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ENZYME-BASED BIODEGRADATION OF HAZARDOUS POLLUTANTS – AN OVERVIEW

Ishtiaq Ahmed[1], Hafiz M. N. Iqbal[2,]*, Kuldeep Dhama[3]

[1]School of Medical Science, Gold coast campus, Griffith University, Southport QLD 4222, Australia
[2]Tecnologico de Monterrey, School of Engineering and Sciences, Campus Monterrey, Ave. Eugenio Garza Sada 2501, Monterrey, N.L., CP 64849, Mexico;
[3]Division of Pathology, ICAR-Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh, India;

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ABSTRACT

There has always been a paramount concern over the widespread occurrences of various pollutant types, around the globe. With ever increasing scientific knowledge, socio-economic awareness, human health problems and ecological apprehensions, people are more concerned about the widespread environmental pollutants. Thus, the occurrences of newly identified pollutants so-called “emerging pollutants – EPs or emerging contaminants – ECs” in our main water bodies is of continued and burning concern worldwide. The undesirable EPs/ECs are being discharged knowingly/unknowingly with/without partial treatments into the aquatic environments that pose serious health issues and affect the whole living ecosystem. So far various approaches have been developed for the degradation of environmental pollutants to decrease their impact on the environment and are divided into three broad categories i.e. (1) physical, (2) chemical and (3) biological. Based on the literature evidence, many previous or ongoing studies have focused on contaminants degradation potentialities of the above mentioned three possible categories. However, the experimental evidence is lacking to enable specific predictions about EPs/ECs mechanistic degradation fate across various in-practice systems. This study overviews the biological degradation at large and enzyme-based degradation of hazardous pollutants in particular. Towards the end, the novel characteristics and unique enzyme system of White Rot Fungi (WRF) are also discussed to present their potentialities and implementation against a broader spectrum of EPs/ECs.

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* Corresponding author

E-mail: hafiz.iqbal@my.westminster.ac.uk; hafiz.iqbal@itesm.mx (Hafiz M. N. Iqbal)

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1 Introduction

The hazardous contaminants/pollutants which are dangerous and toxic in nature mainly originate from various industrial sectors (Asgher et al., 2016; Chatha et al., 2017). Thus, environmental pollution is rapidly expanding with increased development in the entire industrial sector. Water is contaminated by an ample variety of contaminants which is a significant threat to the whole living environment and constituting as impediment to free penetration of light in water hence affecting photosynthesis capability of aquaculture plants (Romero et al., 2006; Asgher et al., 2012a; Asgher et al., 2013; Asgher & Iqbal 2013; Iqbal & Asgher 2013; Bilal et al., 2017a; Bilal et al., 2017b; Bilal et al., 2017c; Bilal et al., 2017d). Owing to their health hazard nature and severe danger to the living ecosystem, research is underway around the globe to address or tackle this problematic issue with utmost care.

Herein, we reviewed the biological degradation at large and enzyme-based degradation of hazardous pollutants in particular. Towards the end, the novel characteristics and unique enzyme system of White Rot Fungi (WRF) are also discussed to present their potentialities and implementation against a broader spectrum of hazardous contaminants/pollutants including emerging pollutants/emerging contaminants (EPs/ECs).

2 Emerging pollutants/emerging contaminants (EPs/ECs)

According to the US Environmental Protection Agency (US EPA), the EPs/ECs are defined as: *"chemicals and other substances that have no regulatory standard, have been recently "discovered" in natural streams (often because of improved analytical chemistry detection levels), and potentially cause deleterious effects in aquatic life at environmentally relevant concentrations"* (Deblonde et al., 2011). There has been increasing apprehension over life-threatening pollutants so-called "emerging pollutants – EPs or emerging contaminants – ECs," which are toxic, carcinogenic, and mutagenic in nature. The reported consequences of many hazardous compounds including EPs/ECs include endocrine disruption, genotoxicity, chronic ecotoxicity, encouragement of antibiotic resistance, and uptake into the food chain, etc. (Ding & He 2010; Arnold et al., 2013; Norvill et al., 2016). In 1996, the Food Quality Protection Act (FQPA) and amendments to the Safe Drinking Water Act (SDWA) have authorized the US Environmental Protection Agency (US EPA) to screen all chemicals in manufacturing or processing where drinking water and/or food supply line could be contaminated (Snyder et al., 2003; Filali-Meknassi et al., 2004; Bolong et al., 2009).

