Roles and applications of enzymes for resistant pollutants removal in wastewater treatment

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Abstract Resistant pollutants like oil, grease, pharmaceuticals, pesticides, and plastics in wastewater are difficult to be degraded by traditional activated sludge methods. These pollutants are prevalent, posing a great threat to aquatic environments and organisms since they are toxic, resistant to natural biodegradation, and create other serious problems. As a high-efficiency biocatalyst, enzymes are proposed for the treatment of these resistant pollutants. This review focused on the roles and applications of enzymes in wastewater treatment. It discusses the influence of enzyme types and their sources, enzymatic processes in resistant pollutants remediation, identification and ecotoxicity assay of enzymatic transformation products, and typically employed enzymatic wastewater treatment systems. Perspectives on the major challenges and feasible future research directions of enzyme-based wastewater treatment are also proposed.

Keywords: Enzymatic wastewater treatment, Resistant pollutants, Transformation products, Toxicity assay, Enzyme-mediator, Enzymatic remediation

1. Introduction

In recent years various pollutants and at alarmingly high levels have been discharged into aquatic environments. As the most widely employed wastewater treatment method, traditional activated sludge can remediate most pollutants effectively, while resistant contaminants like oil, grease, pharmaceuticals, pesticides, plastics are difficult to be eradicated. These pollutants mainly including oil, grease, and organic micropollutants (Fig. 1). Oil and grease containing wastewater are usually produced by dairies, oil mills, slaughterhouses, and food waste (Ahmad et al., 2020; Bustillo-Lecompte & Mehrvar, 2015; Meng et al., 2015). Oil and grease which float on water surfaces will compromise the transfer rate of substrates, products, and oxygen (Cammarota & Freire, 2006). The floating oil and grease may cause a filamentous microorganism bloom and lead to poor performance of activated sludge, resulting in floating sludge, poor sedimentation, and sludge biomass reduction (Cammarota & Freire, 2006). Other pollutants like pharmaceuticals, pesticides, plastics, and personal care products which are all known as micropollutants, are also called emerging pollutants or emerging concern contaminants (Sauvé & Desrosiers, 2014; Teodosiu et al., 2018). These compounds are hard to be tracked or controlled in most situations (Mishra et al., 2020) due to their nanograms-micrograms per liter concentrations in the environment (Virkutyte et al., 2010). They are typically toxic, easy to bioaccumulate, and resistant to natural biodegradation, posing a great threat to the environment and all living organisms (Sauvé & Desrosiers, 2014; Varga et al., 2019).

[Insert Fig. 1]

At present, wastewater treatment methods mainly include physiochemical and biological methods. Physiochemical methods like chemical oxidation, distillation, membrane-based separation techniques, and adsorption have been used for wastewater treatment (Alshabib & Onaizi, 2019). These strategies are treatment-wise, but they are very expensive and may cause

further pollution and damage (Bhatnagar & Anastopoulos, 2017; Mohammadi et al., 2015; Villegas et al., 2016). Biological methods are more eco-friendly and could remove most pollutants in wastewater. These methods employed plants, microbes and enzymes for wastewater treatment (Alshabib & Onaizi, 2019). Organisms applied in these technologies could degrade or assimilate contaminants during the process. However, plants and microbes are sensitive to some toxic pollutants from wastewaters (Ebele et al., 2017; Sharma et al., 2019; Wilkinson et al., 2016), whilst enzymes could work fast and selectively.

Enzyme is an effective biocatalyst which can biodegrade substances specifically under mild conditions (Brandelli et al., 2015; Yao et al., 2020). Enzymes have specific active sites, ones which are able to bind with specific substrates and reduce the activation energy by this approach during enzymatic processes. Thus, these processes have high reaction kinetics and specificity. Furthermore, enzymes could save the time required for substrates' transport into cells, which makes these processes more effective. There are six kinds of enzymes and the most used ones in wastewater treatment are hydrolases and oxidoreductases (Mishra et al., 2020; Varga et al., 2019). These two enzymes can biocatalyst most pollutants in wastewater due to their wide range of substances. At present, enzymes like lipase, laccase, peroxidase have been commercially used. Laccase and peroxidase are widely employed for removing some organic micropollutants (Ji et al., 2016; Stadlmair et al., 2017; Zerva et al., 2017) due to their broad substrate specificities or promiscuity (Varga et al., 2019). Enzyme technology meets the "green chemistry" trend. Compared to conventional chemical processes, enzymatic processes have higher reaction kinetics and need less water and energy, so enzymes will not be consumed by reactions and subsequently can be recycled (Kalia et al., 2013; Summerscales, 2021). Unlike bacteria and other biological methods, enzymes do not compete with other microbial lifeforms (De Cazes et al., 2014; Demarche et al., 2012). Thus, enzymatic processes are promising in removing resistant pollutants like oil, grease, and organic micropollutants from wastewater.

Various enzymes have been studied to treat resistant pollutants in wastewater and many have performed successfully. This is the first review to provide an overview and critical discussion on the roles and applications of enzymes for resistant pollutants removal in wastewater treatment. The paper systematically introduces the influence of enzymes types and sources, studies enzyme-based wastewater treatment, enzymatic transformation products' problems, and typically employed enzymatic systems. Future studies on enzyme for real wastewater with larger-scale applications, enzymatic byproducts monitoring, enzyme ability enhancement, and multiple enzyme systems constructions were proposed to boost the sustainable development of enzymatic processes for green wastewater treatment technologies.

2. Enzyme treatment in different wastewater pollutants

2.1 Selection of enzymes

Enzyme efficiency in wastewater treatment depends strongly on enzyme types and their sources. For one thing, enzymes can only catalyze one or one type of reaction due to their specificity. For instance, lipases could catalyze the degradation of oil, grease, and fat, while protease is able to decompose proteins. The hydrolyzation of ester bonds of carbamates, organophosphates, and other chlorinated organic compounds was catalyzed by carboxylesterases (Cummins et al., 2007). Phosphotriesterases could hydrolyze a wide range of organophosphate (Romeh & Hendawi, 2014), whilst haloalkane dehalogenases should be enabled to cleave the carbon–halogen bond of halogenated aliphatic compounds (Mishra et al., 2020). Despite laccase and peroxidase having a wide range of substances (Catherine et al., 2016; Varga et al., 2019), they still have high selectivity. Relative research showed horseradish peroxidase and *Pleurotus ostreatus* laccase could remove a few pharmaceuticals selectively but effectively. The horseradish peroxidase could transform diclofenac (DCF) and sotalol (STL) completely after 4 hours, and it could converse acetaminophen (APAP) immediately while the *Pleurotus ostreatus* laccase needed 20 min (Stadlmair et al., 2017).

