



Bioremediation of Endocrine Disruptive Chemicals: The Power of Microbial Enzymes

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Authors' contributions

This work was carried out in collaboration between all authors. Authors NHFH and SSS managed the outline and literature search and wrote the first draft of the manuscript. Authors MAK and NAY helped in the writing certain part of the manuscript and revised the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Microbial enzymes have been established as a powerful tool in bioremediation. In this review, applications of microbial enzymes in degradation of endocrine disruptive chemicals are explained. Endocrine disruptive chemicals (EDCs) are a number of pollutants reported bringing negative impacts on human being and wildlife. Exposure to EDCs may cause effect on endocrine system thus detrimental to the health eminently on the developmental and reproductive abnormalities. Physical approaches such as ozonation, activated carbon, nanofiltration have been implemented to remove EDCs in waste water. Over the past decade, bioremediation has been a preference method in EDCs degradation due to its environmental friendly approach. Lignin-modifying enzymes; LMEs have been the attractive candidates in EDC treatment due to their characteristics such as broad selectivity in substrate affinity and ability to degrade either complex, individual

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pollutants or low water solubility compounds. Usage of microbial enzymes either by using the whole organism or free enzymes has been introduced in water treatment. Current technology by immobilizing enzymes to certain matrix allow the enzymatic recycling process thus giving an advantage in operational cost.

Keywords: Microbial enzymes; lignin-modifying enzymes; endocrine disruptor chemical; green technology.

1. INTRODUCTION

All human beings in the world are contributing to the environmental pollution issues. Industrial processes, agricultural activities and not to mention our daily activities like gardening, bathing, household chores contribute indirectly by accidental released of toxic chemicals into the environment. The toxic materials that are released may be soluble and able to sustain in the environment for hundreds of years. Among of the major contaminants released to the environment are the endocrine disruptive chemicals (EDC). EDCs present part of major environmental threat on living organism. The lethal, toxic compounds are commonly released into the environment from chemical industries, chemical by-products, pharmaceuticals and personal care products. Although they may exist in small concentration in the environment, continuous or intermittent long-term exposure to EDCs can cause tremendous of health issues due to the chemicals ability to disrupt/alter normal hormonal functions by binding to the hormone receptors [1].

In order to be degraded by biological mechanisms, the toxic chemicals need to be exposed to a biological agent or catalyst; defined as bioremediation. Bioremediation has been receiving quite a lot of attention in recent years as this technology is cost competitive compared to present technologies. Usage of microbial enzymes as biocatalysts in EDCs degradation has been reported to be used in removing the EDCs efficiently [2-5]. To date, bioremediation by microbes has shown high potential applications in waste treatment due to its metabolic capabilities at extreme environments. In this review, we will explore and emphasize on the several categories of EDCs, microbial enzymes and the applications of the organisms by themselves which have been reported to be used in bioremediation.

2. EDC

Organization of Economic and Cooperative Development (OECD) has defined Endocrine

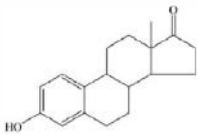
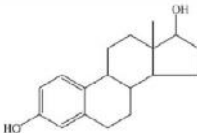
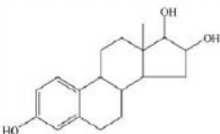
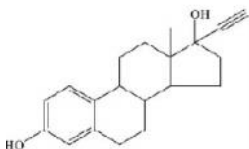
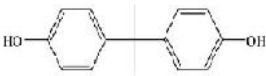

disrupting compounds (EDCs) as “an exogenous substance or mixture that alters the function(s) of the endocrine systems and consequently causes adverse health effects in an intact organism, or its progeny or (sub) populations” [6]. EDCs include a broad spectrum of compounds derived from industrial, agricultural and domestic processes. For example; phthalates and bisphenols (plastic manufacturing), alkyl phenols (detergents and surfactants), polychlorinated and polybrominated biphenyls, dioxins (incinerators), organochlorine pesticides and organohalogens (flame retardants) [7]. In general, these chemicals consist of a wide variety of chemical structures which all of them have the capacity to disrupt normal hormone functions. Due to that matter, they are plausibly toxics to reproductive and immune systems in attributing to carcinogenic activities. Chemical structures of common EDCs found in polluted environments showed structural similarities with endogenous hormones like estrogens [8,9]. Commission of the European Communities has listed more than hundreds of substances classified as EDCs including estradiol, estrones, nonylphenols (NP), bisphenol (BPA) and triclosan (TCS) (Commission of the European Communities, 2001). With the large number of EDCs, one can expect that the effects also vary to the environment, human and wildlife. Table 1 listed the chemical structures and physicochemical properties of common EDCs found in the environment as reviewed by Zhang et al. [10]. In this review, we focus on estrogens, phthalates, and biphenyls.

