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Enzymes in removal of pharmaceuticals from wastewater: A critical review of challenges, applications and screening methods for their selection

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HIGHLIGHTS

- A comprehensive overview of research on enzymatic bioremediation of pharmaceuticals.
- Enzyme screening options with a special focus on mass spectrometry.
- Current gaps between laboratory and large scale research that need to be overcome.
- Perspectives of enzyme-based processes as a future alternative wastewater treatment.

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ABSTRACT

At present, the removal of trace organic chemicals such as pharmaceuticals in wastewater treatment plants is often incomplete resulting in a continuous discharge into the aqueous environment. To overcome this issue, bioremediation approaches gained significant importance in recent times, since they might have a lower carbon footprint than chemical or physical treatment methods. In this context, enzyme-based technologies represent a promising alternative since they are able to specifically target certain chemicals. For this purpose, versatile monitoring of enzymatic reactions is of great importance in order to understand underlying transformation mechanisms and estimate the suitability of various enzymes exhibiting different specificities for bioremediation purposes. This study provides a comprehensive review, summarizing research on enzymatic transformation of pharmaceuticals in water treatment applications using traditional and state-of-the-art enzyme screening approaches with a special focus on mass spectrometry (MS)-based and high-throughput tools. MS-based enzyme screening represents an approach that allows a comprehensive mechanistic understanding of enzymatic reactions and, in particular, the identification of transformation products. A critical discussion of these approaches for implementation in wastewater treatment processes is also presented. So far, there are still major gaps between laboratory- and field-scale research that need to be overcome in order to assess the viability for real applications.

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1. Introduction

Pharmaceuticals and their metabolites are one of the most frequently detected compound classes of emerging trace organic chemicals in the aquatic environment (Rivera-Utrilla et al., 2013a). Knowledge regarding the fate and potential adverse effects of pharmaceutical residues in the aquatic environment are largely unknown. Ecotoxicological effects of some pharmaceuticals have already been reported, including synthetic estrogens like estradiol and ethinylestradiol (Carlsson et al., 2006) or diclofenac (Lonappan et al., 2016). Excretion, bathing, direct waste disposal and veterinary use can be considered as the main sources of contamination of these chemicals into the aquatic environment. Removal of pharmaceuticals during wastewater treatment is currently insufficient (Li, 2014; Richardson and Kimura, 2016; Yang et al., 2017), resulting in the occurrence of pharmaceuticals not only in treated wastewater effluents but also in surface, ground and much less frequently - in drinking water (Caban et al., 2016). Studies imply that concentrations of pharmaceuticals in drinking water are usually significantly below the predicted no-effect concentration (PNEC) level (Luo et al., 2014a). However, due to potential ecotoxicological concerns, there is a great demand for environmentally friendly, cost-effective and efficient removal strategies capable of reducing the discharge to the aquatic environment.

Bioremediation strategies have gained in importance due to their potential energy-saving and environmentally compatible properties than conventional, more resource intensive treatment technologies (Sharma et al., 2018). Those biological approaches exploit the potential of natural systems such as bacteria and fungi for the biotransformation and biodegradation of xenobiotic compounds in water, wastewater, soil or sludge. The conversion of pharmaceuticals by microorganisms can be induced both *in situ* in the aquatic environment and in engineered treatment processes. Regardless of these conditions, the inherent biocatalysts in biological processes are enzymes. However, processes controlling and affecting biological remediation, including metabolic interactions or transport and reaction pathways, are very complex and not sufficiently understood, therefore offering substantial room for improving transformation efficiencies.

The use of isolated enzymes targeting specific chemicals holds promise as a more systematic and controllable alternative to conventional biological treatment processes using a consortium of rather unspecific microorganisms. At present, there is still a great need for research to implement and tailor this methodology to wastewater treatment. Until now, systematic investigations regarding the capability, applicability, and controllability of different enzymes to transform a wide range of pharmaceuticals under environmental conditions are missing. A key issue in this context is the selection and identification of suitable enzymes by means of rapid and versatile analytical tools.

The aim of this review is to (i) provide a comprehensive summary of recent research on enzymatic biodegradation of pharmaceuticals, (ii) present an overview on rapid and targeted enzyme screening options with a special focus on mass spectrometry (MS)-based and high-throughput tools, and (iii) evaluate opportunities to implement enzyme-based processes in wastewater treatment.

2. Pharmaceuticals as emerging trace organic chemicals in wastewater

Pharmaceuticals as emerging trace organic chemicals have gained increasing attention in recent years, due to their continuous release into the aquatic environment (Rivera-Utrilla et al., 2013a) and potential adverse health effects in aquatic ecosystems (Santos et al., 2010). The steady improvement of analytics has played a key role in the growing body of knowledge regarding the occurrence, fate, and effects of these chemicals in the environment and during wastewater treatment. Environmental monitoring of pharmaceuticals and their ecotoxicological impacts is a key component to manage and improve wastewater treatment strategies (der Beek et al., 2016).

About 10,000 pharmaceuticals with up to 3000 ingredients have been approved for usage and are regularly applied by humans (Jelić et al., 2012a; FDA, 2017) In European countries, the consumption rate of pharmaceuticals increased continuously from 2000 to 2015, with the strongest increase for cholesterol-lowering drugs (OECD, 2017). This usage pattern has led to increased concentrations of pharmaceuticals in the aquatic environment (ranging from ng/L to µg/L) (Jelic et al., 2011; Gavrilescu et al., 2015; Petrie et al., 2015). Considering the diversity of pharmaceutical chemicals, it is not feasible to monitor them in their entirety. For this reason, a priority list that classifies the importance of substances according to various criteria such as consumption, toxicity and persistence was created (Voogt et al., 2009).

The pharmaceuticals differ widely in structure and behavior and are usually classified according to their application (Bruce et al., 2010; Rivera-Utrilla et al., 2013b; Tijani et al., 2016). Removal rates from wastewater do not only depend on chemical characteristics, but also on wastewater composition and operating conditions (Jelić et al., 2012b). In addition, pharmaceuticals show different susceptibility to advanced treatment processes (Ziylan and Ince, 2011). All these parameters lead to widely fluctuating concentrations in influent and effluent samples from municipal WWTPs.

Little is known about the conversion products of pharmaceutical compounds, i.e., metabolites or transformation products and conjugates formed during treatment. Recently, studies providing guidance to consider transformation products for environmental