium (pH = 7.6) used in sludge incubations and to feed bioreactors was composed of the following (g/L): K_2HPO_4 (0.25), $NaHCO_3$ (5.0), $MgSO_4 \cdot 7H_2O$ (0.1), NH_4Cl (0.28), $CaCl_2 \cdot 2H_2O$ (0.01), and trace elements (1 mL/L), with a composition described elsewhere, (Cervantes et al., 2000). All chemicals used during the experiments were obtained from either Sigma-Aldrich or Merck. All solutions were prepared with distilled water.

Graphene oxide used in the present study was purchased from Graphene Supermarket®, with the following characteristics: high density and viscosity, concentration of 6.2 g/L in aqueous solution, single-layer > 60%, flake size between 0.5 and 5 μm , C/O ratio 3.95. Commercial bituminous activated carbon (AC) filtrasorb F-400 was obtained from Calgon Inc. Before use, AC was washed with deionized water and then dried at 110 °C for 48 h. Polyacrylonitrile based activated carbon fibers (ACF), with commercial name AW1105, was purchased from KoTHmex and directly used as received from the supplier.

2.2. Source of inocula and activation

Anaerobic granular sludge originated from a full-scale upflow anaerobic sludge blanket (UASB) reactor treating effluents from a candy factory (San Luis Potosí, Mexico) was used as inoculum to assess the biotransformation of IOP. The content of volatile suspended solids (VSS) was 12.52 % based on wet weight. The sludge was acclimated for 30 days in lab-scale UASB reactors (1.5 L working volume) operated under methanogenic and sulfate-reducing conditions at a hydraulic residence time of 1 day and with an organic loading rate of 1 g chemical oxygen demand (COD)/L-d at room temperature (25 °C \pm 2). A mixture of ethanol/lactate (0.5/0.5 in terms of COD) was used as energy and carbon source for the UASB reactors. The reactors achieved COD removal greater than 95% under steady state conditions. For the sulfate-reducing bioreactor, a concentration of 1 g SO_4^{2-} /L was supplemented to the basal medium (added as Na_2SO_4). The sulfate-reducing UASB reactor achieved a constant SO_4^{2-} removal (> 90%) under steady state conditions.

2.3. Chemical reduction of GO

Synthesis of partially reduced GO (rGO) materials was carried out according to Toral-Sánchez et al., (2016). In order to obtain materials with different reduction degrees, reduction kinetics of GO were carried out for 0.5, 1, 1.5, 2, 3 and 4 h. Three samples were selected for use as RM in biological incubations: GO, GO reduced for 2 and 4 h (rGO-2 and

rGO-4, respectively) since they contain a low (60.8 mV), intermediate (329.2 mV) and high (501.9 mV) oxidation reduction potential (ORP) according to previously reported results (Toral-Sánchez et al., 2016), which should be a key factor on the transfer of electrons during the biotransformation of IOP.

2.4. Physical and chemical characterization of GO-based materials

In order to elucidate the importance of intrinsic properties of synthesized GO-based materials (GO, rGO-2 and rGO-4) on the biotransformation of IOP, physicochemical characterization of these carbon materials was performed. Details on the procedure and conditions are described in previous work (Toral-Sánchez et al., 2016). On the other hand, Electrical conductivity measurements of samples were performed in aqueous solution at pH 7.0 in a MICROTRAC Zetatrac NPA 152-31A equipment. In addition, chemical composition of GO-based materials was investigated by X-ray photoelectron spectroscopy (XPS) using a PHI 5000 VersaProbe II equipment with a monochromatic X-ray beam source at 1486.6 eV and 15 kV to scan the surface of the materials.

2.4. Batch experiments for the biotransformation of iopromide

The capacity of GO-based materials to serve as RM on the microbial reduction of IOP was evaluated under methanogenic and sulfate-reducing conditions in 60-mL serum flasks as follows: 5 mg/L of GO and rGO were contacted with basal medium and inoculated with 1 g VSS/L of previously stabilized sludge obtained from both UASB reactors. Subsequently, liquid and headspace were flushed for 3 min with a gas mixture of N₂/CO₂ (80:20, v/v) to ensure anaerobic conditions. An appropriate volume of a mixture of ethanol/lactate was added to the bottles to give a final concentration of 1 g COD/L. Sulfate was provided from a stock solution to obtain a final concentration of 1 g SO₄²⁻/L for experiments performed under sulfate-reducing conditions. Bottles were incubated for 48 h with constant stirring and temperature (125 rpm and 30 °C). After pre-incubation, an extra pulse of ethanol/lactate (1 g COD/L) and sulfate (1 g/L, for the sulfate-reducing incubations) were supplied, and the IOP was added from an anaerobic stock solution in order to obtain an initial concentration of 400 µg/L. The total working volume was 50mL in all incubations. The experiments were incubated in the dark to prevent photodegradation of IOP and metabolites derived from its biotransformation. Control experiments with autoclaved sludge

(sterile control) and sludge-free were performed to evaluate the adsorption of IOP to the sludge and abiotic reduction due to intrinsic reducing compounds, and to assess the stability of the pollutant. All experimental treatments were conducted in triplicate. Samples were analyzed at specific time intervals to monitor the removal of IOP, methane production and sulfate reduction, as described in section 2.5. The same procedures were used to evaluate the biological reduction of IOP using commercial carbon materials as RM (ACF and AC).