However, both enzymes were not susceptible to the transformation of sulfamethoxazole (SMX), carbamazepine (CBZ), ibuprofen (IBP) and naproxen (NAP) (Stadlmair et al., 2017).

In another study, enzyme sources wield significant influence on wastewater treatment performance. Daâssi et al. (2016) used different fungal laccases for the biotransformation of BPA, and the *Coriolopsis gallica* laccase showed a more rapid oxidation rate than other fungus laccases. Also, peroxidase from mesquite could remove over 90% phenol from wastewater within 30 min (Garg et al., 2020), whilst peroxidases from horseradish (Rajesh et al., 2017), soybean, and potato (Al-Ansari et al., 2010) need more residence time to reach the same level of removal. When peroxidases were employed for azo dye methyl orange treatment, 0.5 mL crude soybean peroxidase achieved a maximum 81.4% decolorization under the conditions of 1 h incubation at 30 ℃ using 2 mM of hydrogen peroxide and 30 mg/L methyl orange at pH 5.0. Meanwhile a maximum 75.3% decolorization was observed by 1.5 mL luffa peroxidase under the conditions of 40 min at 40 ℃ using 2 mM hydrogen peroxide, and 10 mg/L methyl orange at pH 3.0 (Chiong et al., 2016). Meng et al. (2017) used three lipases, namely Lipase-I, Lipase-II, and Lipase-III for animal fat, vegetable oil, and floating grease hydrolysis. Results showed that under the hydrolysis conditions of 24 h, 40-50 ℃, and 1000-1500 μL lipase inoculum, Lipase-I and Lipase-II successfully released long-chain fatty acids in these contaminants effectively. Meanwhile a relatively low hydrolysis rate was observed in Lipase-III, so enzyme types and their sources are important for wastewater treatment.

Typically, enzymes are of great significance to life activity, and all live cells could produce enzymes for biocatalysts. Table 1 shows the typical employed enzyme sources and their targeted pollutants. Animals could act as a lipase source (Meng et al., 2017; Ning et al., 2016), but studies employing animals as enzyme sources for wastewater treatment are scarce. Some plants are important enzyme sources for wastewater treatment, especially for peroxidases (Ely et al., 2017; Garg et al., 2020; Torres et al., 2016). Fungi and bacteria

enzymes have been widely employed in wastewater treatment. For instance, hydrolytic enzymes like cellulase, amylase, lipase, and protease produced by hydrolytic microorganisms can decompose carbohydrate, fats, protein and other complex compounds which typically exist in wastewater as simple substances (Liew et al., 2020). Exocellular lipase extracted from *Candida rugosa* proved to be effective in treating triglycerides in palm oil mill effluent (Theerachat et al., 2017). Laccase from *Trametes versicolor* was used for chlorpyrifos degradation (Das et al., 2017). Enzyme production is not only restricted to a single strain. It had been reported that extracellular enzymes extracted from wastewater microbial communities had better catalyzing ability than intracellular enzyme and especially concerning antibiotic biotransformation (Zumstein & Helbling, 2019). The crude exocellular enzyme preparations of two co-cultured yeast, *Candida rugosa* and *Yarrowia lipolytica* rM-4A, could remove 98.5% triglycerides in undiluted palm oil mill effluent in 120 h (Theerachat et al., 2017). Plants, bacteria, and fungi are important enzyme sources for contaminants in wastewater removal, but it still needs more research to find proper enzymes for specific wastewater treatment.

[Insert Table 1]

2.2 Performance of enzyme-driven wastewater pollutants biodegradation

2.2.1 Oil and grease

The world's uncontrolled population growth and its demand for food and resources has led to huge amounts of meat and edible oil, but also in terms of how it is wasted. One report showed that the global oil market was worth US$83.4 billion in 2015, and it would increase to US$130.3 billion in 2024. About 203.8 million metric tons of edible vegetable oils were produced in 2018, with a consumption of 197.3 million metric tons (Luo et al., 2019). Also, animal production, slaughterhouse, and meat production experienced a soaring increase over the past few decades (Cheng et al., 2020). The flourishing meat and edible oil industries are generating a huge amount of oil and grease containing wastewater (Lee et al., 2019; Malomo

et al., 2018). These wastewaters have a high organic and inorganic load (Bustillo-Lecompte & Mehrvar, 2015), which is harmful to aquatic environments (Lee et al., 2019), even surface water (Ma et al., 2015), if discharged without efficient treatment and simply adds to the world's pollution.

Lipases, which have been commercially used, are the most employed enzymes in oil and grease containing wastewater treatment since they could decompose oil and grease into simpler free fatty acids and glycerol (Cheng et al., 2020). The good performance of lipase in oil and grease hydrolysis has been reported, for example a commercial lipase was used for floating fatty wastes from dairy and meat food-processing industries decomposition. Results showed this commercial lipase could promote the release of long-chain fatty acids especially unsaturated acids when the initial pH was adjusted to 7.0 (Pascale et al., 2019). Extracellular lipase produced by *Candida rugosa* could remove 93% triglyceride in palm oil wastewater after 48 h (Theerachat et al., 2017). However, lipase on oil and grease hydrolysis should be regarded as a pretreatment method since the transformation products (TPs) of lipase are simple free fatty acids and glycerol. These enzymatic processes still need to be combined with other methods such as activated sludge or anaerobic fermentation (Meng et al., 2017; Valladao et al., 2009) to complete the treatment.

2.2.2 Pharmaceuticals

Pharmaceuticals constitute a common organic micropollutant in the aquatic environment and have been detected in recent decades (Ternes, 1998). With the extensive use of pharmaceuticals, over 200 different pharmaceuticals active compounds have been reported in water body (Wang et al., 2016b). Anti-infectives, cardiovascular agents, central nervous system agents, metabolic agents, hormones and so on are common pharmaceuticals in wastewater (Varga et al., 2019). At present, the removal of pharmaceuticals by conventional activated sludge systems is insufficient. A report showed that only 4 out of 35 pharmaceuticals achieved over 90% removal by using activated sewage sludge from

municipal wastewater treatment plants, whereas less than 50% removal of 17 compounds was observed (Joss et al., 2006). Another report showed wastewater treatment facilities could only remove 20-90% antibiotic residues passively (Watkinson et al., 2007). Antibiotics like cephalosporins (Guo & Chen, 2015), tetracyclines, and fluoroquinolones (Becker et al., 2016) are difficult to be degraded naturally. Since residual pharmaceuticals in treated wastewater are being constantly discharged into the aquatic environment, pharmaceuticals are prevalent. They have been detected in surface, ground and even drinking water (Caban et al., 2016). Despite the low concentrations of pharmaceuticals in discharged wastewater, their bioaccumulation is minimal at best. Up to 6.5, 0.52, and 0.71 mg/L of ciprofloxacin, norfloxacin, and oxytetracycline were observed in freshwater ecosystems, respectively (Hughes et al., 2013). The ecotoxicity of some pharmaceuticals like DCF, CBZ has been reported (Ferrari et al., 2003; Lonappan et al., 2016), but the effect of most pharmaceuticals in aquatic environment is largely unknown (Stadlmair et al., 2018). The inefficient in insufficient treatment of pharmaceuticals pose a great threat to aquatic environments due to their bioaccumulation and ecotoxicological potential.