2.1 Potential source of various EPs/ECs

Various in-practice processing approaches from different sectors including industrial, pharmaceuticals, cosmeceuticals, nutraceuticals, biomedical, veterinary medicines, nanoparticles from toxic materials and perfluorinated compound among many others are considered as a potential source of various EPs/ECs (Lapworth et al., 2012; Arnold et al., 2013; Marcoux et al., 2013; Brookes et al., 2014; Norvill et al., 2016; Bilal et al., 2017d). More specifically, significant sources include municipal biosolids, landfills and associated leachates, domestics septic systems, aquaculture related systems, elevated fertilizer practices, municipal and industrial wastewater treatment sectors. EP/EC is a broader phrase that refers to a variety of pollutants/contaminants that have recently validated with the advent of highly sensitive and precise analytical techniques which are more reliable both quantitatively and qualitatively. In this regard, the USGS Toxic Substances Hydrology Program (http://toxics.usgs.gov/regional/emc/index.html) has developed and introduced analytical techniques for EPs/ECs. Moreover, the potential environmental matrices including groundwater, surface water, soil, sediment, sludge, and bio-solids have been recommended to investigate to tackle the widespread occurrences of EPs/ECs. As discussed earlier, EPs/ECs include a broader spectrum of human-made chemical compounds e.g. pesticides, cosmetics, fertilizers, household items, dyes or pigments among others, which are requisite for the society of the modern world (Thomaidis et al., 2012; Gavrilescu et al., 2015). In between the 1930s to 2000s, an enormous increase in the chemicals production from 1 million to 400 million tons per each year has been reported in the literature (WWF, TOXIC CHEMICALS; Gavrilescu et al., 2015). According to the careful statistics by EUROSTAT published in 2013, between 2002 to 2011 approximately more than 50% of the chemical production includes environmentally harmful compounds, and among them, over 70% were with sensational environmental concerns (EUROSTAT, 2013). Numerous reports are available that indicate the amounts of chemicals used in various textile processes from preparation to finishing varies from 10% to over 100%. This extensive practice is responsible for the generation of heavy chemicals, which are toxic in nature, into the environment, especially industrial-based original dyes and dyes based toxic wastewater effluents (Bilal et al., 2016; Bilal et al., 2017a; Bilal et al., 2017b; Bilal et al., 2017c; Bilal et al., 2017d).

Some potential EPs/ECs sources includes human-made synthetic compounds, veterinary medicines e.g. antibiotic agents, hormone-based, both, natural and synthetic ones, nanoparticles from nanomaterials with toxic effects, naturally produced toxic compounds by microbes and plants, fertilizer-based compounds, both, synthetic and bio-fertilizers, physical, chemical, and biological reaction metabolites or by-products and medicinal agents from pharmaceuticals, cosmeceuticals and biomedical. The points mentioned above are some examples only. A comprehensive list of EPs/ECs classes along with model substances is available on the EU NORMAN network website (www.norman-network.net).

3 Enzyme-based strategies – A potential to degrade pollutants

In the last few years, researchers have published several research articles and scholarly reviews by highlighting this problematic issue with regard to the design and operation of enzymatic reactors and fluidized bed reactors for the degradation of recalcitrant compounds and wastewater treatments, respectively (Eibes et al., 2007; Bello et al., 2017), biodegradation of industrial pollutants by white rot fungi and their enzyme system (Asgher et al., 2008; Asif et al., 2017), emerging pollutants in the environment and their degradation aspects (Thomaidis et al., 2012; Marcoux et al., 2013; Gavrilescu et al., 2015; Norvill et al., 2016), immobilized ligninolytic enzymes as an innovative and environmental responsive technology to tackle dye-based industrial pollutants (Bilal et al., 2017a), bio-based degradation of emerging endocrine-disrupting and dye-based pollutants (Bilal et al., 2017d). Furthermore, Liu et al. (2009) and Ahmed et al. (2017) discussed major strategies including physical, chemical and biological and their potential to remove emerging contaminants e.g. endocrine disrupting compounds (EDCs) from wastewater. In another review work, Verlicchi et al. (2012) summarized contaminants removal efficacy of conventional activated sludge systems. Recently, Bilal et al. (2017a) have published a review with particular emphasis on the recent achievements of carrier-immobilized lignin-modifying enzymes (LMEs) for the degradation, decolorization, or detoxification of industrial dyes and dye-based industrial wastewater effluents. In the same study, they have discussed various immobilization strategies, physiochemical characteristics of immobilized LMEs and their environmental applications. This study overviews the biological degradation at large and enzyme-based degradation of hazardous pollutants in particular. The novel features and unique enzyme system of White Rot Fungi (WRF) are also discussed to present their potentialities and implementation against a broader spectrum of EPs/ECs. Figure 1 illustrates a schematic representation of enzyme-based degradation of hazardous contaminants.

