A wide perspective of carbon materials as catalysts for bioremediation of emerging pollutants and methanogenesis


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Abstract: Biotransformation of emerging pollutants under anoxic conditions can be accelerated by carbon materials (CM) acting as redox mediators. CM have been also extensively reported as facilitating external electron transfer in methanogenic processes. Here, different CM including magnetic carbon materials (C@MNP), were prepared, characterized and applied as RM on the biological reduction of Acid Orange 10 (AO10) and ciprofloxacin (CIP). CIP could be biologically removed in the presence of CNT and CNT@2%Fe, and AO10 decolourisation rates were 79-fold higher in the assays with CNT@2%Fe. The effect of carbon nanotubes (CNT) on the activity of several pure cultures of methanogens was also investigated, demonstrating that CNT could accelerate up to 17-fold the methane production rate. It is evident from this work that carbon materials with different chemical and textural characteristics can accelerate significantly bioremediation and methanogenic processes. The fact that concentrations as low as 0.1 g/L were used with positive effects, is remarkable in terms of economic feasibility of using CM as efficient catalysts in both processes.

Keywords: Anaerobic bioprocess; Azo dye; Ciprofloxacin; Conductive nanomaterials; Methane; Redox Mediator.

Introduction

Wastewaters containing pollutants released through natural and anthropogenic sources, if not efficiently treated, create serious public health and environmental problems. Anaerobic bioprocesses can be applied to wastewater treatment with simultaneous production of methane, a renewable energy source. However, strategies to accelerate the rate of electron transfer in anaerobic processes are needed to have more efficient and compact treatment systems (Van der Zee et al., 2001). Redox mediators (RM) can act as electron carriers in multiple redox reactions, accelerating the global reaction rate (Van der Zee and Cervantes, 2009).

The exceptional properties of nanomaterials such as the large surface areas, nano-diameters, large scaffold, rich electronic states, excellent chemical, mechanical and thermal stability, make them attractive for diverse applications, in different areas, including the remediation of pollutants as sorbents and as catalysts on biological and chemical removal. For instance, the addition of low amounts (0.1 g L⁻¹) of different carbon materials (CM), namely activated carbon, carbon xerogels and carbon nanotubes (CNT), accelerated the biotic and abiotic anaerobic reduction of azo dyes and aromatic amines (Pereira et al., 2010; 2014; 2016; Pereira et al., 2016b). There are also several reports on the improvement of methane production in bioreactors amended with CM (Martins et al., 2018). C@MNM can be fabricated by combining CM and magnetic nanomaterials (MNM), aiming to increase their catalytic and adsorptive properties and enable their effective separation after
reaction by a magnetic field. Moreover, coating improves their stability and allows introducing additional surface properties and functionalities.

Different C@MNM were prepared and applied as RM on the biological reduction of the azo dye AO10 and the antibiotic CIP. We also studied the effect of CNT on the activity of pure cultures of hydrogenotrophic and acetoclastic methanogens.

Material and Methods

Preparation of nanomaterials
A set of core(ferrite)-shell(carbon) nanocomposites (C@FeO), with a core composed of a ferrite of the type FeO and MFeO (M, a divalent metal cation such as Mn$^{2+}$ or Co$^{2+}$) coated with carbon by chemical vapour deposition (CVD) or hydrothermal method (HdM), were prepared. A composite made of CNT (NC3100$^{TM}$) impregnated with 2 % of iron, CNT@2%Fe, was also prepared as described in Pereira et al. (2017). Single FeO and CNT were also tested. The textural and chemical properties of the materials were characterized as previously reported (Pereira et al., 2017).

Application of nanomaterials on the anaerobic removal of model compounds
All materials were tested as catalysts on the anaerobic biodegradation of AO10 (0.5 mmol L$^{-1}$) by conducting batch assays. CNT and CNT@2%Fe were also applied on the removal of CIP (0.015 mmol L$^{-1}$). The substrate used as the primary electron donor in the assays with AO10 was a VFA mixture (acetate, propionate and butyrate in a chemical oxygen demand COD based ratio of 1:10:10; total COD = 2 g L$^{-1}$) and ethanol (30 mol L$^{-1}$) in the assays with CIP. Controls were made: biological assays without materials, abiotic assays with the materials; biomass and materials; only the nanomaterials, or only the model pollutants; sterile controls by autoclaving the reaction media with biomass. For the assays with CIP, a control without the substrate was also included. The materials reutilization was studied by testing the best nanomaterials in successive cycles of the decolourisation of AO10 (Pereira et al., 2017). The decolourisation of AO10 was followed by spectrophotometry and dye reduction by Ultra High-Performance Liquid Chromatography (UHPLC) as described before in Pereira et al. (2017). Reduction of CIP was also evaluated by UHPLC, as reported in (Silva et al., 2016).