Some researchers have reported the feasibility of enzymatic processes for pharmaceuticals containing wastewater treatment, and oxidoreductases are effective enzymes for removing pharmaceuticals. For instance, Song et al. (2019) used immobilized chloroperoxidase (CPO) for antibiotics levofloxacin and rifaximin in wastewater treatment, and over 88% of both antibiotics were degraded in 30 min when the concentration of enzyme was 20 μg/mL. A laccase along with redox mediator system achieved 95% removal of CBZ under the conditions of 35 ℃, pH 6, 60 U/L laccase concentration and 18 μM mediator concentration (Naghdi et al., 2018a). It is worth noting that the enzymatic process may cause further pollution since pharmaceuticals like CBZ (Naghdi et al., 2018a) and DCF (Li et al., 2017) will be transformed into toxic byproducts. Unfortunately, about 3000 different substances have been used as pharmaceuticals ingredients, and less is known about the

environmental effects of most pharmaceuticals since the biodegradation pathways of pharmaceuticals are complex and not well understood (Stadlmair et al., 2018). The complex structures of pharmaceuticals hinder the application of enzyme-based pharmaceuticals containing wastewater treatment. Consequently, more studies on the mechanisms and pathways of enzyme-based pharmaceuticals transformation are needed.

2.2.3 Pesticides

The widespread nature of pesticides greatly threatens the world's aquatic ecosystems and human health due to their low utilization, great wastage and high residue rate (Maier et al., 2015; Marican & Durán-Lara, 2018). Pesticides are highly toxic and residual pesticides will end up in water bodies via runoff and percolation (Pal et al., 2014), wreck the water quality, impact on the metabolic, regulatory, biochemical processes of aquatic creatures (Villarroel et al., 2009), and disrupt the balance of aquatic ecosystems (Moraes et al., 2007). Currently, organophosphorus and carbamate pesticides are commonly employed pesticides (Jiang et al., 2019; Nguyen et al., 2014c) and they seriously corrupt the environment and animal health since they can act as inhibitors of cholinesterase (Sultatos, 1994). Other toxicities which are not related to cholinesterase but have life-threatening outcomes had been observed (Sultatos, 1994). The use of some high toxic pesticides such as triazophos, methamidophos, and carbofuran are restricted, but they are still in high demand, especially in developing countries (Zhang et al., 2020).

The key enzymes in pesticides decomposition also have been reported. For example, the laccase immobilized on magnetic iron nanoparticles showed more than 99% removal of chlorpyrifos in 12 h at pH 7 and 60 ℃ (Das et al., 2017). The immobilized CPO could completely degrade isoproturon (IPN) with a concentration of 26.7 mol/L within 10 min (Fan et al., 2018). The covalent immobilization of organophosphate degrading enzyme A (OpdA) on nonwoven polyester textiles could degrade small concentrations of organophosphate pesticides effectively (Gao et al., 2014). Other enzymes like organophosphorus acid

anhydrolase (OPAA) and organophosphorus hydrolases (Opns) which could decontamination pesticides had been reported (Schenk et al., 2016). As well as the mono-enzyme based pesticides degradation, the synergistic transformation of multiple enzymes could also achieve a good result. In a double-enzymes reaction for acetylcholine chloride decomposition, acetylcholinesterase could transform acetylcholine chloride (Ach) into choline, while choline was further decomposed by choline oxidase (CHO) into betaine and hydrogen peroxide (Wu et al., 2021). Since many enzymatic TPs of pesticides are not eco-friendly, combining multiple enzymes is a good way to achieve a harmless enzymatic process.

2.2.4 Personal care products

In today's world the personal care products (PCPs) are widely used in shampoos, creams, sunscreens, detergents, UV-filters which are all typical PCPs in millions of people's lives. These PCPs enter the aquatic environment directly and indirectly. On one hand, PCPs enter aquatic environments directly through recreational activities like swimming and bathing outdoors (Balmer et al., 2005). Conversely, the insufficient removal of these contaminants by wastewater treatment plants and human activities like showering, cleaning, laundering leads to an indirect input of PCPs. Triclosan (TCS), UV-filters like octocrylene, benzophenone-3, oxybenzone are commonly used as organic compounds in PCPs (Sendra et al., 2017) which are toxic (Lee et al., 2020; Sheng et al., 2021). It has been reported that *Trametes versicolor* (ATCC 7731), which is an extracellular enzyme extract (predominantly laccase) could degrade oxybenzone effectively in the presence of a redox mediator (Nguyen et al., 2014b). Laccase from white-rot fungus *Coriolopsis polyzona* combined with mediator 2,2′ -azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) could remove TCS effectively after one hour's treatment under the conditions of 40 °C and pH 4 (Cabana et al., 2007). Despite the great potential of enzyme-based PCPs treatment, more studies are needed since reports in this area are still scarce.

2.2.5 Industrial chemicals

2.2.5.1 Phenols and phenolic compounds

As an important material in industry, phenols and phenolic compounds play a significant role in textile, paper, plastic, pharmaceutical, and other industries (Jun et al., 2019). These compounds endanger humans and aquatic organisms, leading to a great threat to the environment, even at a low concentration. At present, enzyme-based phenol and phenolic compounds' removal have been studied. Complete removal of 0.4 mM phenol was achieved by an immobilized laccase after 40 min treatment under the conditions of 35 ℃, pH 4.5 (Fathali et al., 2019). Peroxidases are effective enzymes in the transformation of phenolic compounds since their active sites, namely haem cofactor and redox-activated cysteine/selenocysteine residues, are easy to access (Garg et al., 2020). Research showed that immobilized peroxidases could remove 99.9% 1 mM phenol after 5 h treatment under the conditions of room temperature and pH 7.0 (Wang et al., 2016a). Similarly, Rezvani et al. (2015) immobilized soybean seed coat powder as soybean peroxidase for the removal of phenol from refinery wastewater in a packed bed bioreactor. Up to 97% phenol removal was observed in the best possible conditions, these being 1 mM initial phenol, 56 ℃, 14 mM H_2O_2 concentration, and 5.5 mL/min flow rate.

