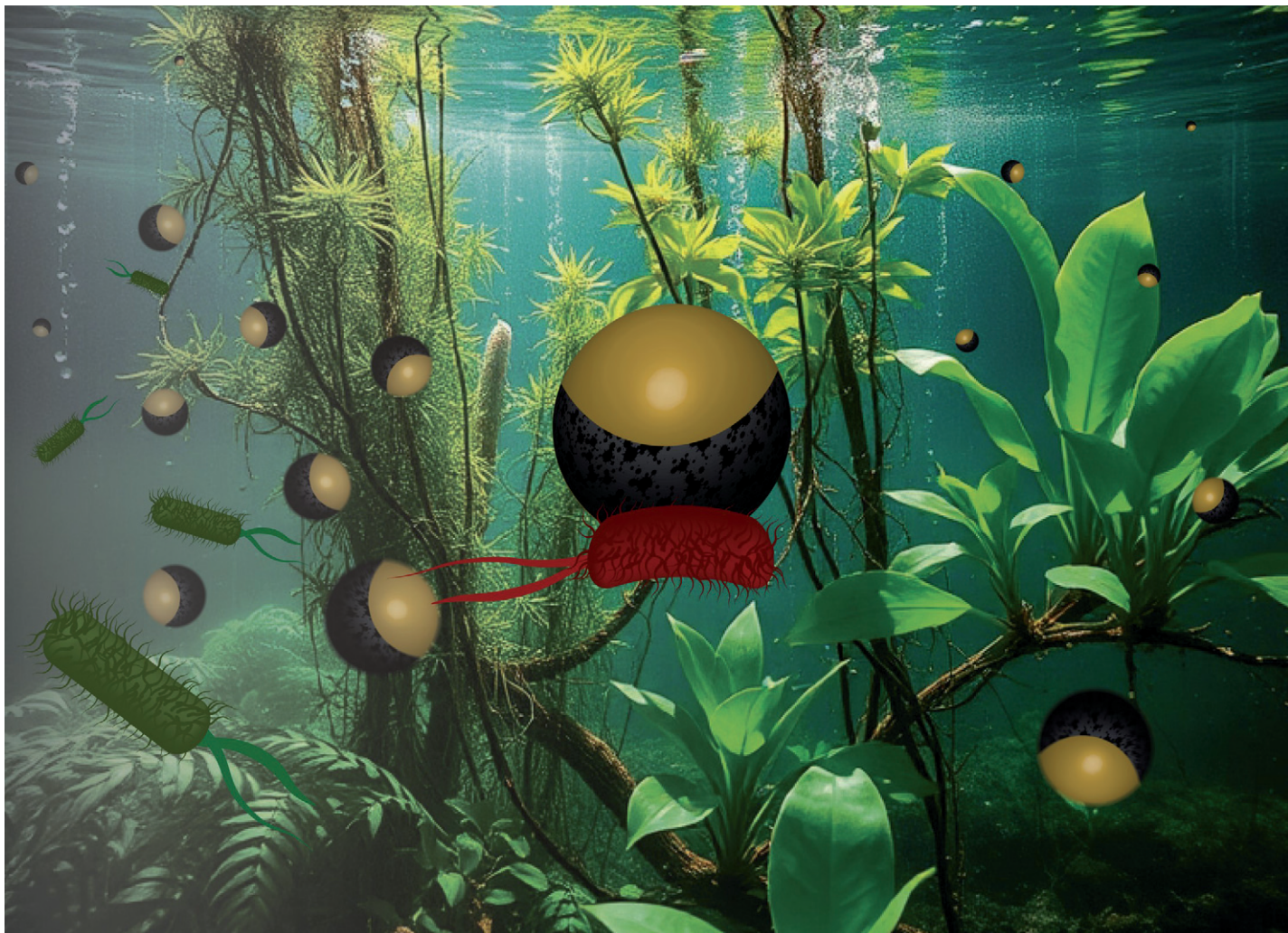




Showcasing research from Carmen Cuntín-Abal, Beatriz Jurado-Sánchez and Alberto Escarpa, Departamento de Química Analítica, Química Física e Ingeniería Química, Universidad de Alcalá, Madrid, Spain.

Micromotors for antimicrobial resistance bacteria inactivation in water systems: opportunities and challenges

The image showcases the main aim of our perspective, which is to provide an updated overview of the opportunity and challenges of micromotors to remove harmful bacteria and antibiotics from water. The picture illustrates a river bottom with a dark part at the left, indicating that the water is contaminated with bacteria (which can be visualized in green). The spheres are micromotors navigating in the water and killing the bacteria, resulting in the clean, bright water.

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# Micromotors for antimicrobial resistance bacteria inactivation in water systems: opportunities and challenges

Carmen Cuntín-Abal, <sup>a</sup> Beatriz Jurado-Sánchez <sup>\*ab</sup> and Alberto Escarpa <sup>\*ab</sup>

The intensive use of antibiotics and the inadequate removal in water treatment plants have contributed to the phenomena of antimicrobial resistance. Bacterial colonies and biofilms present in water distribution and aquatic systems respond to the presence of antibiotics by the generation of resistance genes and other determinants transmitted through the environment. In this perspective, we identify the opportunities and challenges of self-propelled micromotors in the fight against antimicrobial resistance by the elimination of antibiotics and bacteria in water. Recent progress is contextualized in the current scenario in terms of bacteria and antibiotics found in real settings and current removal technologies. As illustrated in this perspective, the unique features of micromotors result in a high surface area to-mass ratio for enhanced degradation capabilities, for both antibiotic removal and bacteria biofilm inactivation, as compared with static current technologies. The autonomous movement of micromotors allows us to reach more volumes of water and even hard-to-access areas, offering great opportunities to reach hard-to-access pipelines, not accessible by current approaches. Yet, as envisioned in this perspective, micromotors are far away from real applications, hampered mainly by the main challenges of the treatment of high-water volumes. We also advocate scientists to include in the proof-of-concept studies real water and the evaluation of a major number of antibiotics and bacteria commonly found in real settings, as will be described in this perspective. Micromotors hold considerable promise as a holistic approach to fight antimicrobial resistance, but cross-discipline collaborations are a must to translate the recent progress into real practical applications.

