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Emerging contaminants bioremediation by enzyme and nanozyme-based processes – A review

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SUMMARY
Due to their widespread occurrence and the inadequate removal efficiencies by conventional wastewater treatment plants, emerging contaminants (ECs) have recently become an issue of great concern. Current ongoing studies have focused on different physical, chemical, and biological methods as strategies to avoid exposing ecosystems to significant long-term risks. Among the different proposed technologies, the enzyme-based processes rise as green biocatalysts with higher efficiency yields and lower generation of toxic by-products. Oxidoreductases and hydrolases are among the most prominent enzymes applied for bioremediation processes. The present work overviews the state of the art of recent advances in enzymatic processes during wastewater treatment of EC, focusing on recent innovations in terms of applied immobilization techniques, genetic engineering tools, and the advent of nanozymes. Future trends in the enzymes immobilization techniques for EC removal were highlighted. Research gaps and recommendations on methods and utility of enzymatic treatment incorporation in conventional wastewater treatment plants were also discussed.

INTRODUCTION
Over the last century, the continuous development of waste management technologies led to a worldwide establishment of engineering and policy tools that can foster effective remediation of environmental pollution.1 However, due to the increased sophistication in the manufacture of synthetic compounds, the release of innumerable novel substances hard to decompose has drastically increased.2 The development of chemical and pharmaceutical industries, as well as the growth of the global population, has increased and diversified market demand. Agrochemicals, microplastics, industrial and building materials, pharmaceuticals, and personal care products are among many substances that have recently prompted proper attention by pollution management actors and scientific community.2 Although frequently found in low concentrations in the environment and water bodies, their high chronic toxicity and bioaccumulation capacity expose ecosystems to significant long-term risks.2 In this way, they pose high fatal risks to living organisms by targeting various organs and systems, such as cardiovascular, neurological, reproductive, and endocrine disorders, as well as cancer and the emergence of multidrug-resistance microbes.3,4 Thus, a paradigm shift resulted from the changed focus from conventional pollutants to these so-called “emerging contaminants” (ECs), which calls for the utmost necessity of their remediation.

Although there is no standard categorization for EC, it generally comprises complex residual substances and their by-products, which are generated from varied origins.2 Agrochemicals, microplastics, hormones, dioxins, phenolics, nanomaterials, and perfluorinated substances are among EC sources. In most cases, wastewater treatment plants cannot reach proper removal efficiency of EC, due to the low biodegradability and high complexity of such compounds. In some cases, conventional biological processes may even present a negative removal, when the contaminant is concentrated during effluent treatment.1 The application of the traditional physical processes of flocculation and sedimentation has reduced removal effectiveness, due to the high polarity and high water solubility of EC.6 Thus, novel research is trying to develop efficient, economical, and environmentally safer remediation techniques.7,8,9 The use of membrane filtration and different oxidation processes has been reported as effective in the removal of EC.6,10,11 However, its materials and energy-intensive processes would require the use of costly compounds and/or energy sources,
eventually raising the cost of treatment system.\textsuperscript{6,9} Besides, many oxidation methods, such as Fenton process and ozonation, can produce harmful by-products.\textsuperscript{12}

Among novel advanced treatment processes that are still in the research phase, the use of enzyme-based process is one of the most propitious methods of EC’s bioremediation. Isolated enzymes catalyze reactions with greater specificity and do not generate toxic side products.\textsuperscript{13} Their use presents a more rapid process with broader configuration options, easier manipulation,\textsuperscript{12} and the elimination of some microbial limitations.\textsuperscript{13} Also, the use of genetically modified microorganisms (GMOs) can be implemented to overcome any wild strain adversities in the production process, such as stress conditions.\textsuperscript{14} In recent years, many works evaluated specific enzymes, such as laccases,\textsuperscript{15} peroxidases,\textsuperscript{16,17} hydrolases,\textsuperscript{18} and so on. To the best of our knowledge, since the work of Bilal et al.,\textsuperscript{3} no work has covered yet the recent innovations in terms of immobilization techniques and genetic engineering in the field of enzyme-based processes for EC bioremediation, with the perspective on how it could be incorporated in worldwide wastewater treatment plants. A comprehensive review about the use of novel nanozyme for EC remediation is also lacking.

Therefore, this work aims to present the state of the art of enzyme-based processes for EC remediation, focusing on recent advances and innovations. The advantages and bottlenecks for the use of immobilization techniques, as well as genetic engineering, to improve the stability and production of enzymes were well described. Also, the advent of the novel compounds called nanozymes and their functionalities and shortcomings were discussed. And lastly, a discussion is presented about which specific step in the addition of an enzymatic treatment could be more useful to wastewater treatment systems.

ECs—CONCERNS AND IMPACTS

Since the Industrial Revolution and the late 19th century, societies have been dealing with the consequences of accelerated industrialization and urbanization, such as the generation of waste streams from anthropogenic activities.\textsuperscript{1} When they are improperly managed, waste can lead to serious ecological problems. Some of them include the damage of ecosystems and irreversible deterioration of environmental quality, which frequently leads to the introduction of constraints on what resources could be safely extracted.\textsuperscript{1,2} For instance, the continuous contamination of water resources raises concerns about the already existing problems of fresh-water scarcity.\textsuperscript{19} Also, the constant increase in global population poses harder challenges to the sustainable development. According to the United Nations,\textsuperscript{20} world population can reach 9.7 billion by 2050, while around likely 80% of the total wastewater generated worldwide is disposed without adequate treatment.\textsuperscript{21} In addition, the introduction of novel chemical products in industries for society consumption has been leading to the widespread occurrence of micropollutants and EC.\textsuperscript{2}

Recent studies have detected EC occurrence in different samples and environment compartments in at least some low concentrations (from ng/L to 1200 mg/L, in some cases).\textsuperscript{22,23} Other reports have shown the specific sources and routes of EC and their interactions with different local characteristics, such as land-use interactions and climate.\textsuperscript{5,24} On the other hand, several research works have reported novel remediation methods of EC in treatment systems.\textsuperscript{8–10,25} Accordingly, EC has attracted increasing international attention from the scientific community. The number of peer-reviewed publications that cited the terms “emerging contaminants” and “emerging pollutants” in the Web of Science database is summarized in Figure 1. It can be seen that, since the beginning of 21st century, a huge increase in publications in high-impact journals has emerged, which proves the increasing interest in the subject.