2.2.5.2 Dyes

With the flourish of textile and apparel industries, more and more dyes containing wastewater are produced due to incomplete fixation on the fabric (Mouni et al., 2018). Printing, leather, plastic industries are also sources for dye containing wastewater production (Zare et al., 2015). The structures of most dyes are complex and have characteristic high stability, which resists biodegradation naturally (Przystas et al., 2018). Synthetic dyes that are highly colored and visibility could affect the food chain, and these compounds could be transformed into harmful products, posing a great threat to aquatic environment and the organisms living in it (Arciniega Cano et al., 2017; Jun et al., 2019). At present, enzyme-based dyes treatment mainly divided into biodegradation, decolorization, and detoxification

(Bilal et al., 2017). Oxidoreductases are the most used enzymes for the treatment of dye containing effluents. Horseradish peroxidase immobilized on cross-linked polyacrylamide gel was effective on an azo dye methyl orange degradation, and the maximum degradation of methyl orange (93.5%) was recorded under pH 6 (Bilal et al., 2018). The laccase could remove two sulfonamides effectively in the presence of 1- hydroxybenzotriazole (HBT) as mediator, and a significantly decreased toxicity of laccase-treated sulfonamide solution was also observed according to a micro toxicity study on the inhibition of bacterial growth (Rahmani et al., 2015). However, research concentrating on real dye containing wastewater is necessary since nearly all wastewater was synthetic and conducted in laboratories, which is not conducive to real-life applications.

2.2.5.3 Plastics

Plastics are prevalent in our lives and especially in packaging which creates a lot of convenience but also a product that cannot be broken down. Polyethylene (PE), polyamide (PA), polyethylene terephthalate (PET), polystyrene (PS), polyvinylchloride (PVC), polyurethanes (PUR) and polypropylene (PP) are commonly used plastics (Danso et al., 2019). However, plastics like ethylene and propylene are derived from fossil hydrocarbons, which resist biodegradation. Thus, they will accumulate rather than decompose in environment (Geyer et al., 2017). The widespread production of plastics and low recycling rate also makes things worse. In 2015, over 6.3 billion tons of plastics have been generated and only 9% was recycled (Geyer et al., 2017). Residual plastics damage the environment significantly due to their toxicity and accumulation characteristics (Zurier & Goddard, 2021). Plastics are widespread in aquatic environments and especially in the world's oceans (Geyer et al., 2017), and even in drinking water (Mintenig et al., 2019). Despite plastics being resistant to biodegradation, enzymes involved in the decomposition of plastics like PET hydrolase and tannase, MHETase have been found (Danso et al., 2019). Unfortunately, the efficiency of enzyme-based plastics degradation is poor. It will take weeks to hydrolyze PET completely by

PETase from *Ideonella sakaiensi* (IsPETase), which is an efficient and specific depolymerase (Zurier & Goddard, 2021).

Recently, studies focusing on increasing the thermostability and enzyme activity of PETase (Son et al., 2019; Ma et al., 2018) have reported great success. At present, enzymes which involved in PA and PET degradation have been found, while less was known about PVC, PP, PE, and PUR hydrolase enzymes, even possible pathways (Danso et al., 2019). However, the degradation of these plastics is possible by enzymes since many microbes which could degrade plastics have been found (Danso et al., 2019). Enzyme-based plastic degradation is feasible and promising, but further research like increasing enzyme diversity and its efficiency is needed for the effective application in plastics that get caught up in wastewater treatment.

3. Enzymatic transformation products monitoring

3.1 Necessity

The pathways of enzymatic processes are complex and sometimes enzymatic TPs pose great threats to the aquatic environment, especially when substrates are micropollutants (Table 2). For instance, CBZ, a recalcitrant pharmaceutical usually detected in wastewater, was transformed into 10,11-dihydro-10,11-dihydroxy-carbamazepine (CBZD), 10,11-dihydro-10,11-epoxy- carbamazepine (CBZE) and acridone by laccase (Ji et al., 2016). CPO could convert CDF into either monohydroxylated or dihydroxylated due to the diverse catalytic activity of CPO (Li et al., 2017). The chlorpyrifos TPs by laccase immobilized on magnetic iron nanoparticles were 2,4-bis(1,1 dimethylethyl) phenol and 1,2 benzenedicarboxylic acid, bis(2-methyl propyl) ester (Das et al., 2017). Thus, the degradation of parent substances does not mean the end of treatment, so the TPs problem are negligible, especially their toxicity potential. The toxicity relationships between TPs and parent substances vary from parent substances and mediators (Table 3). For instance, the TPs of

lincomycin by chloroperoxidase were less toxic than the original lincomycin molecule (Zhu et al., 2020), and the TPs of TCS by soybean peroxidase are nontoxic (Li et al., 2016). The treated effluent of some Trace organic contaminants (TrOCs) biodegraded by laccase with mediator ABTS revealed higher toxicity, whereas the effluent highlighted similar toxicity to influent when employing vanillin (VA) or HBT as the mediator (Ashe et al., 2016). As noted above, the TPs of some compounds contain substances harmful to the environment, whereas many studies only focus on parent pollutants removal and neglected their TPs (Becker et al., 2016; Bettin et al., 2019; Sutar & Rathod, 2016). To date, less is known about enzymatic TPs and the reaction mechanisms of some pollutants. Therefore, enzymatic TPs should be monitored, not only for learning more about pathways and probable reaction mechanisms of enzymatic processes, but also to curtail this threat to the environment.

[Insert Table2]

3.2 Identification methods

3.2.1 Transformation products identification

Typically employed TPs identification methods are shown in Table 2. Nuclear magnetic resonance (NMR), mass spectrometer (MS), and their based methods are common enzymatic TPs detection methods (Stadlmair et al., 2018). NMR is a method which can determine molecular structure accurately (Elyashberg, 2015) but with low sensitivity and flexibility (Lee et al., 2014), whereas MS-based identification methods are the most used technologies since they are able to detect substrates, products, and even ionized intermediates at the same time (Stadlmair et al., 2018). Thus, MS-based methods are more applicable. For instance, two peroxidases - soybean peroxidase and chloroperoxidase were employed for dye thioflavin (ThT) degradation. It was found that the TPs of the two peroxidases were different after High Performance Liquid Chromatography (HPLC) analyses, and this finding was consistent with LC-MS-MS studies (Alneyadi & Ashraf, 2016). One study suggested direct infusion MS to monitor the degradation of some pharmaceuticals and their byproducts by horseradish

peroxidase and laccase from *Pleurotus ostreatus* successfully (Stadlmair et al., 2017). As a fast and sensitive method, MS and MS-based approaches could offer an accurate analysis of enzymatic TPs and the mixture effects, which is of great significance to understand the mechanisms of enzymatic processes (Stadlmair et al., 2018). Of course, other methods like fourier transform infrared spectroscopy (FT-IR), scanning electron microscopy (SEM), and atomic force microscopy (AFM) were also employed for analyzing enzymatic TPs (Fathali et al., 2019).

