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# Innovative Biological Approaches for Contaminants of Emerging Concern Removal from Wastewater: A Mini-Review

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**Abstract**

Over the last few decades, the presence of a group of contaminants, termed as Emerging Contaminants (ECs) in the wastewater stream is imposing a serious concern worldwide. Conventional treatment processes are not capable of removing these micropollutants resulting in their discharge to receiving surface waters, i.e. rivers, lakes and coastal waters. Various alternative approaches have been proposed and developed to remove and/or degrade organic pollutants from contaminated water bodies, including chemical, physical, and biological technologies. The latter offer potential advantages over other methods due to their less expensive cost and their environmentally friendlier nature. The aim of the present paper was to review the state-of-the-art biological processes for ECs removal and to determine future research directions. This mini-review focuses on the type of ECs being removed, the biological treatment applied, and the outcomes achieved. A critical look at current challenges was also provided along with critical comments and perceptive recommendations for further improvement of the performance of the developed methods.

**Keywords:** Biological treatment; Emerging contaminants; micropollutants; wastewater

**Abbrevations:** ECS: Emerging contaminants; EDCs: Endocrine Disrupter Compounds; MFCs: Microbial Fuel Cells; MnP: Manganese Peroxidase; LiP: Lignin Peroxidase; HRP: Horseradish Peroxidase

## Introduction

Emerging contaminants (ECs), also called contaminants of emerging concern, are defined as compounds that are currently not included in routine monitoring programmes nor in existing legislation in the area of water quality, but which are thought to be potentially harmful to environmental ecosystems and human health [1]. This group encompasses a wide range of compounds, including pharmaceuticals, personal care products, pesticides, hormones and several other classes. Conventional treatment processes are not capable of removing these micropollutants resulting in their discharge to receiving surface waters, i.e. rivers, lakes and coastal waters. Although in-depth scientific investigations have been focused on pollutants like nutrients (nitrogen and phosphorus), hydrocarbons, and heavy metals in stormwater and wastewater, only restricted number of studies have reported on the survey of emerging contaminants and even fewer related to the determination of appropriate treatment approaches. The development of innovative processes for the treatment of water contaminated with emerging pollutants is an ongoing challenge and a continuing need. In this regard, various approaches have been investigated and scaled up for the removal and/or degradation of organic pollutants including physical, chemical, and biological strategies. Among the developed approaches, biological technologies continue to attract significant interest of researchers as they have the potential to be less expensive and environmentally friendlier [2]. This mini-review sets out to provide an overview of different innovative biological approaches currently described in the literature for removing a list of emerging pollutants and to determine future research directions.

### Innovative biological treatment processes

The conventional activated sludge processes employed at wastewater treatment plants are inefficient in completely removing the ECs as they are essentially designed to eliminate simple organic material and nutrients from the wastewater [3]. In addition, wastewater treatment plants are not well equipped to monitor the levels of ECs in the source wastewater [4]. Accordingly, the need is becoming obvious to re-design the conventional system in wastewater treatment plants for a better handling and control of the ECs. Some adjustments in the operating conditions, such as hydraulic retention time, vigorous mixing, aeration, etc. have been proposed towards this objective [5]. However, the outcomes are not that satisfactory for the removal of emerging contaminants [6]. In the recent years, numerous of improvements have taken place in the field of ECs treatment. The effectiveness of innovative biological approaches for micropollutants removal Table 1 is reviewed in the following sub-sections.

**Table 1:** Removal efficiency of ECs with biological technologies.

| Removal techniques | EC | Removal efficiency, % | Reference |
|---|---|---|---|
| **Constructed wetlands** | | | |
| Horizontal subsurface constructed wetlands | Ibuprofen | 80 | Zhang et al. [7] |
| | Naproxen | 91 | |
| | Diclofenac | 55 | |
| | Carbamazepine | 26 | |
| Pilot-scale aerated Horizontal subsurface-flow constructed wetland | Atenolol | >75 | Auvinen et al. [8] |
| | Bisoprolol | >50 | |
| | Carbamazepine | <50 | |
| | Diclofenac | <50 | |
| | Gabapentin | <50 | |
| | Sulfamethoxazole | <50 | |
| Full-scale hybrid constructed wetland system | Bisphenol A | >99.9 | Yi et al. [9] |
| | Acetaminophen | 91.4 | |
| | Gemfibrozil | 94 | |
| | N, N-diethyl-m-toluamide | 93.1 | |
| | Caffeine | 80.3 | |
| | Salicylic acid | 77.2 | |
| | Diclofenac | 91.8 | |
| | Clofibric acid | >99.9 | |
| | Sulfamethazine | >99.9 | |
| **Membrane bioreactor** | | | |
| Full scale MBR plant | Diltiazem | 100 | Komesli et al.[12] |
| | Estrone | 100 | |
| | Progesterone | 100 | |
| | Acetaminophen | 100 | |
| Lab-scale MBR | LAS C9-13 | 100 | Bernhard et al. [13] |
| | Ibuprofen | 99 | |
| | SPC C11 | 98 | |
| | 2,4- Dichlorobenzoic acid | 83 | |
| | Bayrepel-acid | 93 | |
| | DEET | 62 | |
| | Diclofenac | 58 | |
| | Clofibric acid | 54 | |
| | MCPP | 50 | |
| | TCEP | 37 | |
| | TCPP | 12 | |
| | Carbamazepine | 13 | |
| | EDTA | 0 | |
| | Atrazine | 9 | |
| | Bentazone | 16 | |
| | Isoproturon | 25 | |