Sources

Briefly, EC reaches the environment and aquatic resources through varied production processes, such as agricultural runoff, landfill leaching, sewage treatment, municipal wastewater, and industrial discharges. Even supposing these novel substances have been discharged from human activities for a long but uncertain time, their identification was just recently allowed by improvements in the sensitivity of analytical techniques for reduced concentrations of chemical compounds (from \(\mu\)g to ng/L), mainly liquid and gas chromatographic techniques.\textsuperscript{72}

Pharmaceuticals and personal care products are among the most relevant EC, due to their widespread uses. The global annual per capita consumption of pharmaceuticals is estimated at 15 g.\textsuperscript{26} Ibuprofen, diclofenac, naproxen, ciprofloxacin, azithromycin, and propranolol are some examples of partially metabolized pharmaceutical products that are excreted by humans. In the case of personal care products, they
comprise disinfectants, fragrances, insect repellents, and UV filters, while benzophenones and chlorinated parabens are some examples.\textsuperscript{27} After consumption, they reach municipal wastewater systems, where inefficient conventional treatments lead to their discharge into the environment.\textsuperscript{2,27} Septic tanks and landfill sites are other significant sources of EC, especially where the groundwater table is shallow and aquifers have a high rate of percolation.\textsuperscript{7} In terms of direct industrial sources, similar routes are taken by various types of dyes, surfactants, additives, and plasticizers from paper, textile, vehicle, detergent, and construction sectors. Due to their incomplete removal, they can escape into environment compartments.\textsuperscript{23}

Otherwise, diffuse pollution sources are harder to detect and control than pointed sources and are normally related to agricultural runoffs and animal farm discharges. Animals excrete around 50–90% of the doses of consumed veterinary medicines for the treatment and growth of livestock.\textsuperscript{23} Normally, their manure is used as fertilizers in agricultural lands. Adding to that, the extensive use of pesticides in global agriculture led to worldwide contamination of natural waters by EC through non-point agents like surface runoffs.\textsuperscript{23}

Recent studies on environmental occurrences and impacts

Due to the presence of ECs in water bodies across worldwide environments, they can lead to the contamination of natural and mineral water resources. Some works proved that bottled drinking water across different countries presented these compounds at low concentrations.\textsuperscript{22} For example, different concentrations for nicotine (7–15 ng/L), triclosan (0.6–9.7 ng/L), and sulfonamides (9–80 ng/L) were found in bottled water brands of different countries, such as in Italy,\textsuperscript{28} China,\textsuperscript{29} and Spain.\textsuperscript{30} This fact shows great concerns about the bottled drinking water production chain.

In research where groundwaters and surface waters were evaluated, higher concentrations and more diversified ECs were found. In South Africa’s groundwater supplies,\textsuperscript{31} 11 ECs were reported, such as efavirenz, nevirapine, carbamazepine, methocarbamol, bromacil, and venlafaxine. Together, they added up to the highest concentration of 593 ng/L at the Jukskei River.\textsuperscript{31} Fram and Belitz\textsuperscript{32} reported 14 different contaminants in the groundwater of California State, in the USA, with higher concentrations for acetaminophen, sulfamethoxazole, and nevirapine. One of the major concerns about pharmaceutical contamination is the emergence of antibiotic- and multidrug-resistance microbes. This is a natural phenomenon in which microorganisms adapt themselves to antimicrobial agents at previously lethal concentrations, withstanding the impact of an antibiotic to which they were previously susceptible.\textsuperscript{33} The consequence is that treating different infections become labored, increasing the risks of diseases spreading.\textsuperscript{34} Thus, the exposition of microbiota to low concentrations of diversified compounds could cause 10 million deaths per year throughout the world by 2050.\textsuperscript{35} Adding to that, the consumption of antibiotics suffered a high increase of 65%, from 21.1 billion daily doses in 2000 to 34.8 billion daily doses in 2015, with an estimated consumption of 42 billion daily doses in 2030.\textsuperscript{35} One proper example is the group of tetracyclines, which are broad-spectrum antibiotics that are highly consumed, with a high incidence in animal manure from...
livestock farms (0.03–183.5 mg kg\(^{-1}\)) and in effluents of hospitals (3–1385 ng L\(^{-1}\)).\(^{36}\) As a comparison, only 0.1–1 mg/kg remarkably raises the gene transfer of tetracycline resistance transposon in liquid manure.\(^{19}\)

Another major concern is the endocrine-disrupting chemicals, which are substances with estrogenic activity that can block natural hormones.\(^{1}\) It impacts the endocrine system that is responsible for the functioning of vertebrate animals and marine organisms. These chemicals can be solvents, plasticizers, pesticides, pharmaceuticals, and personal care products.\(^{2}\) Due to their great capacity to persist in the environment, the endocrine disruptors pesticides atrazine and 2,4-D were found in 99.8% of the total 2,600 water samples collected from Great Australia’s Barrier Reef lagoon.\(^{36}\) Also, bisphenol A and its analogues S and F were detected in the surface waters of Japan, Korea, China, and India at 54–1950 ng/L concentration.\(^{37}\) Lastly, Wang et al.\(^{40}\) investigated the presence of several steroidal and phenolic compounds in the surface water of the Bahe River, China, and found concentrations at 5.2–634.8 ng/L.

**WASTEWATER TREATMENTS AND CONVENTIONAL SYSTEMS**

Among the conventional methods that have been used to treat municipal and industrial wastewater, the application of microbial mechanisms predominates. Some examples are the activated sludge process (ASP) and upflow anaerobic sludge blanket (UASB) reactor.\(^{41}\) Generally, biological processes use fungi, algae, and bacteria to biodegrade high-weight molecular compounds into smaller substances to reach their final mineralization. The main advantages of these systems are that they are easy to use and have a low operating cost.\(^{2}\) These widely used technologies have established efficiencies in the treatment of different wastewater streams, removing nutrients, dissolved organics, particulates, and pathogens. However, they are not originally intended to target EC.\(^{9}\) In developing nations, most conventional wastewater treatment plants do not even monitor EC.\(^{19}\) Normally, they present low efficiency in EC removal. In some cases, a negative contaminant removal occurs, when the effluent presents a higher concentration of the contaminant than the influent, due to concentration during the process.\(^{5}\)