3.2.2 Transformation products toxicity assay

When evaluating the contamination ability in water of enzymatic TPs, toxicity assay is necessary. Bacteria, fungi, algae, and plant seeds are typical employed indicator creatures for toxicity assay (Table 3). The toxicity data is acquired by monitoring the growth inhibition to these bioindicators. Bioluminescence inhibition test, or Microtox assay, is a common toxicity assay method which uses bacteria *Photobacterium phosphoreum* (Marco-Urrea et al., 2009) or *Aliivibrio fischeri* (Becker et al., 2016; Gros et al., 2014) as indicator organisms. Some Gram-positive and Gram-negative bacterial like *Bacillus subtilis*, *Bacillus megaterium*, *Escherichia coli*, and *Saccharomyces* were cultured by treated and untreated solutions, while the toxicity of wastewater was assessed by analyzing the growth of these bacteria (Becker et al., 2016; Rahmani et al., 2015). Except for bacteria, yeast Estrogen Screen assay (YES) is a method employing yeast as a bioindicator for wastewater toxicity assay (Naghdi et al., 2018a). As for other bioindicators, *Daphnia magna* was used as a test organism for dye Cibacron Blue 3GA and its byproducts toxicity assessment (Bayramoglu et al., 2019). The toxicity of TCS in water and its biodegradation byproducts were assessed through growth inhibition of *Scenedesmus obliquus* (Li et al., 2016). Al-Maqdi et al. (2018) used soybean peroxidase for SMX biodegradation and the phytotoxicity studies carried out using *Lactuca sativa* seeds.

The identification and toxicity assay of enzymatic TPs are greatly significant to enzyme-based wastewater treatment, especially when the enzymatic byproducts have toxicity

potential. Despite this subject having drawn many researchers' attention, more studies need to be done for the further application of enzyme in wastewater treatment.

[Insert Table3]

4. Enzyme-related wastewater treatment systems

4.1 Free enzyme systems

In free enzyme systems, enzymes can be divided into crude enzymes and purified enzymes. Both kinds of enzymes performed well in removing pollutants (Lloret et al., 2010; Zhang & Geissen, 2010). However, purified enzymes are expensive since methods like membrane separation, size exclusion chromatography, and column chromatography are required for enzyme purification (Naghdi et al., 2018b; Zhu et al., 2020). Purified enzymes were worse at removing wastewater pollutants than crude enzymes in some cases since crude enzymes contain mediators (Tran et al., 2010). It has been reported that crude laccase extracted from *Trametes versicolor* could remove naproxen (NPX) completely (Tran et al., 2010), whereas 60% removal was achieved by commercial laccase extracted from *Myceliophthora thermophila* (Lloret et al., 2010). However, that does not mean crude enzymes are superior to purified enzymes. The mediators and unspent nutrient in crude enzymes will cause further contamination (Becker et al., 2016; Nguyen et al., 2016). Since enzymes are expensive, the large demand for enzymes which could not be recycled in wastewater treatment makes these systems not feasible, and the remaining enzymes should be removed at the end of treatment. Much work needs to be done to solve the cost and/or contamination problems if using free enzymes for wastewater treatment.

4.2 Immobilized enzyme systems

Immobilized enzymes are often used and studied enzymatic systems. In these systems, enzymes are combined with or physically attached to various matrices, and the catalysis abilities of enzymes are maintained at the same time. Since the enzymes are attached to the matrix, these systems are easy to be controlled because enzymes could separate with

wastewater easily. Also, immobilized enzymes could be reused for many times, which diminishes further pollution and greatly curtails the treatment cost. Nevertheless, immobilization reduces the loss of enzyme activity, prolongs the service life of enzymes, and enhances resistance to pH and temperature rather than cell-free enzymes (Chang et al., 2015; Fan et al., 2018; Quintanilla-Guerrero et al., 2008). It is worth mentioning here that in these systems, immobilization methods and support materials are of great significance to the cost of enzymatic processes, enzyme activity, treatment efficiency, and enzymatic kinetics (Durán et al., 2002; Quintanilla-Guerrero et al., 2008).

Typically employed immobilization methods include adsorption, entrapment, covalent attachment, and cross-linking. These immobilization methods can be divided into physical and chemical methods. Generally, chemical immobilization provides good enzyme stability but will diminish enzyme activity since the covalent bonds formed between enzyme and support materials disturb the native structure of enzyme, whereas physical immobilization and entrapment methods wield less influence on enzyme structure but nonetheless show poor enzyme stability (Durán et al., 2002). However, that finding is not absolute. In physical methods, enzyme stability is improved by modifying the surfaces of carriers. For instance, Naghdi et al. (2017) used a functionalized nanobiochar which was treated by H_2SO_4/HNO_3 (50:50, v/v) as support for laccase immobilization. Improved storage, pH and stability of immobilized laccase were found compared to when using free laccase. Despite physical methods having less influence on enzyme activity, the enzyme activity loss is not to be ignored. It has been reported that 30-40% enzyme activity will be lost in 3 cycles reaction (Naghdi et al., 2018b). As for the immobilization process, chemical immobilization methods are more complex than physical methods since the former one related to chemical reactions. Immobilization methods may affect the performance of the enzymatic process. It had been reported that two immobilized peroxidases had different effects on phenol removal under the same conditions (Quintanilla-Guerrero et al., 2008). There was a similar phenol removal

when the initial phenol concentration was 0.2 mM to 0.8 mM, while the alginate-entrapped turnip peroxidase exhibited better phenol removal than the covalently immobilized turnip peroxidase when the initial phenol concentration was 1.4 mM (Quintanilla-Guerrero et al., 2008). As stated above, immobilization methods should be determined by application demand.