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| | | | |
|---|---|---|---|
| **Biofiltration** | | | |
| Soil filtration | Estrogens | 26 | Carr et al. [17] |
| | 17b-Estradiol | 99 | |
| | 17b-Ethinyl estradiol | 27 | |
| | Triclosan | 90 | |
| | Ibuprofen | 18 | |
| Biological filtration | Cashmeran | 68 | Matamoros et al. [18] |
| | Ibuprofen | 86 | |
| | Benzothiazole, 2-(methylthio) | 66 | |
| | Tributyl phosphate | 22 | |
| | Methyl dihydrojasmonate | 97 | |
| | Tri(2-chloroethyl)phosphate | 2 | |
| | Diazone | 8 | |
| | Caffeine | 49 | |
| | Galaxolide | 89 | |
| | Tonalide | 90 | |
| | Terbutryn | 94 | |
| | Carbamazepine | 5 | |
| | Naproxen | 72 | |
| | Oxybenzone | 89 | |
| | Triclosan | 87 | |
| | Ketoprofen | 99 | |
| | Diclofenac | 93 | |
| **Enzymatic Treatment** | | | |
| Laccase from *Trametes pubescens* | Bisphenol A | >99 | Lassouane et al. [19] |
| Laccase from *Myceliophthora thermophila* | Morphine | 100 | Huber et al. [23] |
| Laccase from *Trametes versicolor* | Orange 2 | 72.8 | Legerská et al. [24] |
| | Acid Orange 6 | 45.3 | |
| Laccase from *Fomes fomentarius* | Remazol Brilliant Blue R | 100 | Neifar et al. [25] |
| Laccase from *Myceliophthora thermophila* | Estrogens | 100 | Lloret et al. [22] |
| | Diclofenac | 100 | |
| | Naproxen | 100 | |
| Peroxidase from *Pleurotus ostreatus* | Bromophenol blue | 98 | Kwang-Soo&Chang -Jin [26] |
| | Methylene Blue | 10 | |
| | Toluidine Blue O | 10 | |
| Bacterial peroxidases | Bisphenol A | 100 | Moussavi& Abbaszadeh Haddad [27] |

## Constructed wetlands

Phytoepuration (constructed Wetlands) are treatment systems that use natural processes involving wetland vegetation, soils, and their associated microbial assemblages to improve water quality. This type of approach have long shown to be efficient, low-cost and simple maintenance and operation treatment system for the removal of organic matter, suspended solids, nutrients and ECs from a wide range of wastewaters. The removal of micropollutants from wastewater by constructed wetlands takes place by the combination/integration of three main processes, i.e. biodegradation a biological process, adsorption - a physicochemical process and oxidation - a chemical process. Several authors have reported some success in applying this technique for ECs removal. Zhang et al. [7] investigated the ability of tropical horizontal subsurface constructed wetlands planted with Typha angustifolia to remove four widely used pharmaceutical compounds, namely carbamazepine, declofenac, ibuprofen and naproxen. The results obtained showed a high removal of ibuprofen (80%)



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and naproxen (91%), while low removal efficiencies have been achieved for carbamazepine and declofenac. Auvinen et al. [8] tested a pilot-scale aerated subsurface-flow constructed wetland treating municipal and hospital wastewater. The atenolol and bisoprolol removal efficiencies were as high as 75% and 50%, respectively, while only limited removal of carbamazepine, diclofenac, gabapentin and sulfamethoxazole was achieved. In another study conducted to assess the removal of target emerging contaminants (i.e. acetaminophen, bisphenol A, clofibric acid, caffeine, crotamiton, diclofenac, N,N-diethyl-m-toluamide, gemfibrozil, lincomycin, salicylic acid, and sulfamethazine) by a full-scale hybrid constructed wetland system, results showed excellent removal efficiencies (>90%) [9].