Toxicity of samples after the CIP treatment was evaluated by the bioluminescent standard method ISO 11348-1 and 11348-3, using Vibrio fischeri, as described in Silva et al. (2016).

Effect of CNT on the methanogenic activity
To evaluate the effect of CM on the activity of methanogenic communities, increasing concentrations of CNT (0.1 g L$^{-1}$, 0.5 g L$^{-1}$, 1 g L$^{-1}$, 5 g L$^{-1}$) were added to pure cultures of acetoclastic (Methanosarcina mazei and Methanosaeta concilii) and hydrogenotrophic (Methanobacterium formicicum and Methanospirillum hungatei) methanogens. Incubations were performed in bicarbonate buffered anaerobic medium supplemented with salts, vitamins and sodium sulfide, as described elsewhere (Stams et al. 1993). For growing hydrogenotrophs, the bottles headspace was composed of H$_2$/CO$_2$ (80 %:20 %, v/v, at 1.7 x 10$^5$ Pa) and for the acetoclasts N$_2$/CO$_2$ (80 %:20 %, v/v, at 1.7 x 10$^5$ Pa) was used instead. Control assays without CNT, abiotic assays, and an assay without addition of sodium sulfide (as reducing agent) were also conducted. Methane production, pH, redox potential (ORP), was monitored during the incubations as described by Salvador et al. (2017).

Results and Conclusions

Application of nanomaterials on AO10 removal
In the absence of nanomaterials, only ~30% of biological decolourisation of AO10 was obtained, at a rate of ~0.2 d$^{-1}$. The presence of 0.1 g L$^{-1}$ of core-shell materials had no effect on the reaction, but at 0.5 or 1.0 g L$^{-1}$, the extent and rates of AO10 biological decolourisation have improved (Figure 1). These results are explained by the low SBET of the core-shell materials. The single MNP had no
effect in the AO10 decolourisation. Among the C@MNP core-shell materials tested, the best were the C@FeO_CVD850 and the C@FeO_CVD.NH₃ composites: over 90% of removal was obtained and the rates increased up to 30-fold. The higher rate with C@FeO_CVD.NH₃ may be related with the fact that doping of CM with heteroatoms (like N) allows rearranging the electrons in the carbon surface and changes the electronic properties of the CM, enhancing their stability and catalytic performance (Figueiredo et al., 2009). Rates of AO10 decolourisation were improved up to 79-fold in the presence of CNT@2%Fe, as compared with the control assay, and as compared with core-shell MNP, probably due to the high surface area and availability of iron (Figure 1A).

In abiotic assays, AO10 decolourisation due to azo dye reduction, also occurred (Figure 1B). Based on these results, a mechanism for the AO10 reduction catalyzed by C@FeO composites is proposed (Figure 1C). In biological assays, electron transfer may occur through: the biological oxidation of the co-substrate (VFA) to AO10 (1), or to the carbon shell or CNT of composites and then to AO10 (2); oxidation of Fe²⁺ carbon shell or CNT of composites, and then to AO10 (3). In the case of abiotic process, only the third mechanism of electron transfer may occur. The results also showed that both C@FeO_CVD850 and CNT@2%Fe can be successfully applied in successive cycles of AO10 reduction (Pereira et al., 2017).

Application of nanomaterials on CIP removal
Biological removal of CIP was observed with CNT and CNT@2%Fe (unpublished work). The obtained results suggest the occurrence of different mechanisms for CIP removal: adsorption on sludge and/or on CM; biological reduction due to the electrons generated by the oxidation of ethanol; abiotic reduction in the presence of CNT@2%Fe, due to electron flow from Fe²⁺ to CNT and then to CIP (adsorbed and on solution), as explained before for AO10. After the anaerobic treatment with and without CM, the products of CIP transformation were significantly less toxic, as assessed by Vibrio fischeri standard method.