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## Environmental significance

Micromotors are micro and nanoscale devices capable of autonomous movement in solution. Among the range of applications, micromotors can offer a new dimension for the removal of pollutants from water systems, accounting for the autonomous movement and enhanced surface-to-area ratio. While their potential has been illustrated in relevant proof-of-concept applications, important challenges remain for practical application. This perspective gives an updated and critical overview of the use of micromotors with emphasis on the holistic treatment of the spread of antibiotic resistance factors propagated in water. Challenges and opportunities in the use of micromotors for antibiotic and bacteria biofilm removal are discussed, with some directions and recommendations for future studies. The topic was selected accounting for the relevance to the well-being of society.

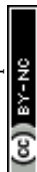
## 1. Introduction

Water contamination with bacteria is a serious concern worldwide. The inadequate recycling of urban wastewaters and anthropogenic activities are among the major causes of such contamination. Indeed, drinking water can become contaminated by bacteria, which can even reach rivers and

other environmental water systems.<sup>1,2</sup> Strict water regulations in Canada, Europe, and the US assure the quality and safety of water for human consumption.<sup>3</sup> Yet, it is becoming increasingly clear that urban wastewater is a key source of antibiotic resistance determinants, *i.e.* antibiotics, antibiotic-resistant (ABR) bacteria, and antibiotic resistance genes (ARGs).<sup>4</sup> Antibiotic resistance is recognized as one of the most important challenges of contemporary medicine and a serious public health problem.<sup>5</sup> This is an extremely dangerous phenomenon, which consequently prevents the effective treatment of bacterial infections, causing epidemic threats and high mortality.<sup>6,7</sup> Protection of our urban water

<sup>a</sup> Department of Analytical Chemistry, Physical Chemistry, and Chemical Engineering, Universidad de Alcalá, Alcalá de Henares, E-28805 Madrid, Spain.  
E-mail: beatriz.jurado@uah.es, alberto.escarpa@uah.es

<sup>b</sup> Chemical Research Institute "Andrés M. del Río", Universidad de Alcalá, E-28805, Madrid, Spain



resources is key to protect our environment, which ultimately, will have a direct effect on human health and the well-being of society.

Two main reasons are responsible for the presence ARB and ARGs in water environments: the intensive use of antibiotics for human, veterinary, and agricultural purposes and the inadequate removal in treatment plants, which generates highly resistant bacteria biofilms. In this context, antibiotics have been crucial as a unique solution to combat pathogenic infections in humans and animals, increasing the overall quality of life. Yet, the inadequate metabolism of such compounds led to their release in wastewater. Current treatment technologies are inadequate to completely remove them in wastewater, not to mention the absence of treatment in some undeveloped countries. The release of such antibiotics in the water environment causes a response in

already existing bacteria biofilms, which tend to survive by releasing ARGs. This can induce a cascade process with horizon gene transfers among different bacteria genotypes in different water compartments, contributing even more to the development of ARB.<sup>8,9</sup> As it is estimated that the use of antibiotics will increase worldwide,<sup>10</sup> new means for adequate treatment are needed to overcome adverse effects.

As already mentioned, biofilms have an important contribution in the generation of ARB. A biofilm is composed of a group of bacteria immobilized in a synthetic matrix known as extracellular polymer substances.<sup>11</sup> This naturally occurring phenomenon is a way of protection and survival of bacteria that show increased resistance to traditional means of treatment. Such biofilms can also colonize surfaces and water systems, being very difficult to remove. In addition, the closeness between bacteria in biofilms allows for communication among them such as quorum sensing, regulating their growth against external factors.<sup>12</sup> Most importantly, this can facilitate ARG and the exchange of factors that create antibiotic resistance.<sup>13</sup> Therefore, it is crucial to develop new strategies and tools for the removal of water contaminants for successful water reutilization, at a low cost, with minimal energy consumption and high efficiency. In this context, emerging nanotechnology applications have added a new dimension to environmental remediation processes.<sup>14,15</sup> In particular, micromotors (MMs) are microscale devices that can convert different energies (magnetic, light, ultrasound fields, etc.) into motion.<sup>16</sup> The self-propulsion of micro/nanomotors, along with the turbulent flows created by their motion, exhibit considerable potential to overcome the diffusion limit of common water treatment operations.<sup>17,18</sup> The continuous movement of such microscale objects imparts significant mixing without



**Carmen Cuntín-Abal**

*Carmen Cuntín Abal obtained a Chemistry degree at the University of Granada in 2019 and a master's degree at the same University, specializing in biological analysis, in 2021. In 2022, she started her PhD, thanks to a grant, at the University of Alcalá focusing on the use of micromotors for the diagnosis and treatment of bacterial diseases under the supervision of Professor Beatriz Jurado and Professor Alberto Escarpa.*



**Beatriz Jurado-Sánchez**

*Beatriz Jurado Sánchez is an Associate Professor at the University of Alcalá. Her research interests are the synthesis of tubular and Janus micromotors based on nanomaterials, the integration of micromotors into portable instrumentation, biosensing with micromotors integrating quantum dots, and the development of micromotor-based environmental remediation approaches. Prof. Jurado has co-authored over 92 scientific papers (H index = 42), 5*

*book chapters, and more than 20 communications at international conferences. Her research has been highlighted as a cover in the top impact journals Angewandte Chemie International Edition, Chemical Science, and Analytical Chemistry. She has served as an Assistant Editor for Microchimica Acta, Associate Editor at RSC Advances and is currently Associate editor for Analytical Methods from RSC.*