2.5. Analytical procedures

The concentration of IOP was measured by high-performance liquid chromatography (HPLC) using a Agilent Technology 1260 series chromatograph as indicated by Toral-Sánchez et al., (2016). Sulfate concentration was determined in previously centrifuged and filtered (0.22 μm) samples by capillary electrophoresis (Agilent 1600A) as previously described (Gallegos-Garcia et al., 2009). Methane production was monitored by analyzing 100 μL of biogas samples in an Agilent Technologies 6890N gas chromatograph under previously reported conditions (Martínez et al., 2013). COD and VSS concentration were determined according to standard methods.

Identification of transformation products of IOP was performed by HPLC coupled to mass spectroscopy (HPLC-MS) in a Varian® 500-MS ion trap mass spectrometer, with electrospray ionization of 90 V and mass-to-charge (m/z) range of 100-2000 m/z .

3. Results and discussion

3.1. Biotransformation of IOP under methanogenic and sulfate-reducing conditions

The role of intrinsic properties of GO-based materials and their use as electron shuttle was explored for the microbial transformation of IOP under methanogenic and sulfate-reducing conditions. Methanogenic conditions mainly prevail in the degradation of recalcitrant pollutants in anaerobic wastewater treatment processes (Cervantes and Santos, 2011). Moreover, industrial wastewaters from different sectors, like pharmaceuticals, contain high sulfate concentrations (Cervantes et al., 2007), therefore sulfate-reducing conditions are also relevant for the reduction of this recalcitrant pollutant.

The catalytic input of GO-based materials on the biotransformation of IOP under methanogenic conditions and the corresponding control experiments are shown in Fig. 1A. It can be seen that biological incubations exhibited a decrease in IOP concentration with removal efficiencies of 64, 75 and 77 % after 11 days, for sludge incubations amended with GO, rGO-2 and rGO-4 as RM, respectively. In contrast, only 20 % of IOP removal occurred in control experiments without RM and methanogenic active biomass during the same incubation period. In addition, the maximum removal rate calculated in assays amended with GO, rGO-2 and rGO-4 were 34.02, 59.12 and 68.76 $\mu\text{g IOP/L-d}$, respectively. Meanwhile, the maximum removal rate achieved in the control experiment incubated in the absence of GO-based materials was only 12.48 $\mu\text{g IOP/L-d}$, which represents a 2.7, 4.7 and 5.5-fold increase in the maximum removal rate of IOP in the presence of GO, rGO-2 and rGO-4, respectively. Also, the same parameter showed an increase of 1.7 and 2.0-fold in the presence of rGO-2 and rGO-4, respectively, with respect to the maximum removal rate calculated for the biological reduction experiment using GO as RM.

Fig. 1B shows the biological reduction of IOP under sulfate-reducing conditions. It can be observed that GO-based materials also promoted a higher reduction of IOP with removal efficiencies of 61, 81 and 86 % in the presence of GO, rGO-2 and rGO-4, respectively. In contrast, the sulfate-reducing control incubated without RM showed an IOP removal of only 38 % in an incubation period of 8 days (Fig. 1B). The maximum removal rate achieved in sulfate-reducing incubations amended with GO, rGO-2 and rGO-4 were 61.38, 73.34 and 90.31 $\mu\text{g IOP/L-d}$, respectively. Biological incubations with sulfate-reducing sludge in absence of GO-based materials showed a maximum removal of 31.2 $\mu\text{g IOP/L-d}$, which indicates an increase of maximum removal rate of up to 2.89-fold in the presence of RM.

Sterilized controls in the absence and in the presence of GO-based materials as RM showed <10% of IOP removal under both relevant conditions. This slight reduction in a sterile environment could be due to the presence of thermally stable intracellular coenzymes such as flavins, which have the ability to act as RM (Prato-Garcia et al., 2013), and/or due to the presence of intrinsic reducing compounds in the biomass (Van der Zee and Cervantes,

2009). In the case of sulfate-reducing incubations, the slight removal observed in sterilized controls could also be due to remaining sulfide accumulated during the activation of sulfate-reducing biomass in the UASB reactor, which could have promoted the chemical reduction of IOP (Toral-Sánchez et al., 2016).