### Membrane bioreactor

Membrane Bioreactor (MBR) which integrates the activated sludge process and membrane separation (i.e. microfiltration or ultrafiltration), has gained a great deal of interest for the treatment of wastewater containing micropollutants and pathogenic micro-organisms [10]. The removal efficiency of MBR is greater than the other biological systems owing to its important biological activity in the immediate vicinity of the membrane surface ensuring a higher removal rate of pollutant. Moreover, due to the membrane sieving effect, contaminants with a molecular weight higher than the molecular weight cut-off of the membranes are retained, bringing them in contact with the degrading microorganisms inside the MBR hence enhances the degradation process [11].

MBR has been deemed to be a promising technology for the treatment of a wide range of micropollutants including Endocrine Disrupter Compounds (EDCs), pharmaceutical compounds, pesticides, etc. Komesli et al. [12] evaluated a full scale MBR plant for EDCs (i.e. diltiazem, estrone, progesterone and acetaminophen) treatment, and using this approach, a complete removal of these molecules has been achieved. In another study by Bernhard M et al. [13], it was found that treatment by MBR results in significant better removals compared to activated sludge treatment, with a removal efficiency of 50- 100% for rapid biodegradable compounds, such as bayrepel-acid, diclofenac and DEET. However, other studies revealed no increase in the removal efficiency of pharmaceutical compounds when using MBR process compared to conventional activated sludge process [14]. Overall, the removal efficiency of different emerging contaminants using MBR system is in the following order: EDCs > beta blockers >pharmaceutical compounds > pesticides [11,15,16].

### Biofiltration

In addition to the above-mentioned technologies, other biological systems (e.g., soil filtration or biological filtration) have been investigated for the removal of ECs with interesting results. Carr et al. [17] examined biological degradation of six pharmaceuticals and personal care products by means of soil microorganisms and it was shown that estradiol derivatives were relatively easier to eliminate, while other compounds like ibuprofen and triclosan were just partially removed. According to these researchers, soils under saturated conditions exhibited faster degradation rates than those remained non-saturated. In another study, the removal efficiency of 18 emerging contaminants has been examined in a biological filtration pilot plant based on Daphnia sp. [18]. Compounds like Ketoprofen, diclofenac and Terbutrin showed high removal rates, while others like carbamazepine, diazinone and tri-(2-chloroethyl)-phosphate achieved the low removal rates.

### Enzymatic Treatment

The chemical structures of several emerging contaminants generally contain an aromatic moiety which can be the target for their modification by oxidative enzymes. The literature survey shows that most of the enzymatic remediation investigations use two families of enzymes, either laccases or peroxidases. These enzymes are very promising biocatalysts for the transformation of toxic organic pollutants into less toxic or non-toxic end products, thereby mitigating or eliminating contamination from the environment. The enzymatic treatments are considered tertiary treatment owing to their ability to transform the recalcitrant compounds that are not removed through secondary processes.

In recent times, laccase-catalyzed systems have shown considerable potential in degrading phenolic compounds, namely, Bisphenol A [19], Triclosan [20] and Nonylphenol [21]. In addition, quite a few studies focused on the efficacy of laccases to remove pharmaceuticals (i.e. antibiotics, anti-inflammatories , narcotic drugs), natural and synthetic hormones (i.e. estrone (E1), 17β-estradiol (E2), estriol (E3) and EE2 (17-α-ethinylestradiol)) as well as various classes of aromatic dyes [22-24]. For instance, Lassouane et al. [19] reported the effectiveness of the immobilized laccase from Trametes pubescens in Bisphenol A removal from aqueous solutions. This biocatalyst was able to remove almost completely 20 mg L-1 Bisphenol A in 2 h. Huber et al. [23] examined the removal of morphine by a laccase from Myceliophthora thermophila both in its free form as well as immobilized on Accurel MP1000 beads.

The results showed complete morphine elimination within 30 min for the free and the immobilized enzymes. Legerská et al. [24] reported color removal efficiencies of 72.8% and 45.3% for Orange 2 and Acid Orange 6, respectively, by employing fungal laccase from Trametes versicolor. In another study performed by Neifar et al. [25], a complete decolorization of the anthraquinone dye Remazol Brilliant Blue R was achieved when using laccase produced by Fomes fomentarius. Lloret et al. [22] examined the degradation of several pharmaceuticals and estrogen hormones by means of the commercial laccase from Myceliophthora thermophila. A complete removal of estrogens was achieved after only 15 min, while 1 h of incubation was required for total removal of diclofenac and 8 h to attain up to 60% of naproxen degradation.