**Alberto Escarpa**

*Alberto Escarpa is a Full Professor at the University of Alcalá. His main research interests are focused on microfluidics and micromotors. He has co-authored more than 200 articles in leading international peer-reviewed journals, and several books, and book chapters. He has given several invited lectures in prestigious forums. He has been included in Stanford University's list of the 2% of the most cited*

*scientists. His research has been highlighted as a cover in Angewandte Chemie, Chemical Science, Nanoscale, and Analytical Chemistry. He is a member of the Editorial Advisory Board of Analytical Chemistry. He is an Editor in Chief for Microchimica Acta.*



external stirring, leading to higher efficiencies and shorter clean-up times in water cleaning and other environmental processes.<sup>19</sup> Most importantly, MMs can exhibit a “tactic” behaviour by the action of a different stimulus, responding to changes in the surrounding environment and moving away or towards specific locations.<sup>20,21</sup> This increases the density of MMs in a defined area, creating new phenomena arising from the influence of the motion of one MM towards surrounding ones.<sup>22</sup> The aim of this perspective is to give an insight into the opportunities of current MM technology as a holistic approach to fight against antibiotic resistance by the removal of antibiotics and bacteria biofilms. The most important strategies will be discussed, with adequate contextualization in the current scenario in terms of specific contaminants and removal strategies. The main challenges facing MM technology in the environmental field and potential solutions for translation into practical applications will be also discussed. While still in its early infancy, the MMs’ scale, strong propulsion ability, and capacity to communicate hold considerable promise for the next generation of tools for combating antimicrobial resistance.

## 2. Engineering micromotor propulsion for antibiotic and bacteria biofilm removal

Energy sources utilized for MM propulsion will exert a strong influence in the final application.<sup>23–25</sup> Material aspects, such as the presence of co-contaminants in wastewater that can poison the catalyst or interfere with the propulsion mechanism, are important to achieve prolonged locomotion of MMs in water environments. A second important aspect is to develop environmentally biocompatible MMs, both in terms of materials and propulsion aspects. While powerful, chemically-propelled MMs usually need toxic fuel and surfactants for propulsion, adding pollution to the water environment. A third aspect is the possibility of recovering the MMs after treatment. This can be achieved by including magnetic layers or using magnetic MMs.<sup>26</sup> Another alternative is the use of self-degrading MMs, as illustrated with magnesium Janus MMs.<sup>27–29</sup> Exploiting the different MM propulsion schemes can be very convenient for pollutant removal. For example, the by-products generated during MM propulsion can contribute to the degradation of the pollutants or the elimination of all of them without another intervention. Relevant examples are the generation of hydroxyl ions for Fenton-like processes, carried out by manganese dioxide catalytic MMs,<sup>30–32</sup> or radical oxygen species (ROS) generation that can be useful for biofilm disruption.<sup>33</sup> The versatility of current synthetic approaches for MM preparation procedures allows for the incorporation of tailored materials for multiple propulsion modes, allowing us to address the potential risk of hampered or incompatible locomotion in complex media. This will be

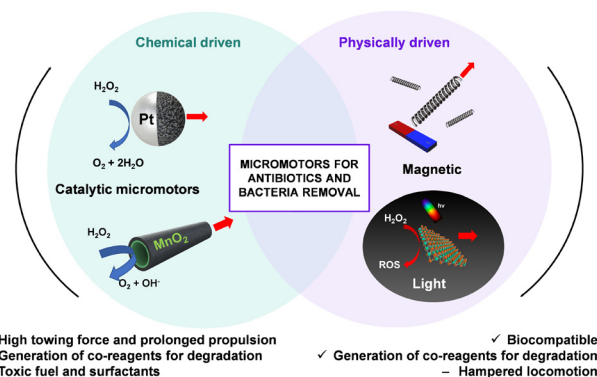


Fig. 1 Schematic of the propulsion and main features of MMs for antibiotic and bacterial inactivation in water.

discussed in more detail in the two following sections when discussing current MM approaches for antibiotic and bacteria biofilm removal. A summary of the relevant propulsion mechanisms, along with the main advantages and disadvantages, is depicted in Fig. 1.

Chemically-driven MMs can convert chemical energy into mechanical energy by local chemical reactions with the presence of fuels in the solution. In brief, the mechanism responsible for bubble propulsion relies on the decomposition of hydrogen peroxide (as fuel) in the platinum/manganese dioxide nanoparticle patch of the nano/micromotors.<sup>34,35</sup> Local generation of oxygen bubbles pushes the MMs forward. This results in strong propulsion and enhanced fluid mixing, improving the likelihood of the pollutant-MM interaction. This has the consequence of higher efficiency as compared with static processes. Yet, high concentrations of fuels are usually required, which can impose toxic and harmful effects on the environment and the human body.<sup>36</sup>

Physically-propelled MMs are powered by external physical stimuli, such as acoustic, magnetic, or electromagnetic radiation.<sup>37</sup> A strong propulsion output required for efficient movement demands a high physical energy input, which is not economical in most cases and may even be harmful to the environment, such as the use of high-intensity ultraviolet (UV) light. Magnetic propulsion has shown considerable promise for environmentally-friendly propulsion, solving the above-mentioned constraints. As a noninvasive and biocompatible form of energy, magnetic fields are used for MM propulsion.<sup>38</sup> In comparison with the extraordinary progress on chemically-propelled MMs, relatively few applications have been described on magnetically-propelled MMs with tactic behavior. Yet, MMs can exhibit magnetotactic behavior towards a magnetic field, which can increase the overall performance of the intended environmental process. Vis-light radiation is also a convenient external stimulant for MM propulsion/directional control. Indeed, light is easily-acquired, renewable, and cheap energy that does not produce any by-products that may be harmful to the environment, as well as showing high



biocompatibility to tissues or any cells. According to the light-triggered motion mechanism, light-driven MMs can be classified according to the type of mechanism responsible for propulsion: the self-electrophoretic effect, self-diffusiophoretic effect, thermophoretic effect, and bubble-induced propulsion. Light-induced self-diffusiophoretic propulsion results from the asymmetrically generated gradients of photocatalytic electrolytes and nonelectrolyte products, while light-induced self-electrophoretic propulsion is enabled by the self-generated electric field from the asymmetric distribution of ions across the bipolar MM system.<sup>39,40</sup> It should be considered here that electrophoretic and self-electrophoretic mechanisms are strongly influenced by the presence of salts and other constituents in water, which can hamper the efficient propulsion of MMs.