Mie`ge et al.\(^{42}\) showed that the ASP system removed fractions of some drugs from wastewater, such as ibuprofen, ciprofloxacin, norfloxacin, and trimethoprim, in the range of 70–85%. However, for other compounds, such as carbamazepine and tetracycline, the removal efficiency reaches a maximum of 38%. Similar results were reported by Ahmed et al.\(^{43}\) ASP processes reached considerable removal efficiency for surfactants (>95%), personal care products (>78%), endocrine disruptors (>75%), and pharmaceuticals (>65%). However, it did not reach proper removal efficiencies for pesticides and β-blockers. Moreover, another bottleneck of biological systems is that EC cannot achieve complete mineralization in the process, especially for pure species. The generation of intermediate degradation products would require extra steps in the treatment system, before fulfilling its complete mineralization. In Figure 2, an example of the proposed degradation pathway of tetracycline by *Stenotrophomonas maltophilia* DT1, *Klebsiella sp*. SQY5, *Klebsiella sp*. TR5, and *Sphingobacterium changzhouense* TC931, with its degradation intermediates, is shown.\(^{7}\) Although the parent compounds present lower molecular weights, there is no unified conclusion on the toxicity of tetracycline biodegradation products. Also, the possibility of product generation with higher toxicity is not discarded.\(^{1}\) Despite the low efficiency of the ASP systems in EC removal, another method, using the anaerobic fluidized bed reactor, has also been applied in the removal of pharmaceutical compounds, such as paracetamol and ciprofloxacin, achieving removal efficiencies above 85%.\(^{43}\)

Otherwise, traditional physical and chemical methods such as coagulation, flocculation, and sedimentation are major processes involved mainly in the removal of suspended solids. However, these processes are not effective in the removal of EC. Researchers have been evaluating some widely used coagulants and/or flocculants, such as ferrous and ferric chloride and aluminum sulfate, for the treatment of EC with insignificant removal rates.\(^{44,45}\) In the case of phenolic compounds and their halogenated derivatives from the drinking water, the removal rate using flocculation with sand filtration reached only 7%.\(^{44}\) In the case of some pharmaceutical products and endocrine disruptors, the use of ferric chloride and aluminum sulfate as coagulants reached only <25% of removal rate.\(^{45}\) Kumar et al.\(^{5}\) critically reviewed worldwide wastewater treatment plants’ efficiencies on EC removal. The conclusion is that conventional wastewater treatment plants showed the highest negative removal for pharmaceuticals, such as carbamazepine and carbadox, and also for pesticides.

Thus, chemical technologies have been evaluated as supplementary steps in hybrid systems. Polishing techniques, based on oxidation reactions with the use of highly reactive chemical species, aim to facilitate
the degradation of EC to less harmful and more biodegradable compounds. Hydrogen peroxide, ozone, chlorine, and their combinations with transition metals and metal oxides are examples of catalysts used in chemical wastewater treatments, in the so-called advanced oxidation processes (AOPs). Generally, the use of catalysts in combination with metals, such as in the Fenton process, is not reasonably compared to removal efficiencies reached by other oxidation processes, such as photo-Fenton and ozonation. Therefore, to improve the removal of EC it would require the addition of extra compounds or different energy sources, such as ultraviolet-visible (UV-vis) radiation, electric current, gamma radiation, and ultrasound. Eventually, it could raise the cost of the treatment system considerably.

Ganzenko et al. reviewed the use of electro-Fenton and solar photoelectron-Fenton in the treatment of hospital wastewaters. Authors evaluated the presence of pesticides and antibiotics with β-blockers activity, such as atenolol, metoprolol, triclosan, sulfamethoxazole, and tetracycline, among others. Removal rates reached 86–100%. Electrochemical treatment systems do not present limitations due to the toxicity of the effluent, but normally it has long reaction times (9-21h). In this case, it could raise operation costs, potentially limiting its application at industrial scale. In Figure 3, a proposed degradation pathway by Zhang et al. of tetracycline by UV/per-sulfate advanced oxidation process is shown. In this case, 95.73% of a 5 mg/L tetracycline solution was removed after 30 min. However, the tetracycline mineralization in the process after 30 min was low, causing insufficient toxicity reduction of the compound and its intermediates, which could be improved by optimizing the process parameters.

The challenge to develop novel efficient strategies is to provide sustainable remediation solutions, in comparison to the current treatment systems that persist. Recently, researchers have tried to use different technologies to find highly effective and cost-effective techniques. The use of adsorption via biochar and activated carbon, which explores intermolecular forces between adsorbents and contaminants, to remove the organic and inorganic contaminants from the water has been studied. Relevant results were reached in the treatment of clofibric acid, naproxen, tetracycline, and diclofenac.

Among these potential novel technologies, enzyme-based processes are considered a prospective choice. In comparison with recent bioremediation technologies applying microbial strains, the use of extracted enzymes present a more rapid process. Nazari et al. reviewed biological technologies for EC degradation, finding works with retention times between 3 and 21 days. Gao et al. evaluated the removal of ciprofloxacin, norfloxacin, and sulfamethoxazole by two fungi strains, reaching removal efficiencies between 63.3% and 73.2%, requiring, however, 2 to 8 days of treatment. As it can be seen in Table 1, higher removal efficiencies in more rapid processes could be reached. The use of enzymes can broaden the reaction conditions, due to the elimination of some limitations posed by microorganisms, and enables the application of
Figure 3. Proposed pathways according to the intermediates from reactions of tetracycline with UV/persulfate advanced oxidation process in deionized water (11). (Copyright (2022) with permission from Elsevier)
more efficient and optimized configurations. Also, enzyme-based treatment avoids the inhibitory effects of antibiotics on microbial growth and reduces the emergence of antibiotic- and multidrug-resistance microbes.15

In addition, the use of enzymes as biocatalysts demonstrates milder reaction conditions in comparison with above-mentioned advanced physicochemical technologies, which demand higher energy inputs. Besides, different kinds of chemical oxidants, such as Fenton’s reagent, potassium permanganate, and ozone, among others, can produce harmful secondary by-products.12 Due to the high enzymes' specificity toward substrates, the occurrence of unfavorable side reactions is minimized, reducing or disabling the generation of hazardous by-products.60 Therefore, the use of enzymes in the bioremediation of EC shows a great capacity as a sustainable booster.