During immobilization processes, reagents toxicity should be considered to avoid environmental problems (Durán et al., 2002). Material cost, toxicity, surface area, and mechanical strength are the important criteria for matrix selection (Naghdi et al., 2018b). Support materials can be divided into three types, namely inorganic matrix, organic matrix, and organic-synthetic matrix. Inorganic supports like silica gel and activated carbon are stable and have good diffusion and flowing characteristics (Hettiarachchy et al., 2018). Also, resistance to microbial contamination and usually affordable are their benefits (Hettiarachchy et al., 2018). Proteins and carbohydrates such as cellulose or chitin belong to the organic matrix, which is inexpensive and available in large amounts (Hettiarachchy et al., 2018). However, these supports are easily contaminated by microbial and with limited diffusion and flowing abilities. Organic-synthetic matrix, supports which are easy to access and hard to be contaminated by microbial lifeforms are widely used in enzymatic procedures (Tischer & Wedekind, 1999). These supports, however, may cause enzyme loss and reduce enzyme activity due to the hydrophobicity or water-repellent property of supports (Tischer & Wedekind, 1999).

Many successful studies have been conducted on immobilized enzymes and their role in wastewater treatment. Immobilized laccase had high decolorization efficiency in anthraquinone dye Remazol Brilliant Blue R (Daâssi et al., 2014). A 90% degradation of bisphenol A (BPA) was observed by using ligninolytic enzymes encapsulated on polyacrylamide hydrogel and pectin after treatment lasting 8 h (Gassara et al., 2013). Sun et al. (2017) used ZnO nanowires/macroporous SiO_2 composite as a carrier to immobilize horseradish peroxidase by *in-suit* cross-linking method for azo dyes decolorization. Results

showed the decolorization rate of Acid Blue 113 was 95.4%, while the Acid Black 10 BX was 90.3% under the conditions of 30 ℃, 35 min treatment, initial dye concentration 50 mg/L.

Primožič et al. (2020) used cross-linked enzyme aggregates and magnetic cross-linked enzyme aggregates for laccase immobilization to remove DCF in wastewater, and their DCF removal capacities were 15.6 ± 0.4 $\mu g/g_{laccase}$ and 13.6 ± 0.4 $\mu g/g_{laccase}$, respectively. Chang et al. (2015) discovered that immobilized horseradish peroxidase could remove 83% 2,4-dichlorophenol in synthetic wastewater in 120 min. However, the preparation of immobilized enzymes needs additional work and analysis of the carrier costs. The matrix may diminish enzymes' activity since they will slightly change their structure and may block the active site (Barrios-Estrada et al., 2018). Some matrices have a positive effect on enzymes. One study indicated the presence of pectin significantly enhanced the activity of ligninolytic enzymes (Gassara et al., 2013). Therefore, immobilization methods should be selected according to the research objective, and more suitable immobilization materials need to be studied to achieve a better system which considers cost, stability, and enzyme activity.

4.3 Enzyme mediators-related systems

Enzyme mediators are stable low weight compounds which could work as an electron shutter between enzyme and substrates (Kim & Nicell, 2006). Mediators could broaden the range of enzyme substrates by producing highly reactive radicals (Naghdi et al., 2018b). Also, these small molecules facilitate the enzymatic process and improve the efficiency in removing target pollutants (Munk et al., 2018). Typically, mediators served to improve the oxidation process when the redox potential of enzyme was higher than that of the substrate (Madhavi & Lele, 2009).

Enzyme mediators can be divided into natural mediators and synthetic mediators. syringaldehyde (SA), AS, VA, acetovanillone, methyl vanillate and 4-hydroxybenzyl alcohol (HBA) are natural mediators, whilst ABTS, HBT, and violuric acid (VLA) are of synthetic

554 origin (Canas & Camarero, 2010). They have the ability to improve enzyme activity and the
555 enzymatic process.

556 Bibi et al. (2019) used laccase for orange G dye decolorization, results showed mediator
557 could improve both laccase activity and orange G dye removal. 61.45% orange G dye
558 removal was achieved at the laccase activity of 156.78 IU/mL, whilst a laccase activity of
559 279.27 IU/mL, 85% orange G dye decolorization, up to 97% orange G dye removed was
560 observed in the laccase-mediator system. Since it is not a reactive substrate for laccase, less
561 than 30% CBZ was degraded by free laccase after 24 h, while more than 82% CBZ removal
562 was achieved in the presence of mediator ABTS (Naghdi et al., 2018a). When using
563 horseradish peroxidase for the removal of tetracycline, the maximal degradation of
564 tetracycline amounted to about 53% in pure enzyme system, whilst more than 95%
565 tetracycline was removed in the mediator added system, and the reaction time of these
566 outcomes fell from 1 h to 0.5 h (Leng et al., 2020). Becker et al. (2016) used immobilized
567 laccase for 38 antibiotics biodegradation. The results showed laccase without the mediator did
568 not remove a significant amount of antibiotics, while 32 out of 38 antibiotics demonstrated
569 more than 50% removal after adding mediator SA.

570 The concentration of mediators has a great influence on pollutants' removal. Naghdi et al.
571 (2018a) showed the CBZ removal was improved from 47% to 95% when the mediator
572 concentration rose from 6 μM to 14 μM. Mediators could improve the efficiency in removing
573 pollutants, but these systems are not always the best. For instance, Ji et al. (2016) used laccase
574 for CBZ removal, and the results showed p-coumaric acid (PCA) as a mediator could remove
575 60% in 96 h, while 71% CBZ removal was observed in a membrane hybrid reactor containing
576 immobilized laccase. Besides, different mediators may have varied effects on a same reaction,
577 leading to a different TPs toxicity (Ashe et al., 2016). The mixture of mediators may also
578 cause poor performance. It has been reported that the separate addition of mediators SA and
579 VLA could improve the decomposition rate of TrOCs by laccase, while it would decrease the

degradation of some pharmaceuticals like CBZ, IBF, and primidone when using their mixture (Asif et al., 2018). It is worth noting here that mediators may change the mechanism of the enzymatic process, leading to different TPs, which brings difficulties to controlling the enzymatic process (Varga et al., 2019). One report showed the addition of mediator SA would increase the effluent toxicity when employing laccase for TrOCs treatment. Despite this being related to the dose of SA, it is not clear whether this phenomenon was caused by the interaction between laccase and mediator or the toxic enzymatic TPs (Nguyen et al., 2016).

Most synthetic mediators are expensive and will cause further contamination (Stadlmair et al., 2018; Varga et al., 2019). Furthermore, the large mediator consumption hinders the application of this method. A research study using ABTS, SA, and acetosyringone (ACE) as mediators for laccase to biodegrade antibiotic SMX showed the consumed mediator to pollutant ratio are 1.1-1.6 (Margot et al., 2015). Despite mediators able to improve the enzymatic process, enzyme activity was inhibited (Ashe et al., 2016; Hata et al., 2010). Nevertheless, the use of mediators may cause new environment problems since they are toxic (Asif et al., 2018; Becker et al., 2016; Grandclement et al., 2017), but this is linked to mediator dose (Nguyen et al., 2014a). Despite the toxicity of mediators, they are necessary when treating compounds like TCS (Cabana et al., 2007) and oxybenzone (Nguyen et al., 2014b). Thus, finding a low-cost and proper mediator concentration to improve the stability and efficiency of enzymatic process are hugely important.