The thermophoretic effect, on the other hand, relies on the asymmetric generation of thermal gradients by a local heat increase in the material, induced by light, with transition metal dichalcogenide MMs ideal for this choice. Collective MM locomotion can also be achieved with light-driven configurations. The MMs can autonomously migrate to and against areas that contain a high concentration of photocatalytic products or more intense light beams, exhibiting an impressive swarming behavior that can increase the overall efficiency of antibiotic removal and bacteria biofilm inactivation.<sup>20</sup>

Bacteria communication mechanisms can also inspire the community to engineer MM propulsion to mimic microorganisms within the contaminated environment. In this context, quorum sensing represents bacteria-to-bacteria cell communication *via* signal molecules called autoinducers. Such substances control the individual behavior of the bacteria and make the population synchronize and unify behaviors. Quorum sensing has been found to play a critical role in converting microcolonies into mature biofilms. Thus, the inactivation of quorum sensing autoinducers by different strategies can be an effective means to control biofilm formation and fight against ABR bacteria.<sup>41,42</sup> In this context, the motion of MMs and their ability to cooperate into swarms can present a convenient strategy to interfere with the quorum sensing mechanism, disabling the chemical responsible for biofilm formation, preventing the formation.

### 3. Current state of micromotors for antibiotic removal: where we are and where we are moving

#### 3.1. Overview of antibiotics in water environments and removal processes

Antibiotics are crucial to treat life-threatening bacterial infections. From 2000 to 2018, antibiotic consumption increased over 46%.<sup>43</sup> This rate increased during the COVID-19 pandemic, and it is estimated that it is going to be even higher, to 200%.<sup>10</sup> Several studies have evaluated the occurrence and fate of antibiotics in the water environment,

**Table 1** Antibiotics present in different water reservoirs and levels

| Water                               | Class of antibiotics                                                                                                   | Levels (ng mL <sup>-1</sup> ) | Ref.     |
|-------------------------------------|------------------------------------------------------------------------------------------------------------------------|-------------------------------|----------|
| Wastewater (hospital)               | Sulphonamides<br>Quinolones<br>Macrolides<br>Tetracyclines                                                             | 10–68 700                     | 46, 47   |
| Wastewater (urban)                  | Sulphonamides<br>Quinolones<br>Macrolides<br>Tetracyclines<br>Trimethoprim<br>β-Lactam<br>Lincomycin<br>Aminoglycoside | 7–400                         | 4, 48–52 |
| River (urban)                       | Tetracycline                                                                                                           | 854                           | 53       |
| River (drinking water)              | Sulphonamides<br>Quinolones<br>Tetracyclines                                                                           | 23–290                        | 54       |
| Drinking water (tap, bottled water) | Sulphonamides<br>Quinolones<br>Macrolides<br>Tetracyclines<br>β-Lactam<br>Florfenicol                                  | 0.0010–0.0089<br>92–666       | 55<br>56 |

with the main identified sources being hospital effluents, municipal water effluents, and agriculture-derived wastewaters.<sup>4,5,44,45</sup> Table 1 shows a brief summary of different environmental water reservoirs and the most common types of antibiotics found, along with the concentration and relevant references.

From the results shown in Table 1, the presence of a high amount of antibiotics is clear, with urban wastewater being the main source with a great variety of families (up to 8). Yet, in hospital effluents, the level of these compounds can reach up to 68 700 ng mL<sup>-1</sup>.<sup>46,47</sup> It is well-known that current wastewater treatment technologies are not efficient for the complete removal of such pollutants. While there is no clear consensus in the literature, some reports reveal the presence of up to 6 families of antibiotics in water. While such reports are focused mainly on China, it is clear that antibiotics are present in tap water and even in bottled water.<sup>55,56</sup> Indeed, common processes used for antibiotic removal in wastewater plants are not efficient for its complete elimination. Ozonation and Fenton processes are more efficient for macrolide removal (up to 86%), but are not efficient for 100% removal.<sup>4</sup> Adsorption-based approaches such as the use of activated carbons have a variable efficiency for removal depending on the type of antibiotic family, ranging from 0 (in the case of some macrolides) to 100% (in the case of tetracyclines). The urgent necessity to develop more efficient techniques to meet the criteria for safe discharge into the environment and to assure safe drinking water consumption is clear. Most importantly, a reduction in the generation of ABR bacteria will be also observed. The enhanced fluid mixing of MMs, the swarming effect, and the myriad of materials and propulsion mechanisms can open new avenues for more efficient processes for antibiotic removal. Once we