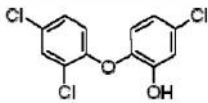
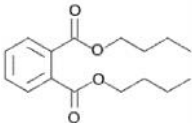
Bisphenol A (BPA) (4,4-isopropylidenediphenol; 2,2-bis(4-hydroxyphenyl)-propane) is one of the most common EDCs found in the environment due to its broad applications especially in polymers production like polycarbonate [11]. Due to its high melting point (~156°C), low adsorption of moisture and high in thermal stability, BPA can be found in a wide range of products such as water pipes, drinking bottles, toys, electronic devices and thermal papers [12]. It is a white crystalline substance with a molecular weight of 228.3 g/cm³ and has low solubility in water (200 mg/dm³ at 25°C) [12]. The existence of BPA

substance in the environment is mainly due to the industrial activities. Several research finding has been reporting the presence of BPA detected at low concentrations ranging from 190 ng/L to 21 mg/L from the water surface and marine water [13]. Although it is present in low

amounts, long-term exposure to low-dose BPA still impart negative impacts on living organisms especially humans. Moreover, BPA has been reported as being able to interact with estrogen, androgen, and thyroid receptors.

Table 1. Physiochemical properties of common EDCs

	Category	Molecular weight (g/mol)	Water solubility (mg/L)	pKa	
	Estrones (E1)	Natural estrogen	270.4	30	10.5
					
	17 β -estradiol (E2)	Natural estrogen	272.4	3.6	10.7
					
	Estriol (E3)	Natural estrogen	288.4	441	10.4
					
	17 α -Ethinylestra-diol (EE2)	Synthetic estrogen	296.4	116	10.4
					
	Bisphenol A (BPA)	Industrial chemical	228.3	120	9.6
					
	Nonylphenol (NP)	Industrial chemical	220.4	5.71	1.03
					

	Category	Molecular weight (g/mol)	Water solubility (mg/L)	pKa
Triclosan (TCS)	Antimicrobial agent	289.54	6.05	7.9
				
Dibutyl phthalate (DBP)	Phthalate	278.35	13	4.72
				

In the same context, nonylphenols (NPs) are widely used as industrial and domestic surfactants, which are added to a variety of products such as dispersants, emulsifiers, detergents, dyes, antioxidants, pesticides, spermicides, and cosmetics [14,15]. Furthermore, due to their characteristics which are slowly degradable under aerobic conditions and persistency at low temperatures, these compounds are accumulating and causing a threat to living organisms [16]. NPs act as competitors to natural hormone E2 as they are able to mimic the structure and bind to the receptor [17]. Chemical structures of NPs consist of the phenolic group attached to the carbon side chain while their high hydrophobicity contributes to the xenoestrogenic activity [8,9].

Besides that, phthalates (PAEs) such as dibutyl phthalate (DBP) and diethyl phthalate (DEP) are the main components in plastics formulation such as PVC. Due to its weak bonds in the covalent bonding interactions, these compounds are easily leached out from the polymers resulting in a harmful form of environmental pollution. Unfortunately, exposure to a small dosage of DBP, however, interferes with reproductive system and human behavioral by disturbing endocrine system [18,19]. It has been reported that PAEs interfere at mRNA level involving a broad range of human genes [20]. Moreover, DBP has been reported to disrupt production of testosterone hormone as well as male reproductive development [21].