Furthermore, methane production was measured during the course of biological transformation of IOP under methanogenic and sulfate-reducing conditions. The results showed poor methanogenic activity in the experiments incubated with active methanogenic biomass (See Fig. S1 of supplementary material (SM) section), which can be explained due to possible inhibitory effects of GO-based materials on the methanogenic consortium (Bianco, 2013; Liu et al., 2011). However, the concentration of GO-based materials used in the sludge incubation was very small (5 mg/L) in comparison with the minimum inhibitory concentration of GO (50 mg/L) reported by Ahmed and Rodrigues (2013). Certainly, there have been reports showing that methanogenic microorganisms are able to achieve the redox biotransformation of recalcitrant pollutants (azo dyes) in pure culture, but not coupled to methane production (Dos Santos et al., 2006). Accordingly, this would suggest that a competition between IOP reduction and methanogenesis for the reducing equivalents available from ethanol/lactate fermentation could have happened in sludge incubations (Colunga et al., 2015). In the case of sulfate-reducing incubations, the methanogenic activity was negligible, suggesting that the methanogenic consortium was probably inhibited either by sulfate or by sulfide (data not shown). On the other hand, sulfate and sulfide concentrations were measured under sulfate-reducing incubations as shown in Fig. 2S of SM. It can be seen that the sulfate-reducing consortium consumed 68% of sulfate over a period of 8 days. Similarly, sulfide production in the same period increased from 1.5 to 6.0 mM, suggesting that the biogenic sulfide generated from sulfate reduction was the main reducing agent promoting the biological reduction of IOP as previously reported for azo dyes and nitrobenzene reduction under sulfate-reducing conditions (Colunga et al., 2015).

The microbial reduction of IOP under methanogenic and sulfate-reducing conditions using commercial carbon materials (ACF and AC) as RM was carried out in order to compare the catalytic activity of these materials with respect to GO-based materials used in this study.

ACF and AC were selected because these carbon materials have been widely used as adsorbents and RM in the adsorption and degradation processes of persistent contaminants, in batch and continuous systems. Fig. 2A shows the comparative microbial reduction of IOP under methanogenic conditions using the different carbon materials tested as RM with respect to control experiments lacking RM. Also, Table 1 shows the summary results of the biological reduction kinetics in both conditions. It can be observed a decrease of IOP concentration in methanogenic incubations of 44 and 59 % for AC and ACF, respectively, in an incubation period of 11 days. In contrast, a higher reduction of IOP is achieved using GO-based materials as RM with removal efficiencies of 64, 75 and 77 % for GO, rGO-2 and rGO-4, respectively (see Table 1). On the other hand, similar behavior was observed under sulfate-reducing conditions (see Fig. 2B and Table 1). IOP removal efficiencies reached 44 and 58 % of IOP using AC and ACF, respectively, which is lower than the efficiencies reported for GO-based materials with values of 61, 81 and 86 % for GO, rGO-2 and rGO-4, respectively. Also, the maximum removal rate achieved under methanogenic incubations using AC and ACF was 26.02 and 35.23 $\mu\text{g IOP/L-d}$, respectively. Under sulfate-reducing conditions, the maximum removal rate reached 31.98 and 39.25 $\mu\text{g IOP/L-d}$ for AC and ACF, respectively, which was lower than those observed for sludge incubation under both conditions in the presence of GO-based materials. These results show that the GO-based materials, specially reduced GO materials, promoted a faster and greater catalytic effect on IOP removal than commercial materials, confirming its potential for application as RM in wastewater treatment systems.

The redox-mediating capacity can be explained due to the concentration of quinone groups (a couple of carbonyl groups (Leon y Leon and Radovic, 1994)) in the carbon materials, which can mediate the transformation of recalcitrant pollutants by enhancing electron transfer (Cervantes et al., 2000; Pereira et al., 2014). Previous studies have reported that the concentration of carbonyl groups in the ACF and AC is 0.775 and < 0.23 milli-equivalents (meq)/g, respectively (Amezquita-Garcia et al., 2013; Nieto-Delgado and Rangel-Mendez, 2012). Also, it was reported by Boehm's acid-base titrations that the concentration of carbonyl groups in the GO, rGO-2 and rGO-4 was 1.23, 1.29 and 1.10 meq/g, respectively (Torral-Sánchez et al., 2016). Therefore, it can be suggested that the GO-based materials had a greater catalytic effect in comparison with AC and ACF due to the higher

concentration of quinone groups and to their availability since these are readily exposed, which contributes to a better electron transferring process towards IOP. Additionally, the results showed that the rGO-4 material improved the biotransformation of IOP. However, it can be seen a slight decrease in the carbonyl concentration of this material. This behavior can be explained due to the increase of electrical conductivity, which resulted in the enhancement of electron transfer through basal plane as will be mentioned later.