5. Future perspectives

As mentioned above, enzymatic processes have great potential in wastewater treatment. Enzyme types and sources play a significant role in enzymatic processes. Thus, more studies are needed to explore the performance of enzymes on specific wastewater. Enzyme-based wastewater treatment has shown its feasibility on resistant pollutants removal. However, many studies were conducted under experimental/laboratory conditions and the wastewater

was synthetic (Table 1), which is not conducive to practical applications. More research on real wastewater and larger-scale experiments are therefore urgently needed. Some resistant pollutants like pharmaceuticals and plastics cannot be degraded well by enzymes, if at all. Mediators are one feasible way to enhance enzymatic performance, but the toxicity of mediators cannot be ignored. Although some researchers are devoted to finding natural mediators which could eliminate the negative outcomes, it is better to minimize the use of mediators. Genetic engineering is a feasible way to improve the catalyst ability of enzymes, which is conducive to the decomposition of these stubborn contaminants. Finding more new enzymes to catalyze the degradation of resistant pollutants is also a feasible way.

It is worth to mention that the enzymatic TPs are of great importance to wastewater treatment due to their toxicity potential. Many researchers only focused on the transformation of parent substrates, while the byproducts were neglected. Fortunately, the TPs have garnered more attention in recent years, which helps us to learn more about enzymatic processes mechanisms, as well as the application of enzymatic wastewater treatment. In enzyme-related wastewater treatment systems, free enzyme systems can achieve good treatment performance under certain circumstances, but these systems may not cost-effective and/or eco-friendly. It is necessary to find high-efficiency and commercial enzymes to reduce the cost. Presently, most studies employed free enzyme systems for wastewater treatment only focused on the removal of targeted pollutant, while the effect of residual enzyme additives is ignored. Hence, measures are needed to eliminate the negative effects of the enzyme additives. Regarding immobilized enzymes, the immobilization carriers and methods are important to how well the immobilized enzyme performs. The immobilization processes are more or less accompanied enzyme activity loss, and immobilized enzymes have a short lifetime. Consequently, finding proper immobilization supports and methods for enzyme immobilization to reduce the loss of enzyme activity and enhance enzyme reusability is crucial. To this point, supports surface modification and functionalization, advanced support materials study are ways to find suitable

carriers. Better understanding of enzyme characteristics such as structures, function mechanisms can also boost enzyme immobilization processes. Specificity is one of the advantages of enzyme-based wastewater treatment, but it is also a limitation since most research only discusses the removal of one or one kind of pollutant from synthetic wastewater by a specific enzyme. In contrast, the real wastewater is complex and one enzyme could not degrade all pollutants, not to mention the enzymatic TPs problems. Therefore, it is important to develop immobilization systems which enable the cooperation of multiple enzymes. Since enzymes only transform one substrate into simpler compounds, this method cannot remove pollutants completely by itself. Thus, the combination of enzymatic degradation with other techniques like activated sludge or anaerobic fermentation is a good option to start with.

6. Conclusion

Enzymes play a significant role in contaminants' decomposition, and enzymatic bioprocesses have demonstrated their priority in wastewater treatment. Enzymatic processes are promising in the biodegradation of resistant pollutants like grease, oil, pharmaceuticals, personal care products, pesticides, and industry chemicals. However, this technology could only act as a pretreatment method and needs to be combined with other methods to achieve complete treatment since enzymes only transform complex compounds into simpler substances. There are currently many obstacles for the application of this technology, and investigations into real wastewater and larger-scale applications, enzymatic TPs monitoring, enzyme abilities enhancement, and multiple enzyme systems constructions are required.

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Figure Captions

Fig. 1. Typical resistant pollutants in wastewater and their classification.

Table Captions

Table 1 Typical employed plants and animals (a), bacteria (b), fungi (c) as enzyme sources and their targeted pollutants in wastewater treatment.

Table 2 Enzymatic TPs of typical resistant pollutants and methods to identify TPs.

1076 Table 3 Enzymatic TPs toxicity and toxicity assay creatures.

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1080 **Table 1**

1081 (a)

Specific enzyme sources	Enzyme types	Targeted pollutants	Wastewater type	References
Prosopis juliflora	Peroxidase	Phenols	Textile, leather	(Garg et al., 2020)
Horseradish	Peroxidase	Phenols, methyl orange, TrOCs	Textile, leather, synthetic	(Bilal et al., 2018; Garg et al., 2020; Stadlmair et al., 2017)
Allium sativum	Peroxidase	Phenols	Leather	(Diao et al., 2011)
				(Diao et al., 2011)
Ipomoea batatas	Peroxidase	Phenols	Leather	(Diao et al., 2011)
				(Diao et al., 2011)
Raphanus sativus	Peroxidase	Phenols	Leather	
Sorghum bicolor	Peroxidase	Phenols	Leather	
Turnip	Peroxidase	Phenolic compounds	Coffee processing	(Torres et al., 2016)
Soybean	Peroxidase	Phenolic compounds, methyl orange dye	Coffee processing, Synthetic	(Torres et al., 2016; Chiong et al., 2016)
Potato pulp	Peroxidase	Phenol	Synthetic	(Kurnik et al., 2015)
Luffa acutangula	Peroxidase	Methyl orange dye	Synthetic	(Chiong et al., 2016)
Pig pancreas	Lipase	Crude fat	Swine slaughterhouse	(Ning et al., 2016)
Porcine Pancreatic	Lipase	Grease	Pig manure	(Meng et al., 2017)

1082 (b)

Specific enzyme sources	Enzyme types	Targeted pollutants	Wastewater type	References
Pseudomonas aeruginosa	Peroxidase	Dye	Textile	(Darwesh et al., 2019)
Yarrowia lipolytica rM-4A	Laccase	Phenolic compounds	Palm oil mill	(Theerachat et al., 2017)
Candida rugosa	Laccase	Phenolic compounds	Palm oil mill	(Theerachat et al., 2017)
Candida rugosa	Lipase	Triglyceride	Palm oil mill	(Theerachat et al., 2017)
Staphylococcus	Lipase	Grease	Slaughterhouse	(Maha Affes et al., 2017)

xylosus				
Pseudomonas aeruginosa UKHL1	Lipase	Oil	Oily	(Patel et al., 2020)
Aeromonas hydrophila	Ligninolytic enzyme, including laccase and LiP enzymes	Crystal violet dye	Synthetic	(Bharagava et al., 2018)
Ideonella sakaiensis	PETase	PET	---	(Ma et al., 2018)