ENZYME-BASED PROCESS BIODEGRADATION

In 1897, after Louis Pasteur reported in the 1850s that fermentation processes are initiated by living organisms, Edward Buchner found that the conversion of sugars was possible even without yeast cells.61 He reported the capacity of enzymes to maintain their catalytic activity outside living cells. Since then, the field of enzymology has elucidated the role of enzyme-substrate complexes in many biochemical processes, representing a projected increasing market value of US $7 billion in 2021.62

Many different enzymes have been used for bioremediation as green biocatalysts, enhancing the rate of reactions by lowering the activation energy of the molecules. The use of enzymes in bioremediation brings many advantages in comparison with other processes.57 A more rapid process in comparison with traditional microbial degradation is reached. Depending on the enzyme used, the configurations of the reaction, and the contaminant, retention times can vary widely between 30 min and 72 h or generally a few hours. For instance, Silva et al.64 reported an 86% removal rate of a reactive textile dye (Remazol Brilliant Blue R) after 13 min of retention time. However, authors used a low dye concentration (10–60 mg/L) and a wide range in enzyme load (5–140 U/mL). In comparison, Nazari et al.52 reviewed biological technologies for EC degradation, reporting retention times between 3 and 21 days. Also, the enzymes are not necessarily consumed during the reaction, which goes on without the generation of toxic residues. Further, wider range of conditions of temperature and ionic strength values, in comparison with the respective microbial strains' tolerable range, can be applied. Finally, enzymes are not able to replicate, which enables a more controlled process in terms of enzyme concentration and cofactors' availability.63

The International Union of Biochemistry proposes seven classes of enzymes: oxidoreductases, hydrolases, transferases, lyases, isomerases, ligases, and translocases. The two main groups of enzymes that have

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Compound removed</th>
<th>Removal efficiency (%)</th>
<th>Optimal conditions (°C; pH; time)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laccase (with ABTS mediator)</td>
<td>Naproxen, diclofenac, indomethacin</td>
<td>100</td>
<td>30°C; 4.5; 12 h</td>
<td>Tran et al.48</td>
</tr>
<tr>
<td>Laccase (with ABTS mediator)</td>
<td>Carbamazepine</td>
<td>82</td>
<td>35°C; 6.0; 24 h</td>
<td>Naghdi et al.49</td>
</tr>
<tr>
<td>Laccase</td>
<td>14 anti-inflammatory</td>
<td>80–95</td>
<td>20°C; 7.5; 16 h</td>
<td>Ba et al.50</td>
</tr>
<tr>
<td>Laccase</td>
<td>Naproxen, acetaminophen</td>
<td>100</td>
<td>20°C; 7.5; 24 h</td>
<td>Ba et al.50</td>
</tr>
<tr>
<td>Laccase</td>
<td>Bisphenol A</td>
<td>99</td>
<td>30°C; 5.0; 2 h</td>
<td>Lassouane et al.51</td>
</tr>
<tr>
<td>Laccase</td>
<td>Diclofenac</td>
<td>92</td>
<td>22°C; 5.0; 24 h</td>
<td>Primožič et al.52</td>
</tr>
<tr>
<td>Laccase</td>
<td>Estrogenic compounds</td>
<td>80–87</td>
<td>26°C; 7.0; 4 h</td>
<td>Lloret et al.53</td>
</tr>
<tr>
<td>Laccase</td>
<td>Morphine</td>
<td>100</td>
<td>25°C; 7.0; 30 min</td>
<td>Huber et al.54</td>
</tr>
<tr>
<td>Laccase</td>
<td>Bisphenol A</td>
<td>93</td>
<td>40°C; 8.0; 3 h</td>
<td>Ghobadi Nejad et al.55</td>
</tr>
<tr>
<td>HrP</td>
<td>Chlorophenolics</td>
<td>83</td>
<td>25-30°C; 6.4; 3.5 h</td>
<td>Chang et al.56</td>
</tr>
<tr>
<td>MnP, LiP and laccase</td>
<td>Bisphenol A</td>
<td>90</td>
<td>25°C; 7.2; 8 h</td>
<td>Gassara et al.57</td>
</tr>
<tr>
<td>MnP</td>
<td>Azo dyes</td>
<td>76–100</td>
<td>27°C; 7.0; 12–24 h</td>
<td>Samir Ali et al.58</td>
</tr>
<tr>
<td>Peroxidase</td>
<td>Azo dyes</td>
<td>42–95</td>
<td>30°C; 5.5; 48 h</td>
<td>Joel et al.59</td>
</tr>
<tr>
<td>Peroxidase</td>
<td>Textile phenolics</td>
<td>91.1</td>
<td>30°C; 5.5; 2 h</td>
<td>Joel et al.59</td>
</tr>
</tbody>
</table>
shown better biodegradation ability are oxidoreductases and hydrolases. Some examples are shown in Table 1.

### Oxidoreductases

As redox reactions are one of the most important mechanisms involved in bioremediation processes, the oxidoreductases’ biodegradation acts mainly through the catalysis of oxidation-reduction reactions. In these processes, the electron donor is usually the pollutant, and the electron acceptor is commonly oxygen or peroxide.

Peroxidases are an enzyme group that shows high potential as green catalysts, degrading aromatic contaminants by using hydrogen peroxide as the electron acceptor and a mediator. Its classification is divided into heme (complex with Fe$^{3+}$) and non-heme-containing enzymes; non-animal peroxidases account for 87% of them. Among them, the extracellular class II heme peroxidases, including lignin peroxidase (LiP) and manganese peroxidase (MnP), are the most widely applied. However, novel peroxidases have also been researched for bioremediation processes.

LiP (EC 1.11.1.14) is a monomeric hemoprotein that functions at acidic pH (~3.0), with a high redox potential up to 1.4 V. LiP has been known to oxidize several aromatic phenolic and non-phenolic compounds, as well as, many other organic compounds, such as xenobiotics. LiP was extracted and purified from Kocuria rosea MTCC 1532. In terms of industrial dyes’ removal, it was able to treat the wastewater of eleven structurally different kinds of dyes after 5 h, such as azo, heterocyclic, polymeric, triphenylmethane, and metal-complexes. Although the enzyme has presented low optimum pH value of 3.0, removal rates of 60–100% were reached in neutral pH, which shows the versatility of LiP. Other works that have evaluated LiP as green biocatalysts for EC bioremediation are shown in Table 1 and Table 2.