Table 1 shows that the microbial reduction of IOP was higher under sulfate-reducing conditions than under methanogenic conditions (86 % vs 77 % with respect to rGO-4), which could be explained due to more prevailing reductive conditions (Colunga et al., 2015). Also, biogenic sulfide generated under sulfate-reducing conditions may increase the IOP removal, since the conditions involve chemical and biological mechanisms that favor the reduction of IOP.

3.2. Biotransformation pathway of IOP

Previous studies have reported the biotransformation pathway of IOP under methanogenic conditions using different removal strategies (Cruz-Zavala et al., 2016; Pat-Espadas et al., 2015). However, the biotransformation pathway of this pollutant using GO-based materials as RM has not previously been elucidated. Furthermore, the biotransformation pathway of IOP under sulfate-reducing conditions by any removal strategy has not been reported yet. Accordingly, it is important to elucidate the mechanisms that take place during the microbial reduction of IOP under these two relevant conditions. In order to propose the biotransformation pathway of IOP, samples derived from biological reduction of IOP in the presence of rGO-4 as RM were analyzed by HPLC-MS. The molecular weights (m/z) of 13 principal transformation products (TP) were identified in sludge incubations under methanogenic conditions. Proposed structures for these TP and their elemental composition, and the suggested biological transformation pathway are shown in Fig. 3S of SM. The chemical structure modification of IOP includes several chemical reactions, which will be briefly described in the following lines.

The proposed structure of TP with m/z of 788.85 indicates the loss of two hydrogen atoms at either side chain A or B. The structure of TP 724 suggests the loss of a molecule of H₂O,

demethylation and decarboxylation in side chain B and N-demethylation in side chain A of IOP molecule. The structure of TP 775 implies the loss of a molecule of H₂O at side chain B of IOP molecule. The structures of TP 711 and 647 come from TP 788.80, involving demethylation and decarboxylation reactions in side chain A and dehalogenation of TP 647 (removal of one iodine atom, HI). The structure of TP 599 suggests the loss of a molecule of H₂O and demethylation in side chain A of TP 647. The cleavage of the C-N and amide bonds at the side chain A and B, respectively, and the removal of one iodine atom (HI) of TP 711 results in the formation of TP 375. Moreover, N-demethylation in side chain A of TP 599 results in the formation of TP with *m/z* of 578. The cleavage of amide bond in side chain B, deiodination of the aromatic ring (HI), demethylation and subsequent decarboxylation of side chain C from TP 578, yields TP 317 and 306.8. Finally, the proposed structure of TP with *m/z* of 772.86, 753 and 733, represents a slight modification in side chain A and B of TP 775, which include demethylation, N-demethylation and dehydration reactions.

The biotransformation pathway of IOP under sulfate-reducing conditions was also elucidated and the proposed structure of TPs can be observed in Fig. 4S of SM. Ten TPs were identified and biotransformation of IOP includes several chemical reactions similar to those observed under methanogenic incubations. Structure of TP 778.70 suggests that decarboxylation in side chain B was carried out. TP with *m/z* of 746.7 indicates removal of two molecules of water and demethylation of side chain B. The structures of the TPs identified as TP 736 and 720.70 result from N-demethylation in side chain A, and demethylation and elimination of one molecule of water of TP 778.70. Furthermore, structures of TP 328.28, 322.90 and 316.9 suggest the cleavage of amide bond in side chain A, dehalogenation (elimination of two iodine molecule of the aromatic ring) and elimination of the side chain B ramification, with sequential removal of molecules of H₂O and demethylation in side chain C. Moreover, TPs identified with *m/z* of 532 and 390.8 indicate the amide bond rupture in side chain A and B, and sequential deiodination (HI) of aromatic ring from TP 746.7. Finally, TP with *m/z* of 254.70 was identified as a result of deiodination of aromatic ring and C-N bond rupture in side chain B from TP 390.8. The mechanisms of the biotransformation pathway and *m/z* of TPs proposed in this work under both conditions are consistent with previous studies (Cruz-Zavala et al., 2016; Gros et al.,

2014; Lütke Eversloh et al., 2014; Pat-Espadas et al., 2015; Pérez et al., 2006; Schulz et al., 2008; Toral-Sánchez et al., 2016)

On the other hand, results obtained from microbial incubations performed without RM and active methanogenic biomass revealed that the main TPs produced were 646.6, 714.8 and 782.7. For sulfate-reducing incubations in the absence of RM, the main TPs with m/z identified were 578.9, 662.6, 720.8 and 782.7. According to these results, it can be concluded that the presence of GO-based materials as RM promoted a higher extent of IOP biological transformation (involving dehalogenation, dehydration, demethylation and decarboxylation reactions) in both conditions, which was evidenced by the formation of TPs with low m/z as compared to control incubations performed in the absence of these materials. The biological transformation of IOP under methanogenic and sulfate-reducing conditions and distinct capacities of GO-based materials to act as RM can be explained by their surface chemistry and physical properties as discussed in the next section.