**Table 2** MM approaches for antibiotic removal according to the propulsion mechanism

| Chemically-driven micromotors                                                              |                                   |                                          |      |
|--------------------------------------------------------------------------------------------|-----------------------------------|------------------------------------------|------|
| Micromotor (mechanism)                                                                     | Antibiotic (removal efficiency)   | Antibiotic levels (ng mL <sup>-1</sup> ) | Ref. |
| CoFe <sub>2</sub> O <sub>4</sub> Janus (Fenton)                                            | Tetracycline in wastewater (84%)  | 50 000                                   | 57   |
| MgAl/MnO <sub>2</sub> tubular (Fenton)                                                     | Oxytetracycline in DI water (88%) | 50 000                                   | 58   |
| Pollen MnO <sub>2</sub> /Fe <sub>3</sub> O <sub>4</sub> (Fenton)                           | Tetracycline in DI water (60%)    | 80 000                                   | 59   |
| Poly (aspartic acid)/Fe <sub>3</sub> O <sub>4</sub> /MnO <sub>2</sub> tubular (Fenton)     | Tetracycline in DI water (90%)    | 30 000                                   | 60   |
| Fe–MnO <sub>2</sub> core–shell (adsorptive bubble separation and Fenton)                   | Tetracycline in DI water (80%)    | 50 000                                   | 61   |
| CuS@Fe <sub>3</sub> O <sub>4</sub> /Pt Janus (light-enhanced Fenton)                       | Tetracycline in DI water (81%)    | 40 000                                   | 62   |
| Hydrogel Mn <sub>3</sub> O <sub>4</sub> /CoFe <sub>2</sub> O <sub>4</sub> Janus (sorption) | Erythromycin in DI water          | 200 000                                  | 63   |
| Graphene/Pt Janus (sorption)                                                               | Tetracycline in DI water (96%)    | 10 000                                   | 64   |
| MgAl-LDH/Mn <sub>3</sub> O <sub>4</sub> /molecular imprinted polymer tubular (sorption)    | Doxycycline                       | 250 000                                  | 65   |
| Physically-driven micromotors                                                              |                                   |                                          |      |
| Light-driven micromotors                                                                   |                                   |                                          |      |
| ZnO/enzyme (enzymatic degradation)                                                         | Oxytetracycline                   | 0.04                                     | 66   |

established the current scenario and challenges in antibiotic removal, we will contextualize in the next section the current state, opportunities, and challenges of MMs in this field.

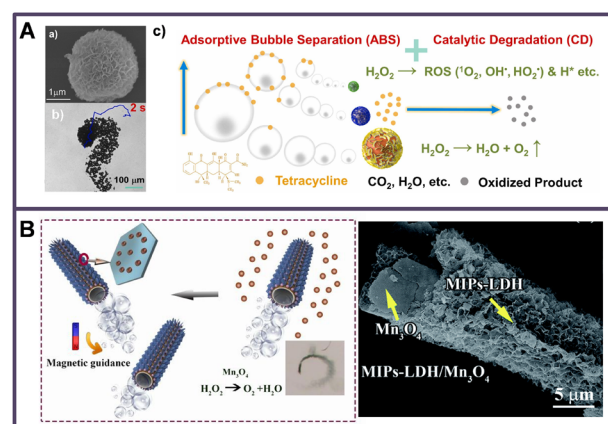
### 3.2. Overview of micromotor approaches for antibiotic elimination in water

Table 2 summarizes the current existing approaches using MMs for antibiotic removal in water. At first glance, the field is still in an early development stage. Most strategies focus on the removal of tetracycline as a model antibiotic, without exploring the suitability for the removal of other families. Most strategies focus on catalytic designs, with the use of MnO<sub>2</sub> as a catalyst to exploit Fenton-like degradation approaches. Surprisingly, to the best of our knowledge, only one report explores the use of physically-propelled MMs, using ZnO MMs for the enzymatic degradation of oxytetracycline.

Regarding the degradation mechanisms, MM-based approaches mimic the commonly used approaches in real water treatment plants: Fenton-like processes and sorption. To help non-specialized readers of this perspective, Fig. 2 shows a summary of representative approaches for antibiotic degradation with the MMs, focused on the description of the mechanism and the role of the material employed. One important and distinctive aspect of the MM-based approach is the high concentration used to test the removal efficiencies. Using tetracycline as a reference, and comparing the real levels found in water from Table 1 (854 ng mL<sup>-1</sup>)<sup>53</sup> with the levels tested using MMs (10 000–80 000 ng L<sup>-1</sup>),<sup>57,59–62,64</sup> it is surprising how MMs improve the removal efficiency from 12 to 94 times. A key point is the combination of multiple materials and functions in the MMs, along with enhanced fluid mixing. For example, Fig. 2A illustrates an example of a core-shell MnO<sub>2</sub>/Fe Janus MM for tetracycline degradation. In the presence of hydrogen peroxide, MMs are propelled by catalytic decomposition on the MnO<sub>2</sub> layer. The bubbles generated during the MM motion interact with the tetracycline in water, bringing them closer. At the same time, the

decomposition of the hydrogen fuel on the catalyst later generates ROS that induce the degradation of the antibiotic, reaching up to 80% removal.<sup>61</sup> While 100% removal was not achieved, please note the relatively high amount of pollutant used for the study (50 000 ng mL<sup>-1</sup>) in comparison with the levels that are normally reported in water (Table 1).

Regarding adsorption-based approaches, as a representative example, MgAl-LDH/Mn<sub>3</sub>O<sub>4</sub> MMs were decorated with a highly specific molecularly imprinted layer for targeted removal of doxycycline. Mn<sub>3</sub>O<sub>4</sub> acts as a catalytic layer for hydrogen peroxide decomposition and autonomous motion, enhancing the interactions with the pollutant for highly efficient removal of very high antibiotic concentrations (250 000 ng mL<sup>-1</sup>). High selectivity is achieved due to the specific recognition of the outer layer. Please note the rough surface in the SEM images in



**Fig. 2** Representative examples of MMs for antibiotic elimination in water. A) Fe–MnO<sub>2</sub> core–shell catalytic MM combining Fenton degradation and adsorptive bubble separation: a) scanning electron microscopy (SEM) image showing the rough morphology of the MM surface; b) time-lapse image of catalytic MM propulsion and c) schematic of the degradation mechanism. B) MgAl-LDH/Mn<sub>3</sub>O<sub>4</sub>/molecular imprinted polymer tubular MM for tetracycline removal by adsorption: the mechanism on the left and the SEM image showing the rough MM morphology on the right. Reprinted with permission from ref. 61 copyright 2022, Elsevier (A) and ref. 65 copyright 2020, Royal Society of Chemistry (B).