MnP (EC 1.11.1.13) are glycosylated heme-containing extracellular enzymes that utilize Mn$^{2+}$ as an electron donor. They oxidize Mn$^{2+}$ into Mn$^{3+}$ by multistep reactions, which is a highly reactive compound that cleaves an array of substrates. A MnP was isolated from white-rot fungus Trametes sp. 48424 showing a high decolorization capability (85.0–94.6%) of different dyes after 4–14 h of reaction time. The dyes, such as indigo, anthraquinone, azo, and triphenylmethane, were also evaluated even in combination with metal ions and organic solvents, without enzyme inhibition. Also, polycyclic aromatic hydrocarbons, such as fluorene, fluoranthene, pyrene, phenanthrene, and anthracene, were degraded, in the range of 89.9–95.3%, after 12 h. These results represent a higher removal rate when compared with MnP from previous studies, such as the removal rates reported by Acevedo et al., which were >15% for fluoranthene and phenanthrene hydrocarbons. Kalsoom et al. isolated an

### Table 2. Examples of immobilized enzymes applied in biodegradation and bioremediation of emerging contaminants

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Source</th>
<th>Immobilization method and matrix</th>
<th>Compound removed</th>
<th>Efficiency of removal (%)</th>
<th>Reusability</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laccase</td>
<td>Myceliophthora thermophila</td>
<td>Covalent binding in epoxy functionalized silica</td>
<td>Catechol</td>
<td>95</td>
<td>61% after 5 cycles</td>
<td>Mohammadi et al.</td>
</tr>
<tr>
<td>Lignin peroxidase</td>
<td>Schizophyllum commune IBL-06</td>
<td>Cross-linking with 2.5% glutaraldehyde</td>
<td>Diclofenac</td>
<td>89.6, 81. 5, 79.6</td>
<td>62.3% after 3 cycles</td>
<td>Parveen et al.</td>
</tr>
<tr>
<td>Lignin peroxidase</td>
<td>Ganoderma lucidum</td>
<td>Entrapment in Ca-alginate beads</td>
<td>Sandal-fix Red C4BLN dye</td>
<td>93</td>
<td>41% after 7 cycles</td>
<td>Shaheen et al.</td>
</tr>
<tr>
<td>Dye-decolorizing peroxidase</td>
<td>Bacillus amylobioficus</td>
<td>Chitosan-coated, halloysite nanotube porous microspheres</td>
<td>Dyes mixture</td>
<td>93.9</td>
<td>57.6% after 6 cycles</td>
<td>Ren et al.</td>
</tr>
<tr>
<td>Laccase</td>
<td>T. versicolor and P. sanguine CS43</td>
<td>Cross-linking with 4% glutaraldehyde</td>
<td>Bisphenol A</td>
<td>100</td>
<td>–</td>
<td>Barrios-Estrada et al.</td>
</tr>
<tr>
<td>Manganese peroxidase</td>
<td>Anthracophyllum discolor</td>
<td>Magnetic nanocomposite Fe$_3$O$_4$/chitosan</td>
<td>Methylene blue dye</td>
<td>96</td>
<td>85% after 5 cycles</td>
<td>Siddeeg et al.</td>
</tr>
<tr>
<td>Manganese peroxidase</td>
<td>Anthracophyllum discolor</td>
<td>Magnetic nanocomposite Fe$_3$O$_4$/chitosan</td>
<td>Reactive orange 16 dye</td>
<td>98</td>
<td>86% after 5 cycles</td>
<td>Siddeeg et al.</td>
</tr>
<tr>
<td>Horseradish peroxidase</td>
<td></td>
<td>Functionalized biochar with 1% glutaraldehyde</td>
<td>Phenolics</td>
<td>90</td>
<td>79% after 4 cycles</td>
<td>Petronijević et al.</td>
</tr>
</tbody>
</table>

shown better biodegradation ability are oxidoreductases and hydrolases. Some examples are shown in Table 1.
MnP from a solid-state culture of *Aspergillus flavus*, showing maximum catalytic potential at 35°C and pH 4.0. The enzyme catalyzed 94% decolorization of Direct red 31 and 85% of Acid black 234, while 100% and 92% of decolorization were achieved with immobilized MnP, respectively, after 24 h. In summary, the MnP extracted from *Trametes* sp. 48424 showed higher capacities than other enzymes recently evaluated from other strains for hydrocarbons remediation, such as *Anthracophyllum discolor* and *Nematoloma frowardii*. Also, high biocatalytic activity, thermal stability, and recyclability were reported with the application of immobilization techniques in MnP enzymes.

Horseradish peroxidases C (HrP, EC 1.11.1.7) are among the most abundant peroxidase isoenzymes in nature and are extracted from the roots of horseradish. HrP are heme-containing peroxidases that mainly catalyze the oxidation of phenolic acids, aromatic phenols, and non-aromatic amines and have been applied in wastewater treatments for this purpose. HrP showed high efficiency in phenolic compounds degradation. Lu et al. reported a removal rate of 51.7% for bisphenol A, 55.4% for *p*-chlorophenol, 95.4% for paracetamol, 95.0% for 2,4-dichlorophenol, and 99.3% for 4-methoxyphenol. Even removal rate of 100% was reported by Besharati Vineh et al., applying immobilized HrP in effluents with a high phenol concentration of 2.5 g/L. Recently, Petronijević et al. evaluated a HrP immobilized in biochar for the removal of phenolics. The immobilized enzyme showed the highest activity at 30°C and pH 7.0, reaching 90% of phenol removal after 2 h.

A novel group of non-heme peroxidases with a high ability to decolorize certain industrial dyes were found and were called dye-decolorizing peroxidases (DyP). Ren et al. isolated DyP from *Bacillus amyloliquefaciens*. In their tests of immobilization into halloysite nanotube porous microspheres, after a 72 h incubation period, the decolorization of a dyeing effluent mixture reached 93.9%. Also, the chemical oxygen demand was removed by 52.8%. Also, Athamneh et al. reported an efficient degradation of 6 EC (furosemide, paracetamol, 2-mercaptobenzothiazole, salicylic acid, sulfamethoxazole, and methylparaben) through the biocatalytic action of DyP.

Laccases (EC 1.10.3.2) are a group of copper-containing polyphenol oxidases, which utilize molecular oxygen as the final electron acceptor. Recently, researchers showed some laccase applications in oxidative processes of EC, in comparison to their traditional use in conventional lignin depolymerization. Due to the use of atmospheric oxygen as an oxidant instead of hydrogen peroxide, the application of laccases can potentially have a more cost-efficient process. A wide range of heterocyclic and phenolic compounds are catalyzed by laccases’ oxidation reactions. Mohammadi et al. reported a rapid process of 2 h with 95% removal efficiency of catechol. Many other works reported high efficiencies (83–100%, 8–18 h) for pharmaceutical and personal care products degradation, such as triclosan, diclofenac, acetaminophen, and carbamazepine. In terms of the endocrine disruptors degradation, Zdarta et al. evaluated different operational parameters of temperature, pH, and initial pollutants concentration, reaching 100% of removal efficiency after 24 h for bisphenol A (pH 5, 30°C) and F (pH 5, 40°C) and 40% for bisphenol S (pH 4, 30°C). Also, after 10 h, significant efficiency was reached (~80%) for bisphenol A and F.