Effect of CNT on the activity of pure cultures of methanogens
The addition of CNT reduced the lag phases preceding methane production, and increased the initial methane production rate (IMPR) of all methanogens tested (Table 1, Salvador et al., 2017). However, the improvement of the methanogenic activity was even more evident with the hydrogenotrophic methanogens. For example, the IMPR of *M. formicicum* increased up to 17 times with CNT when
compared with the control assay without CNT. Increasing CNT concentrations resulted in the decrease of the ORP of the reaction medium, which was directly correlated to the increase of the IMPR. Nevertheless, although low ORP are beneficial for methanogenesis, it was not the major factor affecting the methanogenic activity. Even though higher ORP were measured in assays performed without reducing agent, the beneficial effect of increasing concentrations of CNT was maintained (Table 1). Therefore, CNT enhances the activity of pure cultures of methanogens by yet unknown mechanisms. These results may explain the acceleration of anaerobic digestion processes when CNT and other CM are provided.

<table>
<thead>
<tr>
<th>CNT (g L⁻¹)</th>
<th>Methanogen:</th>
<th>M. formicicum</th>
<th>M. formicicum (without reducing agent)</th>
<th>M. hungatei</th>
<th>M. concilii</th>
<th>M. mazei</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>IMPR (mmol L⁻¹ d⁻¹)</td>
<td>0.14 ± 0.04</td>
<td>No growth</td>
<td>0.7 ± 0.16</td>
<td>0.21 ± 0.02</td>
<td>0.14 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>ORP (mV)</td>
<td>-301 ± 18</td>
<td></td>
<td>-331 ± 30</td>
<td>-302 ± 14</td>
<td>-298 ± 6</td>
</tr>
<tr>
<td>0.1</td>
<td>IMPR (mmol L⁻¹ d⁻¹)</td>
<td>0.20 ± 0.02</td>
<td>No growth</td>
<td>0.22 ± 0.01</td>
<td>0.22 ± 0.06</td>
<td>0.23 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>ORP (mV)</td>
<td>-310 ± 13</td>
<td></td>
<td>-327 ± 25</td>
<td>-310 ± 18</td>
<td>-307 ± 6</td>
</tr>
<tr>
<td>0.5</td>
<td>IMPR (mmol L⁻¹ d⁻¹)</td>
<td>0.97 ± 0.12</td>
<td>1.00 ± 0.06</td>
<td>1.97 ± 0.45</td>
<td>0.23 ± 0.03</td>
<td>0.28 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>ORP (mV)</td>
<td>-313 ± 2</td>
<td></td>
<td>-349 ± 37</td>
<td>-316 ± 15</td>
<td>-314 ± 9</td>
</tr>
<tr>
<td>1</td>
<td>IMPR (mmol L⁻¹ d⁻¹)</td>
<td>1.20 ± 0.12</td>
<td>1.41 ± 0.14</td>
<td>3.55 ± 0.69</td>
<td>0.27 ± 0.02</td>
<td>0.19 ± 0.01</td>
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<tr>
<td></td>
<td>ORP (mV)</td>
<td>-340 ± 20</td>
<td></td>
<td>-380 ± 28</td>
<td>-322 ± 17</td>
<td>313 ± 10</td>
</tr>
<tr>
<td>5</td>
<td>IMPR (mmol L⁻¹ d⁻¹)</td>
<td>2.34 ± 0.09</td>
<td>1.75 ± 0.06</td>
<td>3.87 ± 0.35</td>
<td>0.15 ± 0.02</td>
<td>0.08 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>ORP (mV)</td>
<td>-360 ± 13</td>
<td></td>
<td>-402 ± 9</td>
<td>-346 ± 11</td>
<td>-334 ± 12</td>
</tr>
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</table>

Here we demonstrate that CM are advantageous to increase the efficiency of removal of organic compounds and water detoxification, and to increase the methane production rates in methanogenic processes. Magnetic materials, e.g., CNT@2%Fe, can be easily separated from the treated water by applying a magnetic field, which is an advantage comparatively to other soluble materials.

### References