Fig. 2B. While the MM performance is comparable with the commonly used static process in real water treatment plants, some drawbacks still exist to transfer these innovations towards practical applications:

i) Volume of treated water: in most MM studies, volumes not higher than 10 mL can be treated. While MMs have a high towing force, the small size and poor synthesis yield obtained per batch prevent the movement and treatment of high-water volumes. Collaborations among different disciplines including chemistry, nanotechnology, and environmental engineering are needed to transfer MM technology into real water treatment plant settings.

ii) Operational cost. MMs are prepared in some cases using expensive materials. The cost of preparation, poor synthesis yields, and the need for specialized personnel in the synthesis process are obstacles to translation from the research laboratory into practical applications. Multidisciplinary collaborations, as specified in (i), are also needed.

iii) Most studies are tested using DI water, with only one exploiting this technology in wastewater. This is crucial, because in real water settings the presence of co-contaminants and other compounds can interfere with the propulsion/degradation mechanisms, hampering adequate pollutant removal. As a potential solution, current scientists in the field must expand and include real water samples from different environmental compartments in their studies.

iv) Narrow exploration of the full antibiotic's family. Most studies selected tetracycline as a model compound, with only two studies exploring erythromycin and doxycycline. While it is expected that the existing procedures will work with other antibiotic families, as most strategies are directed to Fenton degradation and sorption, potential research must include and expand the range of applications to selected compounds belonging to different antibiotic families, considering the current scenario reflected in Table 1.

v) Most designs rely on catalytic models based on chemically-driven propulsion. While normally the Fenton process requires the addition of hydrogen peroxide, this is extremely necessary in the case of the use of chemically-driven MMs. High levels of such toxic compounds, along with surfactants, are required for efficient operation, introducing an additional source of pollution in water that should be avoided. As a solution, physically-driven MMs are being explored. Yet, only one work based on light-driven propulsion is reported. Scientists are encouraged to explore more configurations in this direction, exploring magnetic propulsion, relying on the previous knowledge acquired in the use of catalytic models. Also, the relatively low levels of antibiotics present in real water compared with the high concentrations that MMs can remove show their potential as highly efficient removal tools.

All in all, despite being promising, the field of MMs for antibiotic removal towards combating ABR bacteria is still in the very early phase, with mostly proof-of-concept applications which are far away from real practical application in water treatment plants.

## 4. Current state of micromotors for bacteria (biofilms) inactivation: where we are and where we are moving

### 4.1. Overview of bacteria and biofilms in water environments and removal processes

Wastewater treatment plants are essential for the previous treatment of pollutant water from hospitals, industry, urban residues, *etc.* before releasing into the environment. In this water, antibiotics, microplastics, and bacteria can be present. We have previously discussed the link between antibiotics and the generation of ABR bacteria.<sup>67</sup> The treatment of wastewater involves a primary treatment for the physical removal of suspended particles, a secondary treatment for the removal of organic matter, and a tertiary advanced treatment to further eliminate organic matter, nutrients, *etc.*<sup>68</sup> In all these steps, bacteria number and population are greatly reduced, but some are inefficient for the removal of ABR bacteria,<sup>69,70</sup> which are detected in wastewater effluents, as summarized in Table 3. Biofilms, which are promoted by the presence of organic matter and other substances in wastewater, are present in some processes of wastewater remediation, with the undesired effect of generation of antimicrobial-resistance genes by bacteria.<sup>71</sup>

Regarding drinking water, chlorination and related treatments are applied to ensure the safe consumption in most countries and the absence of pathogenic bacteria. Yet, the generation of biofilms and their adhesion to the water distribution systems are well-known problems that can contribute to ABR bacteria generation.<sup>82</sup> Several surveys of different countries in Europe, China, *etc.* have revealed the presence of bacteria and biofilms within the pipelines and distribution systems of drinking water treatment plants, as summarized in Table 3. To prevent biofilm generation, different strategies are currently adopted, including the maintenance of fixed disinfectant levels (chlorine, chloramine) in the water distribution system, the reduction of the amount of organic matter and the use of materials

**Table 3** ABR bacteria and biofilms present in water and wastewater

| Water                                        | Bacteria                                                                                                                                                                                            | Ref.  |
|----------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------|
| Wastewater                                   | <i>Enterobacteriaceae</i><br><i>Aeromonas</i><br><i>E. coli</i><br><i>P. aeruginosa</i>                                                                                                             | 72–77 |
| Well and household drinking water            | <i>E. coli</i><br><i>Enterococcus</i>                                                                                                                                                               | 5     |
| Drinking water pipelines/distribution system | <i>Acinetobacter</i><br><i>E. coli</i><br><i>Enterococcus</i><br><i>P. aeruginosa</i><br><i>Klebsiella</i><br><i>S. aureus</i><br><i>Gammaproteobacteria</i><br><i>Bacilli</i><br><i>Salmonella</i> | 78–81 |



with biofouling properties. Yet, biofilms are difficult to eliminate, and new alternative methods are needed for successful removal.<sup>81</sup> MMs are very suitable alternatives for biofilm elimination, especially in pipelines and hard-to-access areas with traditional means, acting as moving fighters for the deactivation of bacteria. Indeed, as will be described in the next subsection, a myriad of promising MM-based approaches for bacteria biofilm removal and elimination have been proposed.

#### 4.2. Overview of micromotor approaches for bacteria and biofilm elimination in water

Table 4 summarizes the currently existing approaches using MMs for bacteria biofilm and bacteria removal in environmental water. Compared with strategies for the elimination of antibiotics, vast progress with a myriad of efficient applications has been made. The high efficiency of the procedures is remarkable, with a removal efficiency close to 100%. It should be noticed that in most studies, the biofilms used are grown from commercial strains in the laboratory, as it is difficult to isolate and grow real bacteria present in water treatment plants, and safety reasons should be also considered. It should be also noticed that early strategies relied on catalytic designs, but great progress is being made toward the exploration of physically-propelled MMs, particularly photocatalytic designs that can generate ROS and other species during movement, highly efficient for biofilm removal.