In Figure 4, three biotransformation tetracycline pathways proposed by Han et al. for a bacterial laccase from *B. amyloliquefaciens* are shown. The enzyme was immobilized as laccase-inorganic hybrid nanoflowers. After the proposed sequential reactions, the TC8 compound is formed (Figure 4). The proposed final compound presented lower molar weight when compared to initial tetracycline. In terms of efficiencies, the proposed process decomposed tetracycline, doxycycline, and tigecycline, with an efficiency higher than 79%, after 1 h of treatment.

Together with the exceptional physicochemical properties of laccases, they present high reusability in immobilized form, not necessitating expensive cofactors, which could improve process cost efficiency. In general, oxidoreductases could be evaluated in mixtures of enzymes, as evaluated by Zhang et al., which combined laccase with MnP, with improved rates reached. The use of more than one enzyme in reaction medium could combine different enzymes specificities and advantages to treat more diversified contaminants in the same treatment.

Hydrolases

Depending on their substrate specificity, there are esterase hydrolases and lipases that act on ester bonds (EC 3.1), glycosylases such as amylases and cellulases that act on glycosyl bonds (EC 3.2); hydrolases that
act on ether bonds (EC 3.3); and proteases that act on peptide bonds (EC 3.4). Due to their hydrolytic characteristics, they are a powerful tool to decrease soil and water pollution. Hydrolases can convert large toxic molecules into small subunits, reducing or eliminating their toxicity. The main advantages of the use of hydrolase-type enzymes in bioremediation are their easy availability, low cost, environmentally friendly, and tolerance to the addition of water-miscible solvents.

Organophosphate pesticides can be detoxified through enzyme bioremediation by applying organophosphate hydrolases, which hydrolyze phosphodiester bonds. The most important step in the detoxification of organophosphate compounds is degradation through hydrolysis of P-O-alkyl and P-O-aryl bonds. For this reason, hydrolases have been gaining wide acceptance as a strategy in enzymatic bioremediation. Enzymes such as organophosphate hydrolases, organophosphate acid anhydrolases, and methyl parathion hydrolases have been applied as catalysts in the degradation and decontamination of organophosphate compounds. Mali et al. achieved the heterologous expression of an organophosphate hydrolase gene from the bacterium Arthrobacter sp. HM01. The results showed an increase of more than 10 times in the catalytic capacity of the enzyme, being highly resistant to organic solvents and heavy metals. Furthermore, through site-directed mutagenesis, it was confirmed that the catalytic site presented a highly conserved metal-bridged residue (Lys-127) that was of great importance in the degradation of organophosphates. Another component known for its toxicity is cyanide, being highly harmful to human health. Cyanide-derived compounds can be removed by two pathways: the first has two steps involving the enzymes nitrile hydratase and amidase, while the second has a simple pathway that involves the nitrilases enzyme hydrolyzing the cyanide components into carboxylic acids and ammonium.

Due to their high catalytic power, proteases are applied in the degradation of proteins, being widely used for the bioremediation of wastes from the food industry and domestic wastewater. In addition, some proteases can degrade α-ester bonds and lipase γ-ω bonds, thus being quite useful in the depolymerization of polymers such as poly(hydroxybutyrate) and others. One of the main applications of proteases in bioremediation is the hydrolysis of protein residues resistant to degradation, such as insoluble keratin. This type of protein, present in most animals, represents abundant biomass in domestic wastewater and poultry waste. Keratinase is commonly used in bioremediation because it hydrolyzes the peptide bonds of keratin, releasing amino acid subunits that can be reused in feed additives and fertilizers for plant growth. Akram et al. managed to produce a keratinase from a thermotolerant strain of Bacillus sp. NDS-10, reaching a
maximum production of 92 U mL\(^{-1}\). Keratinase has a high tolerance to pH 6–10 and temperatures of 20–60°C.\(^9\) In addition, it has a high rate of bioconversion of chicken feathers of 97% in 20 h of incubation with an initial concentration of 0.8% (w/v) at 45°C. This keratinase was also evaluated in the degradation of skin and hair in the production of animal leather, eliminating these residues in just 6 h of treatment, showing a high potential for its use in bioremediation and the leather industry.

On the other hand, lipases belonging to serine hydrolases have the ability to hydrolyze ester bonds, which are present in lipids such as triglycerides, releasing fatty acids and glycerol.\(^9\) The catalytic reaction of these enzymes occurs in a lipid-water interface where the lipid substrate forms a balance between monomeric, micellar, and emulsified states. The lipolytic enzymes used in bioremediation are mainly those that degrade pollutants such as fossil fuels, domestic effluents, and domestic oils residues.\(^6\)

**INNOVATIVE APPROACHES**

**Enzymes’ immobilization**

The main bottlenecks in the use of enzymes for bioremediation are their higher cost when compared to conventional wastewater treatment methods and poor reusability and stability.\(^3\) Enzymes’ immobilization techniques can be applied to overcome these gaps. It consists of the attachment and/or entrapment of the enzyme molecule to or in a matrix. This process can change enzymes’ conformation, improve their stability, and allow their use in several reaction cycles and further recovery from the media.\(^7\) In wastewater treatment systems, in which toxic compounds may hinder enzyme activity, immobilization techniques can bring huge advantages as the applied matrix can protect the molecule.\(^8\) Thus, immobilization’s matrix and technique need to be well chosen (Figure 5). Each method presents its characteristics and properties that may influence enzyme conformation, activity, and stability.\(^9\) Information about enzyme particularities, such as their structure, optimal reaction conditions, and reaction parameters, need to be well defined. Besides, optimization of process conditions, such as temperature, pH, and enzyme concentration, need to be conducted for maximum activity, recovery, and yield.\(^9\)

Immobilization can be also made in enzymatic membrane reactors (EMRs)—systems that apply membranes for the retention of the enzyme. The membrane unit can be either separated from the reactor with further enzyme recycling, coupled inside the reactor with the enzymes being retained in the membrane matrix, or entrapped in microcapsules.\(^9\) These systems enhance industrial continuous processes, reducing the time of reaction and enabling better operations. On the other hand, co-immobilization of enzymes is a
technique that allows more than one enzyme in a system. Also, the co-immobilization of mediators can enhance enzymatic activity. Therefore, systems of multiple enzymatic reactions can be created for the degradation of various compounds simultaneously. Pylypchuk et al. evaluated the adsorption of LiP and HRP onto magnetite nanoparticles and sol-gel encapsulated in a surface silica layer. The removal efficiency of carbamazepine and diclofenac by sol-gel LiP composites at pH 5 after 3 days were 68% and 64%, respectively, while the total removal was reached at pH 3 after the same time. Examples of different immobilized enzymes applied for biodegradation are presented in Table 2.