4 Unique enzyme system of White Rot Fungi (WRF)

The WRF have shown great potentialities to produce two novel enzyme families with industrial and biotechnological interests i.e. (1) peroxidases that include lignin peroxidase (LiP; E.C. 1.11.1.14), manganese peroxidase (MnP; E.C. 1.11.1.13), and versatile peroxidase (VP; E.C. 1.11.1.16) among others, and (2) phenol oxidases that mainly comprised of laccases (E.C. 1.10.3.2). Both peroxidases and phenol oxidases have been or are being evaluated extensively for various applications (Iqbal et al., 2011; Asgher & Iqbal 2011; Asgher & Iqbal 2013; Asif et al., 2017).

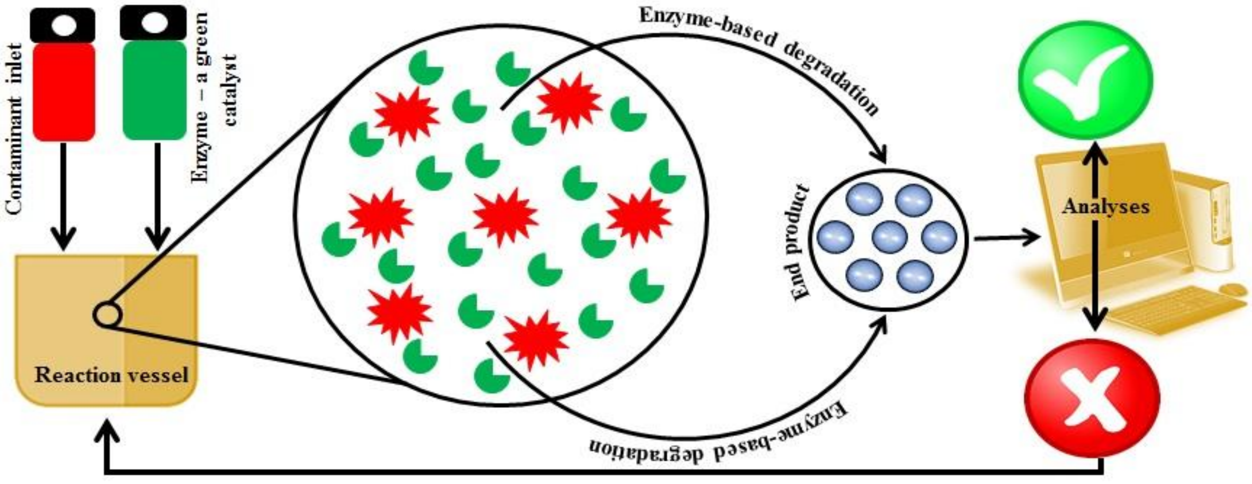

Figure 1 Enzyme-based degradation of hazardous contaminants.

The strong oxidative capabilities, low substrate specificity, and no steric selectivity render the WRF-based peroxidases and phenol oxidases, particularly fascinating for environmental exploitability (Ashger et al., 2014).