The natural and synthetic estrogens are commonly found in polluted environments due to their broad usages in pharmaceutical products, livestock commodities and human organs like cholesterol, testis, ovary, and placenta [22]. Not

only that, plants are also reported to be able to secrete phytoestrogens like genistein and β -sitosterol [9]. Generally, estrogens are not very soluble in water with 17 α -Ethinylestra-diol (EE2) being the least soluble. At pH 7, the order of increasing aqueous solubility is Estrones (E1) (one OH group) to 17 β -estradiol (E2) (two OH groups) followed by EE2 with the added ethinyl groups at 17 α -position on the D ring [23].

3. CURRENT TECHNOLOGIES IN EDC REMOVAL

Presence of EDCs in water sources has raised great attention to the community due to their effects on health, behavioral and development of living organisms. In order to overcome these issues, revolutionizing effective methods and innovative technologies are structured to combat the issues. Currently, mass spectrometry is the most favored technique in identification and quantification of EDCs substances in a natural environment such as gas chromatography (GCMS/MS), liquid chromatography (LCMS/MS) as well as the time of flight (TOF). Even so, due to the fact concentration of EDCs in the environment may not reach to part per million, the detection of EDCs in most laboratories is not an easy task [24].

EDCs removal methods fall into three categories: physical removal, chemical advanced oxidation (CAO) and bioremediation. Physical removal has been a method of choice for the effective removal of EDCs from the water. However, the inconsistency in the removal of EDCs has brought a disadvantage to this method. Furthermore, adsorption method using activated carbon is able to remove EDCs up to 95%, however, it is highly influenced by operational

factors like kinetic constants, contact time, solubility and the carbon skeleton type [25]. Granular activated carbon adsorber was shown able to remove nonylphenols effectively in drinking water treatment plan [25]. Membrane separation technique is greatly affected by the matrix and usually insufficient in EDCs removal [26]. In addition, the chemical advanced oxidation (CAO) method involve the mineralization of pollutants in waste water to carbon dioxide or transfer of pollutants to other products through oxidation reduction reactions. In order to increase the removal effect, some combinations are applied such as UV/O₃, UV/H₂O₂, UV/Fenton which are widely applied to the removal of EDCs. In such a way, generation of the hydroxyl radical is obtained (redox potential 2.80 V) [27]. Although this is the preferable choice, CAO brings several disadvantages such as time consuming, high in cost and toxic byproducts from the treatment [12].

Additionally, bioremediation has been receiving much attention in recent years. Recently, microbial degradation of aqueous EDC has been the limelight among researchers. Bisphenol A (BPA), which is recalcitrant in anaerobic treatment is degraded to 4-hydroxybenzoic acid and 4-hydroxyacetophenone by gram-negative bacteria under aerobic conditions [28]. The enzymatic degradation of aqueous EDCs with oxidases have also been reported; the manganese peroxidases from *Phanerochaete chrysosporium* was found to degrade bisphenol A [2]. The enzymatic-degrading microorganisms are inoculated or stimulated in the polluted sites by supplying the required growth nutrients. However, both inoculation and growth control of the microorganisms may cause an increase of the biochemical oxygen demand in the aquatic environment [29]. Hence, treatment with enzymes is expected to diminish defects of the bioremediation by microorganisms since enzymes are not greatly affected by the supply of nutrients in the environment.

4. LIGNIN-MODIFYING ENZYMES (LMEs)

Lignin-modifying enzymes are a group of enzymes which are involved in lignin breakdown from biomass. Fungi belonging to mainly *Basidiomycetes* and a few *Ascomycetes* termed as white rot fungi are generally known as the best LMEs producer as these fungi naturally colonize lignocellulose material [30].

Lignin consists a complex structure of polymer which generally is the main component of lignocellulose materials. Lignin decomposition is a crucial step in order for the fungi to gain their carbon sources from polysaccharides which resides within the lignocellulose complex. Due to that, the extracellular enzymes consisting of several LMEs are essential for lignin degradation. The main LMEs are lignin peroxidases (LiP; E.C. 1.11.1.14), manganese-dependent peroxidases (MnP; E.C. 1.11.1.13), versatile peroxidases (VP; E.C. 1.11.1.16) and laccases (Lac; E.C. 1.10.3.2) [9,31].