1083 (c)

Specific enzyme sources	Enzyme types	Targeted pollutants	Wastewater type	References
Phanerochaete chrysosporium	Lignin peroxidase	CBZ and DCF	Synthetic	(Zhang & Geissen, 2010)
Trametes versicolor	Laccase	Phenol, SMX and IPN, CBZ, chlorpyrifos, BPA	Synthetic	(Fathali et al., 2019; Ji et al., 2016; Das et al., 2017; Margot et al., 2015; Daâssi et al., 2016)
Aspergillus oryzae	Laccase	DCF	Synthetic	(Sutar & Rathod, 2016)
Coriolopsis gallica (BS54)	Laccase	BPA	Synthetic	(Daâssi et al., 2016)
Bjerkandera adusta (11B)	Laccase	BPA	Synthetic	(Daâssi et al., 2016)
Aspergillus oryzae	Laccase	TrOCs	Synthetic	(Asif et al., 2018; Nguyen et al., 2015)
Pleurotus ostreatus	Laccase	TrOCs	Synthetic	(Ashe et al., 2016; Stadlmair et al., 2017)
Coriolopsis gallica	Laccase	Synthetic dyes	Synthetic	(Daâssi et al., 2014)
Pycnoporus sanguineus	Laccase	BPA	Synthetic	(Barrios-Estrada et al., 2018)
Caldariomyces fumago	CPO	Lincomycin	Synthetic	(Zhu et al., 2020)
C. fumago	CPO	Pesticide, antibiotics	Synthetic	(Fan et al., 2018; Song et al., 2019)
Aspergillus	Lipase	Grease	Pig manure	(Meng et al., 2017)
Candida	Lipase	Grease	Pig manure	(Meng et al., 2017)
Thermomyces lanuginosus	Lipase	Tributyrin	Synthetic	(Jurado et al., 2008)

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Table 2

Compounds	Enzyme	Mediator	TPs	Identification methods	References
Lincomycin	CPO	---	3-chloro-1,4-dimethylpyrrolidine-2-carboxamide; 2-(1-amino-2-hydroxypropyl)-6-(methylation) tetrahydro-2H-pyran-3,4,5-triol	HPLC-MS	(Zhu et al., 2020)
CBZ	Laccase	PCA	CBZD, CBZE, and acridone	LC-MS	(Ji et al., 2016)
Cibacron Blue 3GA dye	Laccase	---	11 different compounds	MALDI-ToF-MS	(Bayramoglu et al., 2019)
TCS	Soybean peroxidase	---	Seven different compounds	LC-MS	(Li et al., 2016)
TCS	Horseradish peroxidase	---	Seven different compounds	LC-MS	(Li et al., 2016)
ThT	Soybean	HOBT	3-methylhex-4-enoic	LC–MS-MS	(Alneyadi

	Peroxidase		acid		& Ashraf, 2016)
ThT	CPO	---	Chlorinated ThT	LC–MS-MS	(Alneyadi & Ashraf, 2016)
BPA	Laccase	HBT	Tartaric acid, Pyroglutamic acid	GC-MS	(Daâssi et al., 2016)
BPA	Laccase	---	β-hydroxybutyric acid	GC-MS	(Daâssi et al., 2016)
CBZ	Laccase	ABTS	CBZD and CBZE	LDTD- MS and LDTD-MS-MS	(Naghdi et al., 2018a)
Chlorpyrifos	Laccase	---	2,4-bis(1,1 dimethylethyl) phenol and 1,2 benzenedicarboxylic acid	GC-MS	(Das et al., 2017)
DCF	Laccase	---	4-(2,6-dichlorophenylamino)-1,3-benzenedimethanol	NMR	(Marco-Urrea et al., 2010)

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1124 **Table 3**

Compounds	Enzyme	Mediator	TPs toxicity	Toxicity assay creatures	References
SMX	Peroxidase	---	Less toxic	*Lactuca sativa* seeds	(Al-Maqdi et al., 2018)
Phenols	Mesquite peroxidase	---	Non-toxic or less toxic	*Brassica juncea* seeds	(Garg et al., 2020)
Orange G dye	Laccase	*Punica granatum* peel extracts	Less toxic	*Trigonella foenum-graecum* L seeds	(Bibi et al., 2019)
TCS triclosan	Soybean perxoidase	---	Non-toxic	*Scenedesmus obliquus*	(Li et al., 2016)
Tartrazine	Laccase	ABTS	Less toxic	*Pseudokirchneriella subcapitata*	(Blanquez et al., 2019)
Lincomycin	CPO	---	Less toxic	*Chlorella pyrenoidosa*	(Zhu et al., 2020)
SMX and IPN	laccase	ABTS or SA or AS	Less toxic	*Pseudokirchneriella subcapitata*	(Margot et al., 2015)
CBZ	Laccase	ABTS	Less toxic	Yeast	(Naghdi et al., 2018a)
Ketoconazole	Laccase	HBT	Less toxic	*Pseudokirchneriella subcapitata*, *Candida albicans*, *Cryptococcus neoformans*, and *Saccharomyces cerevisiae*	(Yousefi-Ahmadipour et al., 2016)
CBZ	Laccase	PCA	Less toxic	*C. marina* (CMPL01) and *M. aeruginosa* (PCC 7806)	(Ji et al., 2016)
TrOCs	Laccase	---	Non-toxic	Bacterial Luminescence Toxicity Screen	(Nguyen et al., 2016)
TrOCs	Laccase	SA	Non-toxic, more toxic, or less toxic, varies from the dose of SA	Bacterial Luminescence Toxicity Screen	(Nguyen et al., 2016)
TrOCs	Laccase	ABTS	more toxic	*Photobacterium leiognathi*	(Ashe et al., 2016)
TrOCs	Laccase	VA or	The variation of	*Photobacterium*	(Ashe et al.,

		HBT	toxicity was not significant	*ietognatni*	2016)
STZ and SMZ	Laccase	HBT	Less toxic	four Gram-negative bacterial strains and two Gram-positive bacterial strains	(Rahmani et al., 2015)
Methyl orange	Horseradish peroxidase	---	Less toxic	*A. salina* and *D. magna*, erythrocyte, *T. aestivum*	(Bilal et al., 2018)
Phenolic compounds	Horseradish peroxidase	---	Less toxic	Human cervix carcinoma cells, human hepatoma cells and human breast adenocarcinoma cells	(Wang et al., 2016a)
Cibacron Blue 3GA	Laccase	---	Initial TPs are more toxic	*Daphnia magna, Chlorella vulgaris*	(Bayramoglu et al., 2019)