3.3. Mechanisms involved in the biotransformation of IOP mediated by GO-based materials

Several studies have reported that carbonaceous materials can enhance electron transfer in reductive reactions of organic compounds by serving as RM (Larsen et al., 2000; van der Zee et al., 2003). It has been reported that the zigzag edges of rGO materials can accelerate the reduction reactions of recalcitrant pollutants due to the reactivity of their nonbonding π -electrons (Enoki et al., 2007; Gao et al., 2011). Moreover, the presence of GO-based materials can enhance extracellular electron transfer process in electroactive microorganism (Logan and Rabaey, 2012; Reguera et al., 2005), which is a promising pathway for biological reduction of pollutants (Summers et al., 2010; Wang et al., 2014). In addition, the high electrical conductivity of basal plane of rGO materials provides an excellent opportunity to improve the biotransformation performance of organic pollutants (Lu et al., 2014; Wang et al., 2014). Accordingly, it is expected that different mechanisms are involved in the electron transfer towards IOP mediated by GO-based materials.

As mentioned above, the basal plane of GO sheets has very high electrical conductivity that depends on the presence of epoxy and hydroxyl groups and it is generally proportional to

the C/O ratio (Toral-Sánchez et al., 2016). In order to determine the amount of carbon and oxygen on GO-based materials, XPS analysis was performed as can be seen on Fig. 3. The spectra show a peak corresponding to C-C stretching at 284.5 eV. Also, it can be observed that the intensity of the C-O and O-C=O peaks gradually decreases as the reduction degree of the samples is higher. In contrast, the intensity of C=O peak and its content ($\approx 5.36\%$) remain constant due to their greater stability in comparison with the other oxygenated groups as reported previously (Toral-Sánchez et al., 2016). Furthermore, XPS analysis indicated that the carbon content in GO, rGO-2 and rGO-4 was 41.6, 48.8 and 52.2 %, respectively, which indicated an increase in the C/O as the reduction degree advanced. On the other hand, Fourier transform-infrared spectra performed in our previous work showed a removal of mainly epoxy and hydroxyl groups when GO was chemically reduced by L-ascorbic acid (Toral-Sánchez et al., 2016). Hence, it was observed that the electrical conductivity of GO-materials increased as the reduction degree was higher, which was reflected in an increased biotransformation of IOP (see Fig. 1A and B).

In order to demonstrate this hypothesis, the electrical conductivity of GO-materials was measured. The results showed that this parameter increased as the reduction degree of GO-based materials was higher with values of 43.33 ± 1.2 , 72.3 ± 3.2 and $116.6 \pm 0.54 \mu\text{S/cm}$ for GO, rGO-2 and rGO-4, respectively. Furthermore, oxygenated functional groups in carbon materials, like quinone groups, have great redox activity (Montes-Morán et al., 2004) serving as acceptors and electron donors (Leon y Leon and Radovic, 1994), which improve the electrical conductivity of carbon materials, making best mediator for electron transfer (Fu and Zhu, 2013).

Finally, it has also been reported that the non-bonding π -electrons localized at the zigzag sites of graphene sheets are able to interact strongly with H, OH or halogenated groups (Jiang et al., 2007). IOP molecule has hydroxyl and halogenated (iodinated) groups in its chemical structure, which might well interact with the non-bonding π -electrons of graphitic sheets (Toral-Sánchez et al., 2016), favoring the partial rupture of this molecule during biological incubations as it can be seen in the biotransformation pathway (see Fig. 3S and 4S of SM).

3.4. Environmental relevance

Major efforts have been made to develop wastewater treatment systems integrating materials with redox mediator capacity and specific properties in order to improve efficient redox transformation of recalcitrant pollutants. Carbon-based materials, such as activated carbon and activated carbon fibers have been widely applied in chemical and biological reduction of a variety of contaminants, including azo dyes (Emilia Rios-Del Toro et al., 2013; Pereira et al., 2014; Van der Zee and Cervantes, 2009), nitroaromatic compounds (Amezquita-Garcia et al., 2013) and poly-halogenated contaminants (Fu et al., 2014). Recently, the use of graphene materials, like GO, has been studied as a promising material in the reduction of recalcitrant pollutant due to their physical and chemical properties. However, the use of these carbon nanomaterials in the removal of pharmaceutical compounds has been poorly studied. Also, the applications of graphene-based nanomaterials have a great interest in many research areas, which gives to this material an excellent opportunity to be used in the biological and chemical treatment of different pollutants.