The catalytic cycle of extracellular peroxidaes from WRF is based on two-electron oxidation process in the presence of H_2O_2. The H_2O_2, as a primary oxidant, yield compound following the oxidation of the enzyme. In the next step, electron donor substrates return the oxidized form of the enzyme into the intial state following two sequential reduction steps. The wider spectrum of extracellular peroxidaes including glyoxal oxidase, pyranose oxidase, and aryl alcohol oxidase generate H_2O_2 through direct reduction of O_2 (Dosoretz & Reddy, 2007). Laccases are the widest oxidoreductases in WRF that catalyze the four-electron reduction of O_2 to water (H_2O). Likewise, H_2O_2-dependent peroxidases or Mn-dependent peroxidaes, laccase do not require H_2O_2 or Mn(II), respectively, for its catalytic role. The unique catalytic system of laccase offer diversity in oxidizing phenolic and non-phenolic compounds via O_2-dependent oxidation and free radical based oxidation, respectively (Dosoretz & Reddy, 2007; Hofrichter et al., 2010; Rodgers et al., 2010). The oxidative mechanisms are difficult to establish because of the high reactivity of the free radicals usually involved during the breakdown processes. The unique extracellular enzyme system from WRF has shown incredible potential for removing recalcitrant pollutants from wastewater, dyes, xenobiotics, toxic aromatic hydrocarbons and endocrine disrupting chemicals EDCs (Cabana et al., 2007; Eibes et al., 2007; Bilal et al., 2017d).

5 Biological degradation of hazardous contaminants

An efficient degradation of the contaminants mentioned above i.e. EPs/ECs is an urgent and requisite concern and yet to be solved. Based on the literature evidence, many previous or ongoing studies have focused on contaminants degradation potentialities of various physical, chemical, and biological approaches. Among them, bio-based or biological approaches have been the focal point of recent investigations related to the dye-based contaminants and/or EPs/ECs, as the current physiochemical techniques are environmentally unattractive, and expensive (Asgher et al., 2012a; Iqbal & Asgher, 2013; Saratale et al., 2013). Biological degradation systems that include enzyme-based degradation facilities have been widely recognized as a powerful tool for the contaminants degradation purposes (Gupta et al., 2015; Bilal et al., 2017a; Su et al., 2017). In this context, many studies have focused on direct microbial culture either fungal or bacterial and/or enzyme-based biodegradation of these contaminants effectively (Chen, 2006; Asgher et al., 2008; Asgher et al., 2009; Asgher et al., 2012a; Asgher et al., 2012b; Asgher et al., 2012c; Asgher et al., 2012d; Irshad et al., 2012; Asgher & Iqbal 2013). Under biological treatment systems, the biodegradation involves certain enzyme-based catalysts and ultimately leads to the biotransformation of hazardous pollutants (Guieysse & Wuertz, 2012; Norvill et al., 2016). Moreover, bio-based or biological approaches (bioremediation) offers considerable advantages over physio-chemical technologies including overall cost-effective ratio, environmental friendlier process, mild reaction conditions, energy saving potentialities and wasteful protection and de-protection steps. Bioremediation is a process where removal of pollutants and xenobiotic is achieved using biological systems (including micro-organisms and their enzyme system), to degrade or at least reduce the concentration of hazardous waste (Molina-Guijarro et al., 2009; Lin et al., 2010; Asgher et al., 2012a; Asgher et al., 2012b; Asgher et al., 2012c; Asgher et al., 2012d; Waghmode et al., 2011; Yang et al., 2011; Manikandan et al., 2012; Asgher et al., 2013; Asgher et al., 2016; Bilal et al., 2016; Bilal et al., 2017a; Bilal et al., 2017b; Bilal et al., 2017c; Bilal et al., 2017d). Over the past decades, many microorganisms including bacteria, fungi, yeast, and algae have been reported for the degradation purposes (Dilek et al., 1999; Novotný et al., 2001, Asgher et al., 2008; Asgher et al., 2009; Molina-Guijarro et al., 2009; Lin et al., 2010; Ramaya et al., 2010; Asgher et al., 2012a-d; Waghmode et al., 2011; Yang et al., 2011; Manikandan et al., 2012; Asgher et al., 2013; Asgher & Iqbal 2013; Iqbal & Asgher 2013; Asgher et al., 2016). However, the efficacy of microbial degradation strongly depends on the adaptation and activity of the selected microorganisms. The enzyme-based catalytic engineering can be applied to the wider spectrum of various contaminants including real dyes or dye containing industrial effluents and EPs/ECs (Asgher et al., 2013). Other significant points include a complete degradation and mineralization with low environmental impact and without the use of potentially toxic chemical substances (Robinson et al., 2001).