Shaheen et al. evaluated LiP isolated from *Ganoderma lucidum* IBL-05 entrapped in Ca-alginate beads at pH 5.0, 55°C, and 4% Na-alginate. Decolorization efficiencies were in the range of 80–93%, in comparison with free enzyme removal rates of 48–66%. Also, the cytotoxicity of dye solutions was reduced up to 2.1–9.2%, by treatment with Ca-alginate immobilized LiP. High reduction rates were also observed in water quality parameters, such as biological (66.4–98.2%) and chemical oxygen demand (81.3–98.8%), and total organic carbon (80.2–97.8%) values. Parveen et al. immobilized LiP from *Schizopyllum commune* IBL-06 in glutaraldehyde cross-linked enzyme aggregates reaching 89.6, 81.5, and 79.6% of degradation of Sandal-fix Black CKF, Sandal-fix Turq Blue GWF, and Sandal-fix 26 Red C, respectively, at pH 4.0, 60°C, and 6 h of treatment. Also, Jankowska et al. evaluated HRP immobilized into electrospun fibers to dyeing effluent treatment; the highest dye removal efficiency of 70% was reached at pH 7.0, 25°C, after 1 h. However, further works must evaluate the efficiency of such enzymes on different EC removal.

Laccases also showed high removal efficiencies through immobilized enzymes processes, such as in the case of Barrios-Estrada et al. (Trametes versicolor and *Pycnoporus sanguineus* CS43 enzymes, 4% glutaraldehyde, 24 h, pH 5.4: 100% degradation of bisphenol A) and Lassouane et al. (*T. pubescens* enzyme, glutaraldehyde cross-linking and Ca-alginate beads entrapment, 2 h, pH 5.0: 99% degradation of bisphenol A). Yavaşer and Karagözler immobilized *T. versicolor* laccase in functionalized polyacrylamide-alginate cryogel, reaching 70% removal of phenolics from olive mill wastewater, and 93.3–99.1% decolorization of selected synthetic dyes. In the case of real textile wastewater, 55.6% of dyes were removed.

The enzymatic immobilization is a promising technique that enhances the enzyme reusability and stability to harsh conditions, which can reduce operational costs. However, the majority of these systems have been developed at laboratory scale and applied in simulated residues. Besides, the current techniques developed for enzymatic immobilization can present high cost for implementation, discouraging their use. Therefore, technical and financial analyses could be conducted in order to evaluate the balance between cost saving due to enzyme reusability and loss due to the immobilization technique price. Furthermore, studies at large-scale applications of immobilized enzymes and in real wastes need to be developed for the assessment of techniques and further application in an industrial manner.

**Genetic engineering for modified enzymes**

A vast variety of wild strains secrete key enzymes for the bioremediation of industrial contaminants. However, occasionally, wild strains have some limitations or mild-to-low efficiency. These restrictions can occur due to extreme physical conditions, such as temperature, salinity, pH, and chemical contaminants. Adding to that, the degradation of industrial pollutants usually requires a huge amount of purified enzymes, and their high costs limit the economic feasibility and practical application. To overcome these factors, the genetic engineering approaches can design robust strains to withstand stressful conditions and achieve high production specificity and efficiency. Even though the process involves complex genomic analyses and is time consuming, studies show how the use of GMOs is promising to treat pollutants.

Ma et al. expressed CueO laccase from *Escherichia coli* K12 to *Pichia pastoris* GS115. Higher results were achieved reaching 556 mg/L expression level and 41,000 U/L enzyme activity. *P. pastoris* is an efficient secretory expression host and is being employed in other works. Also, it has a more accurate post-translational system and expresses high yields of recombinant proteins. Zhuo et al. expressed the laccase rLAC-EN3-1 from *Ganoderma sp. En3* to *P. pastoris*, concluding that purification was facilitated. Also, a better performance was reached. Another recombination possibility is the clone of multiple enzymes in the same organism. Liu et al. cloned ligninolytic enzymes (laccase, MnP, and LiP) from *Aspergillus* sp. TS-A to *P. pastoris* GS115, reaching better removal of Congo Red dye, due to synergism between enzymes. In the case of hydrolases, Janatunaim selected PETase and MHETase from *Ideonella sakaiensis* 201-F6 to express in *E. coli* BL21 to degrade polylethylene terephthalate (PET) plastic. MHETase gene was
constructed into a plasmid and was successfully inserted by heat shock. The update of microbial genes in databases facilitates the applicability of the use of enzymes. Liu et al. made a list of genes for constructing GMOs from different species that degrade pesticides, such as atrazine chlorohydrolase gene from *Pseudomonas sp*. ADP and organophosphorus hydrolase from *Burkholderia cepacia*.

GMOs are more efficient overproducers than wild strains and have improved catalytic performance and stabilization, reaching robustness on hazardous industrial waste reduction. Novel techniques such as functional genomics, proteomics, and metabolomics might assist in their viability. Additionally, some strategies seek deeper efforts to boost the use of enzymes in open systems and large scales, such as the discovery of novel genes for EC and the construction of a diverse metagenomic library.

**Advanced nanozymes**

Nanozymes can be defined as nanomaterials with the ability to catalyze reactions in a way similar to that carried out by biological enzymes. These nanocatalysts are becoming concurrent and possible substitutes for natural enzymes since the enzyme micrometers have marked advantages concerning biological catalysts, among which stand out the control of the enzymatic activity, high stability, and multifunctionality. The activity of nanozymes is affected by several factors such as size, morphology, structure composition, surface valence state, and surface modification (Figure 6). Different morphologies may present different amounts of dangling bonds and variability in the arrangement of atoms.