The removal strategy proposed in the present study represents the first step toward a simple and viable alternative to apply GO-based materials in wastewater treatment systems to enhance the reductive transformation of recalcitrant pollutants. Also, the results suggest that the redox catalyst could be applied in the treatment of industrial effluents of pharmaceutical sector. This work has demonstrated that the combination of catalytic activity of GO-based materials and the biological activity of two relevant processes (methanogenic and sulfate-reducing conditions) involved in anaerobic bioreactors, promote the biotransformation of IOP more efficiently than the capacity observed separately. To our knowledge, this is the first study that demonstrates the effectiveness and successful application of GO-based materials as electron shuttle in the biotransformation of IOP. In addition, the information derived from the present study suggests that the GO-based materials could significantly promote dehalogenation and further biotransformation reactions of IOP, which could be relevant for similar emerging pollutants. Derived from this work, new technological challenges are the synthesis of novel GO-based materials for

immobilization and application as RM in wastewater treatment systems, in order to improve the biotransformation of recalcitrant pollutants.

4. Conclusion

GO-based materials were demonstrated, for the first time, as effective RM stimulating a higher biotransformation of IOP under methanogenic and sulfate-reducing conditions. The results showed the importance of the intrinsic properties of GO-based materials in the biotransformation of IOP. The presence of quinone groups, electrical conductivity and extracellular electron transfer processes appeared as key drivers in the biotransformation of IOP. Engineered GO-based materials combined with biological activity of relevant processes prevailing in anaerobic treatment systems could be applied to enhance the redox conversion of recalcitrant pollutant in industrial effluents.

Acknowledgments

The authors greatly acknowledge the financial support from the Council of Science and Technology of Mexico (Grants SEP-CB-2014-237118 and SEP-CONACYT 155656) and from the Marcos Moshinsky Foundation. In addition, the authors also appreciate the help provided by Laboratorio Nacional de Biotecnología Agrícola, Médica y Ambiental (LANBAMA, IPICYT) and Laboratorio de Investigaciones en Nanociencias y Nanotecnología (LINAN, IPICYT), as well as the technical support of A. Aguilar-Aguilar, A. Colunga-Álvarez, D. Partida-Gutiérrez, G. Vidriales-Escobar, J.P. Rodas-Ortiz, B. Rivera-Escoto, M. Martínez-Pérez, G. Labrada-Delgado, M. Bravo-Sánchez, Ma. del C. Rocha-Medina, A. Quezada-Rentería and I. Bueno-López.