Numerous studies have reported as well on the effective and extensive use of peroxidases in the oxidation of a vast variety of emerging compounds, including azo dyes, nonsteroidal


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anti-inflammatory drugs (NSAIDs), hormones, antibiotics, and pesticides. These enzymes are extensively distributed in nature, particularly in plants, animals, and microbes. The white-rot fungus Pleurotus ostreatus produced an extracellular peroxidase that can decolorize several recalcitrant dyes including triarylmethane, heterocyclic azo, and polymeric dyes. The enzyme was more efficient in decolorizing Bromophenol blue (98 %), while heterocyclic dyes, Methylene Blue and Toluidine Blue O were least decolorized (only 10%) [26,27] utilized bacterial peroxidases-mediated bioprocess under H2O2-infusion for treating bisphenol A as a toxic endocrine disrupting compound. The complete biodegradation of 100 mg/L bisphenol A was achieved within 54 h reaction time at the optimum H2O2 bisphenol A molar ratio of 10. Pizzul et al. [28] examined the ability of pure Manganese Peroxidase (MnP), laccase, Lignin Peroxidase (LiP) and Horseradish Peroxidase (HRP) to degrade the widely used herbicide glyphosate, and achieved a complete degradation of glyphosate with MnP, MnSO4 and Tween 80, with or without H2O2.

### Biological treatment processes: Issues and outlooks

Although biological treatment approaches proved satisfactory results for emerging pollutants removal, they are, however, still inefficient to some extent. In fact, many compounds, when present in relatively high concentrations, may complicate and even inhibit the treatment. For instance, in a study performed by Seyhi et al. [29], it was found that excessive bisphenol-A concentrations (> 5 mg/L) disrupted the bacterial activity and bisphenol-A removal processes in a submerged membrane bioreactor [29]. In another study examining the impact of four commonly occurring pharmaceutically active compounds (ketoprofen, naproxen, carbamazepine and gemfibrozil) on nitrogen removal were shown to inhibit microbial activity of Nitrosomonas europaea at concentrations of 1 and 10 μM [30]. On another hand, previous studies reported that biological treatment processes work well for easily degradable compounds, but not at all for recalcitrant ones. Research investigating three biological treatment processes including conventional activated sludge, biological nutrient removal, and membrane bioreactor have reported that only easily biodegradable ECs (i.e. caffeine, diclofenac, trimethoprim) can be removed, while no removal was achieved regardless of the treatment processes for the recalcitrant ECs (e.g., sulpiride, metoprolol, bezafibrate) [31].

Another concern in biological degradation is the production of transformation products that could be more toxic than the original pollutant, such as perfluoroalkyl acid derivatives (e.g., perfluorooctanoic acid and perfluorooctanesulfonic acid) [32]. Accordingly, following up on the toxicity of the biologically treated effluent can be as crucial as the analytical quantification of the parent pollutants. The research for alternative biological treatment approaches continues and some promising processes have emerged. For example, bio electrochemical systems, such as Microbial Fuel Cells (MFCs) and Microbial Electrolysis Cells (MECs), have been proposed as the next generation for simultaneous wastewater treatment and energy production [33]. In bio electrochemical systems, biological oxidation of the organic contaminants occurs at the anode by bacteria forming a biofilm layer, with transfer of electrons from the microorganisms to the electrode surface. The electrons pass then through an external electrical circuit to the cathode where a reduction reaction may occur. By using bio electrochemical systems, Harnisch et al. [34] & Werner et al. [33] reported high efficiency in the removal of some selected contaminants. Currently, there is not a sole technology that is able to transform an entire family of ECs into friendly sub-products, and several pre- and post- treatment processes have to be involved to achieve high conversion rates for ECs. Further researches are needed to improve the performance of the current methods and to develop combined technologies for efficient ECs transformation.

## Conclusion

In recent years many studies have been done on the development of innovative sustainable technologies for the removal of ECs in wastewater. Among them, as highlighted in the mini-review, biological processes have been proved to be suitable for the degradation of some organic micropollutants, whereas, some extreme recalcitrant contaminants still undegraded or only partially removed. The emphasis should be given to the development of more efficient innovative processes and to the investigation of the use of coupled systems which can bridge the deficiencies in existing single biological technologies for the removal of these complex pollutants present in the water environment.

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