Most degradation approaches are innovative, but also based on previous knowledge and commonly used strategies for bacteria biofilm inactivation. In some studies, methicillin-

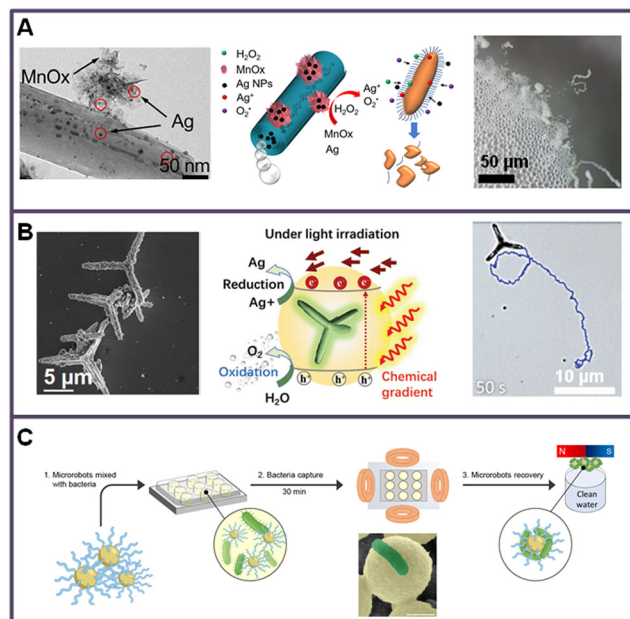
resistant bacteria are even evaluated.<sup>91,93</sup> As in the previous discussion with antibiotics, to help non-specialized readers of this perspective, Fig. 3 shows a summary of representative approaches for bacteria degradation with the MMs. In all strategies, only 3 bacterial species have been evaluated: *P. aeruginosa*, *E. coli*, and *S. aureus*. In Table 3, other species can be detected in water mainly as biofilms. Yet, as the strategies in most cases are based on the generation of radicals and silver release, which can target a broad range of bacteria, it is expected that these novel MM proof-of-concept applications can be applied to real settings.

Regarding the degradation mechanisms, the vast majority of MM-based approaches have been devoted to exploring chemical and light-driven MMs. This is due to the ease of generation of radicals and other species for bacteria inactivation during MM propulsion, either by the generation of catalytic by-products or UV-triggered corrosion/release. As an example, Fig. 3A illustrates this concept using catalytic MnO<sub>x</sub>-Ag/halloysite tubular MMs. The inner MnO<sub>x</sub> catalyst serves as a propulsion layer, generating OH<sup>•</sup> species and ROS, while the Ag particles in the MM surface are released during movement. The enhanced movement of the MM swarm (see the time-lapse images in the figure) enhanced their interaction with the bacteria for highly efficient inactivation, reaching percentages of almost 100%.<sup>85</sup> To avoid the use of toxic fuel, the catalyst can be replaced by water-propelled magnesium MMs that can move with a pitting corrosion mechanism and do not require additional fuel. Silver nanoparticles can be introduced into the structure, inducing their release as antibacterial and bacteriostatic agents. Indeed, all chemically-driven MMs are based on the release of Ag as an antibacterial agent, with

**Table 4** MM approaches for bacteria (and biofilms) elimination according to the propulsion mechanism

| Chemically-driven micromotors                                                                        |                                        |      |
|------------------------------------------------------------------------------------------------------|----------------------------------------|------|
| Micromotor (mechanism)                                                                               | Bacteria (removal efficiency)          | Ref. |
| Zeolite/Pt/Ag Janus (Ag release)                                                                     | <i>P. aeruginosa</i> biofilms (99.97%) | 83   |
| PEDOT/MnO <sub>2</sub> @Ag tubular (Ag release)                                                      | <i>E. coli</i> (95%)                   | 84   |
| MnO <sub>x</sub> -Ag/halloysite tubular (Ag release)                                                 | <i>E. coli</i> (97%)                   | 85   |
| Chitosan/alginate/PLGA Mg micromotor (contact with chitosan)                                         | <i>E. coli</i> (96%)                   | 86   |
| Ag/Mg Janus (Ag release)                                                                             | <i>E. coli</i> (95%)                   | 87   |
| Ag/Mg Janus (Ag release)                                                                             | <i>E. coli</i> (100%)                  | 88   |
| Physically-driven micromotors                                                                        |                                        |      |
| Light-driven micromotors                                                                             |                                        |      |
| Galacto-oligosaccharide Janus (light-induced NO generation/self-electrophoresis)                     | <i>P. aeruginosa</i> biofilms (99.97%) | 89   |
| Polypyrrole-zeolitic imidazole 8 metal organic framework Janus (NIR triggered phototaxis/Zn release) | <i>S. aureus</i> (98.99%)              | 90   |
| Ag <sub>3</sub> PO <sub>4</sub> tetrapod structure (light-induced Ag generation)                     | <i>E. coli</i>                         | 33   |
|                                                                                                      | <i>S. aureus</i> (99.99%)              |      |
| Ag <sub>3</sub> PO <sub>4</sub> (light-induced Ag generation)                                        | <i>P. aeruginosa</i>                   | 91   |
|                                                                                                      | <i>S. aureus</i> (83–99%)              |      |
| MoS <sub>2</sub> and WS <sub>2</sub> flakes (photophoretic induced generation of ROS and collisions) | <i>E. coli</i>                         | 92   |
|                                                                                                      | <i>S. aureus</i> (88%)                 |      |
| ZnO/Ag (Ag generation/self-electrophoretic motion)                                                   | <i>P. aeruginosa</i>                   | 93   |
|                                                                                                      | <i>S. aureus</i> (80%)                 |      |
| Magnetic-driven micromotors                                                                          |                                        |      |
| Tea buds derived chitosan-modified FeONPs Janus loaded with ciprofloxacin (antibiotic treatment)     | <i>P. aeruginosa</i> biofilms          | 94   |
|                                                                                                      | <i>S. aureus</i> biofilms (80%)        |      |
| Magnetic beads modified with methacrylamide polymer (adsorption and UV irradiation)                  | <i>P. aeruginosa</i> (100%)            | 95   |