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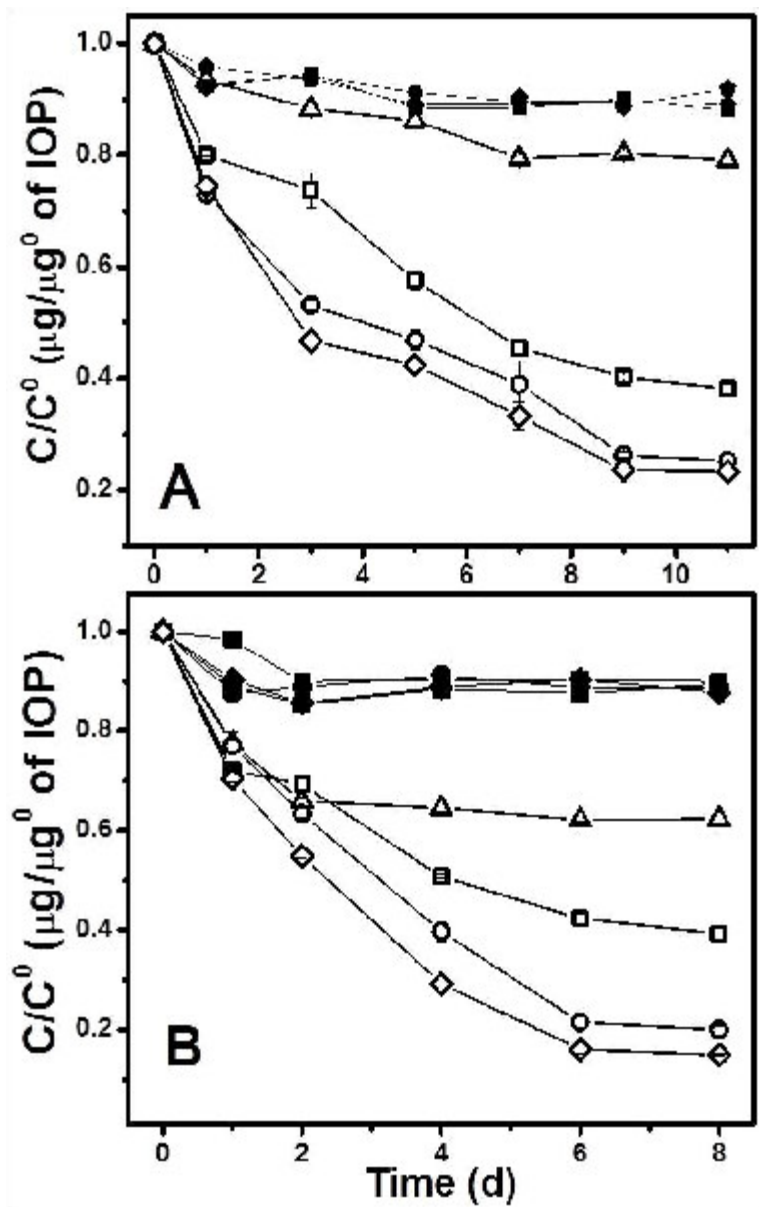
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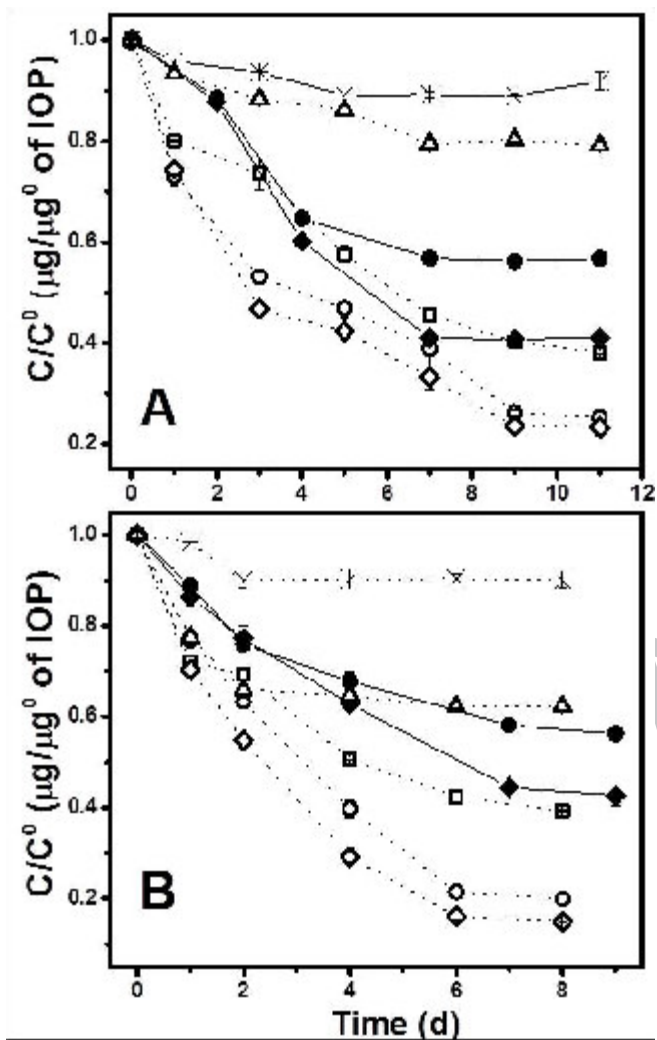
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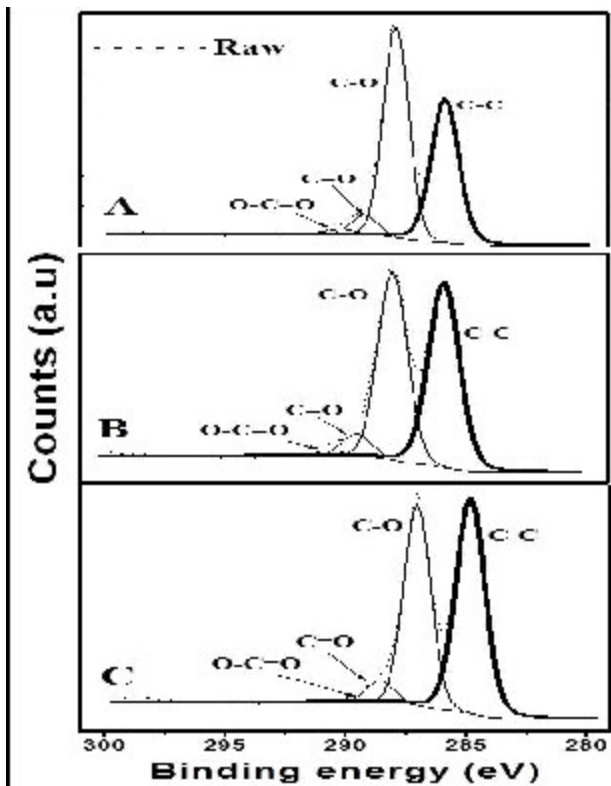
Figure 1. GO-based materials catalysis of biological reduction of IOP by anaerobic sludge under methanogenic (A) and sulfate-reducing conditions (B). Sterilized controls with and without GO-based materials (full symbol, Fig. A and B). Incubations in the absence of GO-based materials and activated biomass (open triangles, Fig. A and B). Biological reduction with GO-based materials as RM (Fig. A and B): GO (open squares), rGO-2 (open circles) and rGO-4 (open diamonds).