The structural composition of the catalytic agent is also essential to improve the performance of the nanozyme. The strategy widely used is the mixture of nanomaterials of lower activity (Ag and Au) with nanomaterials of higher activity (Ir and Pt). These hybrids can adjust catalytic activities through doping and alloying techniques. Furthermore, the activity of enzyme mimetics is also related to their valence state and oxygen vacancies. In some cases, using some coatings on the surface of the nanozyme can improve the rate of reaction catalysis by increasing the active sites of the nanozyme.

Enzyme mimetics are also classified into two broad groups, as are natural enzymes. The oxidoreductase family includes oxidase, peroxidase, catalase, superoxide dismutase, and nitrate reductase. The hydrolase family includes nuclease, esterase, phosphatase, protease, silicatein, and urease. For example, gold nanoparticles can exhibit enzyme activities similar to superoxide dismutase and oxidases; a similar case occurs with Ag nanozymes. According to Wang et al., Au nanozyme has the ability to catalyze the generation of hydroxyl radicals (•OH) from the decomposition of H₂O₂ under acidic conditions.

In general, nanozymes with smaller size tend to be more effective due to the greater specific surface area. For instance, Luo et al. tested different sizes of Ag nanozymes. Smaller nanozymes showed greater catalytic activity (13 nm > 20 nm > 30 nm > 50 nm). A similar situation was observed by Jv et al., where 34 nm

![Figure 6. Schematic illustration of nanozymes' structure activity](image-url)
Au nanozymes showed greater catalytic activity than 48 nm size. Other types of nanozymes are shown in Figure 7.

Nanozymes are being used in the detection of EC and also in their treatment. Liu et al.\textsuperscript{108} proposed an easy method to detect copper ions (Cu$^{2+}$) using histidine-modified gold nanoclusters (His-AuNCs). The presence of histidine in the surface of nanoclusters promotes the binding of nanozymes with the substrate, which increases the catalytic activity of the nanozyme. However, due to the high affinity between Cu$^{2+}$ and histidine, the peroxidase activity carried out by the nanozyme is reduced in the presence of copper ions. The activity of nanozymes is also influenced by the presence of metal ions, similar to natural enzymes.\textsuperscript{109} It can be evaluated as they catalyze the reactions of hydrogen peroxide and substrates, such as ABTS (2,2’-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) in colored complexes, so they are easily measured at different wavelengths.\textsuperscript{104} Gold nanoclusters modified with glutathione exhibit activity similar to peroxidases.\textsuperscript{110} Thus, the presence of Pb$^{2+}$ ions can be detected by inducing aggregation of gold nanoclusters due to binding with glutathione. ECs, such as pesticides, have also been detected using enzyme mimetics. Singh et al.\textsuperscript{111} evaluated palladium-gold nanorod as nanozyme (PdOuNa) for malathion detection, which exhibited high enzymatic activity similar to peroxidases, in a pH range of 2–6 and a temperature range of 4–70°C.

Enzyme mimetics have also been used to degrade EC. According to Wei et al.,\textsuperscript{112} nanocery nanozyme was applied to hydrolyze the pesticide methyl-paraoxon to p-nitrophenol. Cerium oxide, or nanocery, is considered a critical metal oxide due to its unique ability to switch oxidation cation states between (+3) and (+4).\textsuperscript{113} Boruah et al.\textsuperscript{114} applied Fe$_3$O$_4$ nanoparticles decorated and functionalized with reduced graphene oxide sheets and degraded 98% of triazine pesticides. Sadaf et al.\textsuperscript{115} synthesized the nanozyme CNPs (cellulose incorporated magnetic nano-biocomposites) using cellulose as a base material, which exhibited enzymatic activities similar of peroxidase enzyme. The nanozyme was able to degrade the compound methyl orange at concentrations of up to 50 ppm. Another dye, methylene blue, was also degraded using W18O49 nanozyme, which exhibits activities of a peroxidase.\textsuperscript{116} Also, a range of multivalent cerium-based metal-organic frameworks with laccase-mimicking activity were recently designed by Liang et al.,\textsuperscript{117} showing superior stability and recyclability toward the oxidation of phenolic compounds than the natural enzyme.

**INTEGRATED PROCESS**

Due to the low efficiencies of EC removal frequently observed in conventional biological wastewater systems and their widespread uses of them, integrated systems combining the advantages of different established technologies with advanced ones could be a proper solution to the removal of EC in wastewater treatment plants. Thus, integrated treatment systems could combine the benefits of existing technologies with the advancements...
of novel technologies to reach high ECs removal. As was already discussed, conventionally used steps in wastewater treatment plants are widely used to remove a variety of impurities, such as nutrients, dissolved organics, colloidal and suspended particulates, and pathogens from waste streams. Combining conventional treatments with novel advanced technologies could dispose of lower rates of pollutants in the higher costs steps, which otherwise could suffer a decrease in its removal efficiencies. Generally, wastewater treatment plants comprise a series of different treatment steps, such as primary, secondary, and tertiary treatments. These treatments involve multiple technologies including chemical and biological reactions and physical separations, among others. For instance, Melo-Guimaraes et al.118 evaluated biological processes separately and in combination with flocculation and ultra-filtration membrane systems. The authors reported that pharmaceutical compounds were removed more efficiently in activated sludge as compared with other treatments; however, the use of cationic flocculants enhances the biodegradability of the compounds. So, the integration approach of these different processes improved their total effectiveness.

In Figure 8, we proposed an ideal step where an enzymatic bioreactor could be placed into a wastewater treatment plant, to reach the polishing of the final effluent and EC removal from municipal wastewater. The ideal treatment plant was conceived according to a real wastewater treatment plant.119 The proposed system consisted of a flotation tank for grease and oil separation, followed by an aerated activated sludge unit as primary treatment, a secondary clarifier, the enzymatic bioreactor, and a final chlorine disinfection unit. In that way, it is expected that the influent of the enzymatic bioreactor reaches this specific step already clarified, with minimum presence of impurities, to the maximization of EC removal efficiency.

CONCLUSIONS
Oxidoreductases and hydrolases present a suitable biocatalyst for the bioremediation of EC, with high removal efficiencies reported. It was shown that laccases and peroxidases can be improved in terms of process efficiency and enzyme reusability through immobilization techniques. The development of novel supporting materials and their evaluation in large-scale experiments could be important to reach maximum recyclability of enzymes. Also, the performance of enzymatic processes must be well evaluated with real wastewater effluents and integrated system contexts.

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