**Fig. 3** Representative examples of MMs for bacteria biofilm elimination in water. A) Catalytic  $\text{MnO}_x$ -Ag/halloysite tubular MM releasing Ag for *E. coli* inactivation: SEM images showing the morphology of the MM (left), mechanism (middle), and propulsion (right). B)  $\text{Ag}_3\text{PO}_4$  tetrapod light-driven MM for *E. coli* and *S. aureus* removal: SEM images (left), mechanism (middle), and propulsion (right). C) Magnetic beads modified with methacrylamide polymer for *P. aeruginosa* capture and magnetic removal: schematic of the set-up and details of captured bacteria on the MM. Reprinted with permission from ref. 85 copyright 2022, Elsevier (A); ref. 33 copyright 2024, Wiley (B) and ref. 95 copyright 2024, American Chemical Society (C).

only one approach dealing with the introduction of chitosan by direct capture and contact with the MMs.<sup>86</sup> All processes are highly efficient, requiring from 30 min to a couple of hours for biofilm inactivation. Interestingly, the MM size and high towing force are promising to navigate in pipelines and hard-to-access areas where the biofilm tends to proliferate. This is a unique MM feature unexploited in the field. Silver can be replaced by chlorine or other agents for controlled release.

Light-driven MMs are highly versatile and rely on the generation of photocatalytic products that can readily deactivate bacteria. In a very innovative approach, *N*-nitrosamines as nitrogen sources have been incorporated in galactooligosaccharide Janus MMs. Under light irradiation, NO radicals are generated for highly efficient inactivation. In another set of strategies, Ag releasing MMs have been also exploited. As an example, Fig. 3B shows an  $\text{Ag}_3\text{PO}_4$  tetrapod MM that can deactivate up to 99.9% of *E. coli* and *S. aureus* biofilms.<sup>33</sup> Under light irradiation, the Ag composing the MMs is reduced into  $\text{Ag}^+$  and liberated into the media. A chemical gradient of ROS is also generated, for self-electrophoretic propulsion (see the time-lapse microscope image showing the motion in the figure). Other designs have explored Zn liberation using zeolitic imidazolate-based MMs, uniting degradation and motion.<sup>90</sup> In most cases, a potential drawback of these designs is the self-

electrophoretic mechanism, which can be hampered due to the presence of constituents, contaminants, *etc.* in the water. As a potential solution, photophoretic  $\text{MoS}_2$  and  $\text{WS}_2$ -based MMs display efficient propulsion even in a bacteria culture for biofilm inactivation. This is achieved *via* collisions and ROS generation, although not complete removal of biofilms is achieved.<sup>92</sup> In the case of magnetic MMs, fewer approaches have been described, due to the difficulties in generating inactivation agents during motion, thus requiring functionalization with antibacterial agents and lower MM speed, which reduces the towing force. Promising efforts in this direction described the capture of bacteria by polymer-modified magnetic beads (see Fig. 3C), with highly efficient magnetic pulling and removal.<sup>95</sup>

As already described when discussing the progress in MMs for antibiotic elimination, important challenges need to be overcome to translate these innovations towards practical applications for bacterial removal:

i) Efficiency on tons of water. The existing MM applications are still in the proof-of-concept stage, and scientists perform the evaluation in microarray plates or test tubes with volumes not higher than 10 mL. It is unclear if this performance will remain in water treatment plants and along pipelines. To address these challenges, collaboration among different disciplines is a must.

ii) Operational cost. This is a common drawback shared with the MM approaches used for antibiotic treatment. MM production is expensive due to the required specialized personnel and costly reagents needed, as well as equipped laboratories. The treatment necessary to eliminate these MMs after their use is also an expensive challenge.

iii) The necessity of exploring more bacteria families and relevant bacteria found in biofilms. The main challenges are the difficulties in culturing these bacteria in the laboratory and safety issues. Scientists are encouraged to include and evaluate additional bacteria in future studies.

The use of MMs for bacteria biofilm inactivation is a particularly promising field for real applications. The versatility and enhanced motion of MMs allow the motion mechanism to adapt. For example, the strategies can be re-designed to perform passive bacteria inactivation instead of active. MMs can mimic the bacteria, and with the adequate functionalization, interfere with the quorum sensing of the bacteria biofilm, allowing for its inactivation.<sup>96,97</sup> There is still plenty of room at the bottom to explore.

## 5. Conclusions

We have presented here an up-to-date perspective of the current progress and opportunities on the use of MMs for antibiotic removal and bacterial inactivation in water systems. Nanotechnologies such as MMs present great opportunities for water remediation approaches. As a first unique feature, the micrometre size of MMs results in a high surface area-to-mass ratio for improved adsorption capabilities. A second feature is the relatively large reactive surface, with a high density of edges



and reactive atoms, increasing the catalytic performance for degradation. A third feature is the mobility of MMs in solution allowing us to reach more volumes of water and even hard-to-access areas. The first two features are reflected in current MM-based approaches for antibiotic and bacteria biofilm elimination, with remarkable performance compared with existing technology and the current levels of such pollutants found in real settings. The third feature remains a challenge and maybe the main drawback that prevents the translation of MMs into real water treatment: the challenge of treating high water volumes. The next steps should be directed to evaluate MMs in the context of real water settings: evaluation of the performance in high water volumes of environmental waters, and evaluation of several families of antibiotics and bacteria. All in all, MMs offer great opportunities to solve the great social challenge of antimicrobial resistance from an environmental approach.

## Data availability

No primary research results, software or code have been included and no new data were generated or analysed as part of this perspective.

## Author contributions

C. Cuntín-Abal: conceptualization and writing – review & editing. B. Jurado-Sánchez: conceptualization, funding acquisition, project administration, resources, supervision, writing – original draft, and writing – review & editing. A. Escarpa: conceptualization, funding acquisition, project administration, resources, supervision, and writing – review & editing.

## Conflicts of interest

There are no conflicts to declare.

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