From a critical point of view, for an efficient biodegradation of the target substances, the following point needs to be considered with utmost care i.e. (1) overall cost-effective ratio, (2) higher percent degradation ratio with respect to the processing time, (3) maintenance of optimal reaction environments, (4) controlled parameters i.e. pH of the reaction, incubation temperature, availability of co-reactant molecules, and target substances, (5) mild and energy saving environment, (6) recyclability of the system, (7) limited need for wasteful protection and de-protection steps, (8) competitive removal mechanisms with limited or no irreversible sorption of the target substances, and (9) no or less toxicity of the end product, if any.

Despite many useful aspects, the reaction medium to high sorption affinities of various small hydrophilic and bioavailable molecules e.g. antibiotic agents may help them in stabilization and ultimately prevents them from biodegradation (Li & Zhang 2010; Michael et al., 2013). Even though the sorption could be a preliminary and

Table 1 A list of enzyme-based biodegradation of hazardous contaminants or pollutants.

Enzyme	Source	Contaminant	% removal	Reference
Horseradish peroxidase	Horseradish roots	Remazol Brilliant Blue R	82.17	Bilal et al., 2017b
Horseradish peroxidase	Horseradish roots	Reactive Black 5	97.82	Bilal et al., 2017b
Horseradish peroxidase	Horseradish roots	Congo Red	94.35	Bilal et al., 2017b
Horseradish peroxidase	Horseradish roots	Crystal Violet	87.43	Bilal et al., 2017b
MnP	*Ganoderma lucidum* IBL-05	Nonylphenol	96	Bilal et al., 2017d
MnP	*Ganoderma lucidum* IBL-05	Triclosan	75	Bilal et al., 2017d
MnP	*Ganoderma lucidum* IBL-05	Sitara textile (SIT-based) effluent	100	Bilal et al., 2017d
MnP	*Ganoderma lucidum* IBL-05	Crescent textile (CRT-based) effluent	95.5	Bilal et al., 2017d
MnP	*Ganoderma lucidum* IBL-05	K&N textile (KIT-based) effluent	88	Bilal et al., 2017d
MnP	*Ganoderma lucidum* IBL-05	Nishat textile (NIT-based) effluent	84.2	Bilal et al., 2017d
MnP	*Ganoderma lucidum* IBL-05	Dye-based effluents	>94	Bilal et al., 2017e
Laccase, MnP	*Trametes versicolor* IBL-04	Remazol Brilliant Yellow-3GL	100	Asgher et al., 2016
Laccase, MnP, LiP	*Schizophyllum commune* IBL-06	Solar brilliant red 80	100	Asgher et al., 2013
Oxidoreductive enzymes	*Lysinibacillus* sp. RGS	C.I. Remazol Red	100	Saratale et al., 2013
Laccase	*Pleurotus ostreatus* IBL-02	Drimarine blue K2RL	100	Asgher et al., 2012a
Laccase	*Coriolopsis polyzona*	Nonylphenol	>95	Cabana et al., 2007
Laccase	*Coriolopsis polyzona*	Bisphenol A	100	Cabana et al., 2007
Laccase	*Coriolopsis polyzona*	Triclosan	65	Cabana et al., 2007
Ligninolytic enzymes	*Pleurotus ostreatus, Irpex lacteus*	Remazol Brilliant Blue R	93	Novotný et al., 2001
Ligninolytic enzymes	*Pleurotus ostreatus, Irpex lacteus*	Bromophenol blue	100	Novotný et al., 2001
Ligninolytic enzymes	*Pleurotus ostreatus, Irpex lacteus*	Cu-phthalocyanine	98	Novotný et al., 2001
Ligninolytic enzymes	*Pleurotus ostreatus, Irpex lacteus*	Methyl red	56	Novotný et al., 2001
Ligninolytic enzymes	*Pleurotus ostreatus, Irpex lacteus*	Congo red	58	Novotný et al., 2001

important stage in the biodegradation of some pollutants (Esparza-Soto & Westerhoff, 2003; Urase & Kikuta, 2005; Shi et al., 2010; Norvill et al., 2016). Earlier, Nguyen et al. (2014) reported the removal of 30 different trace organic contaminants (TrOC) by comparing the role between biosorption and biodegradation. Overall, 20 to 90 % degradation of four phenolic and seven non-phenolic TrOC was achieved in the presence of a redox mediator relate to the enzyme system of WRF.