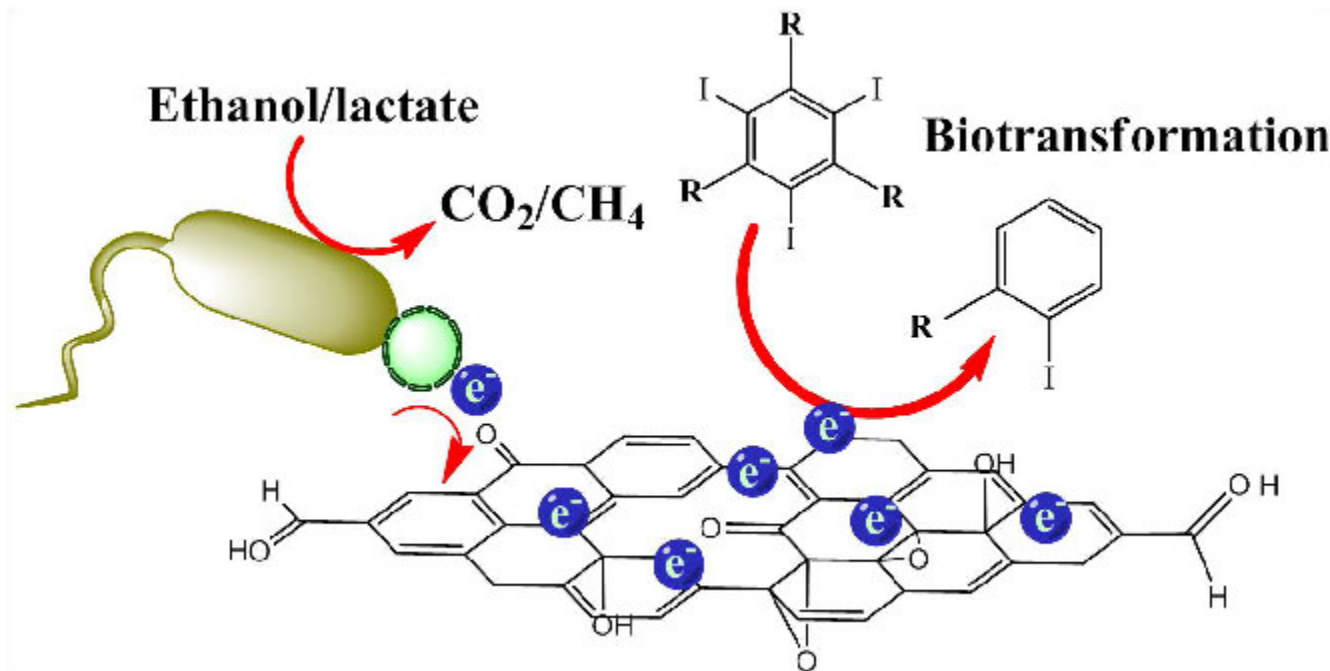
Figure 2. Comparative biological reduction of IOP using different carbon materials as RM by anaerobic sludge under methanogenic (A) and sulfate-reducing conditions (B). Sterilized control (asterisks, Fig. A and B). Incubations in the absence of GO-based materials and activated biomass (open triangles, Fig. A and B). Biological reduction with commercial materials and GO-based materials as RM (Fig. A and B): AC (full circles), ACF (full diamonds), GO (open squares), rGO-2 (open circles) and rGO-4 (open diamonds).

Figure 3. X-ray photoelectron spectra of GO-based materials with different reduction degrees: (A) GO, (B) rGO-2 and (C) rGO-4.









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Tables

Table 1. Summary results of biological reduction of IOP using different carbon materials as RM under methanogenic and sulfate-reducing conditions and concentration of carbonyl groups in these materials.

Treatments	Carbonyl groups (meq/g)	IOP reduction (%)	
		Methanogenic	Sulfate-reducing
SB + IOP*		< 10.0	< 10.0
AB + IOP*		20.0	38.0
AB + IOP + AC	< 0.23	44.0	44.0
AB + IOP + ACF	0.775	59.0	58.0
AB + IOP + GO	1.23	64.0	61.0
AB + IOP + rGO-2	1.29	75.0	81.0
AB + IOP + rGO-4	1.10	77.0	86.0

SB: sterile biomass, AB: active biomass. *Control experiments

- First application of GO-based materials in the biotransformation of iopromide.
- GO-based materials have properties to promote the biotransformation process.
- GO-based materials enhanced up to 5.5-fold the biotransformation of iopromide.
- Deiodination, decarboxylation, demethylation, and N-dealkylation occurred.
- Redox catalyst suitable for promoting degradation of emerging pollutants.

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