6 Enzyme-based degradation of hazardous contaminants

The unique characteristics of WRF and their novel enzyme system with novel structural and catalytic features enables them to biodegrade a huge plethora of resistants contaminants-pollutants including original dyes or dye-based effluents, polychlorinated biphenyls, polycyclic aromatic hydrocarbons and many others as described in earlier section (Hai et al., 2008; Hai et al., 2009; Gao et al., 2010; Asgher et al., 2013; Chen et al., 2015; Lopez-Echartea et al., 2016; Asgher et al., 2016; Ahmed et al., 2017; Asif et al., 2017; Bilal et al., 2017d). Recently, Su et al. (2017) developed a novel enzyme-based biocatalyst with a remarkable thermal stability and pH tolerance which are considered critical parameters for industrial exploitations.

In a recent study by Asgher et al. (2016), a statistical correlation between the LMEs secretion from *Trametes versicolor* IBL-04 and Remazol Brilliant Yellow-3GL dye degradation has been proposed (Asgher et al., 2016). In the same study, researchers have also optimized various physicochemical and nutritional parameters including nutrient media, pH, incubation temperature, carbon sources i.e. glucose, glycerol, starch, wheat bran, and lactose, and nitrogen sources i.e. ammonium oxalate, maize gluten meal (MGM) 30%, MGM 60%, and corn steep liquor (CSL) to enhance the efficiency of *T. versicolor* IBL-04 for maximum dye degradation. Furthermore, enzyme-based biodegradation of emerging endocrine disrupting contaminants e.g. nonylphenol and triclosan and dye-based pollutants has been investigated in the presence of MnP-based packed bed reactor system (Bilal et al., 2017d). Following careful optimization and characterization, an up to 80% biodegradation of both EDCs has been recorded. Whereas, the percent degradation/decolorization of dye-based effluents was varied between 84.2% to 100% in the following order, 100% for Sitara textile (SIT-based) effluent, 95.5% for Crescent Textile (CRT-based) effluent, 88.0% for K&N textile (KIT-based) effluent and 84.2% for Nishat textile (NIT-based) effluent (Bilal et al., 2017d). Table 1 summarized a comprehensive list of enzyme-based biodegradation of hazardous contaminants/pollutants. Various authors have exploited diffent enzymes or enxyme-based immobilized constructs for degradation purposes.

7 Considerable merits, demerits and considerations/ recommendation

7.1 Merits

- ✓ Environmental friendlier
- ✓ Mild and energy saving reaction environment
- ✓ WRF facilitate a facile processing environment
- ✓ Immobilized enzymes are more effective as compared to the free counterparts
- ✓ Easy to adopt and inexpensive as compared to the purified or immobilized enzymes

7.2 Demerits

- × Overall mass transfer limitation
- × Instability against higher pH and temperature
- × Denaturation of enzymes beyond the optimal environment
- × Tedious work prerequisite for enzyme extraction and purification
- × Though efficient but overall immobilization processing is expensive
- × Enzymes and enzyme-related mediator washout with the treated substances

8 Considerations/recommendation

- ➢ Catalyst washout can be avoided via immobilization engineering
- ➢ Instability feature can be enhanced/improved via immobilization engineering
- ➢ Denaturation of enzymes at higher pH and temperature conditions can be avoided via immobilization engineering
- ➢ Membrane-based bioreactors coupled with engineered enzymes can be developed for efficient degradation potentialities
- ➢ Using advanced molecular approaches highly active and efficient enzymes systems can be engineered as future catalysts with maximal degradation and reusability features

Concluding remarks

In conclusion, the above-reviewed data suggests that biological degradation at large and enzyme-based degradation of hazardous pollutants, in particular, are of supreme interest. Enzyme-based degradation has already been recognized as a powerful tool for the treatment or biodegradation of various types of existing or emerging contaminants. A plethora of hazardous pollutants has been efficiently treated/eliminated though using different methodologies, worldwide. However, environmental pollution is rapidly expanding with increased development in the entire industrial sector of the modern world. Owing to this heavily in practice human-made industrialization and synthetic manufacturing processes, the continuous disposal/discharge of toxic compounds with/without partial or insufficient treatments is among a major a cause of environmental contamination, both terrestrial and aquatic systems. Nonetheless, more wide-ranging and all-inclusive efforts are desirable to interpret the transformation products generated during the treatment procedures.

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Disclosure statement

Authors declare that no conflict of interest could arise.

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