5. LMEs IN EDCs DEGRADATION

LMEs have been the attractive candidates in EDCs treatment due to their broad selectivity in substrates affinity, ability to degrade either complex or individual pollutants with low water solubility substances [32]. The growing attention on the removal of EDCs from environmental matrices is making this group of enzymes as attractive candidates in the bioremediation [1,5,9,31,33-35]. Moreover, LMEs can be introduced to the polluted environments either by using the fungi itself, free enzymes or enzyme immobilization to a specific matrix. Enzyme immobilization technique is the most recommended as the enzymes (catalysts) can be retained within the system, reusable and offer a rapid, cost-efficient technique [36].

In addition, laccases are a class of various extracellular ligninolytic enzymes produced by fungi to degrade lignin and other polyphenolic compounds in woods. This group of enzymes is one of the versatile LMEs that can degrade a wide range of EDCs such as BPA, nonylphenols, and estrogens [36-39]. Laccase from *Trametes versicolor*, *Trametes vilosa*, and *Coriolopsis polyoma* are among the widely studied due to its strong degrading activity towards BPA, NPs and triclosan [1,40-41]. Immobilization of laccases from *T. versicolor* and *Myceliophthora thermophila* was reported to be able to remove EDCs (NPs and BPA) in artificial mixtures (99% removal after 24 h) as well in real waste water (82% removal after 24 h) [38]. Immobilized enzymes displayed better removal performance compared to the free enzymes. In this study, significant removal of EDC was detected by free laccase for *T. versicolor* (44%) as well as for *M. thermophila* (54%) after 24 h. Elimination of BPA and NPs by immobilized laccase on acrylic beads showed much high removal rates (97% after 24 h for both immobilized enzymes). Dâassi

et al. reported the ability of different fungal laccases; *T. versicolor*, *Coriolopsis gallica* and *Bjerkandera adusta* to remove BPA completely after treatment at 45°C for 24 h with *C. gallica* that gave the highest oxidation rate (91%) compared to others; 85% for *B. adusta* and 72% for *T. versicolor* [37]. Analysis using GC-MS/MS on degradation products of BPA showed *C. gallica* produced different by-product peak profile compared to the two other laccases; suggesting *C. gallica* laccase may have different catalytic and kinetic properties. Besides that, the lac-bioTiO₂ particles, a titania matrix triggered on the surface of magnetic particles in the presence of laccase was able to catalyze the removal of bisphenol A, 17 α -ethinyloestradiol, and diclofenac in a mixture matrix and retained 90% of activity after five reaction cycles and 60% after 10 cycles of reaction [36].

Removal of BPA was also achieved by using manganese peroxidase (MnP) from *Pleurotus ostreatus* O-48 [42]. The complete removal was achieved after 21 days in the culture medium. Proposed degradation pathway which was outlined in this study showed several compounds were generated as the byproducts of BPA degradation. These include a hexestrol; an estrogenic compound other than phenol and 4-isopropenylphenol. Over 95% of NP and 82% of TCs were eliminated from the solution after 3 h of treatment with native MnP from *Trametes versicolor* IBL-04 [43]. They showed remarkable potential to eliminate toxic EDCs with good degradation efficiency at 40°C in aqueous solution. A multiple P450 monooxygenase proteins from *P. cladosporium* were reported to be involved in degradation of nonylphenols (NP) in both nutrient-limited cultures and nutrient-sufficient cultures [44]. Interestingly, using genomic approaches a total of 17 genes were shown to be upregulated in limited nutrient condition while 18 genes were induced under nutrient-rich conditions where all the upregulated genes were associated with nonylphenols degradation. Pezzella et al. reported *T. versicolor* was the most efficient fungus in degradation of different classes of EDCs; phenols, paraben and phthalate compared to two other fungal; *P. ostreatus* and *P. chrysosporium* [45]. Further investigation using *T. versicolor* to degrade mixtures of EDCs was performed in bioreactor has shown that the fungus was able to completely degrade the EDCs (up to 21 mg/L) in 4 days of incubation. Furthermore, *T. versicolor* was also found to be able to remove EDCs without any additional nutrients; thus lowering the

cost and gave advantage in a water treatment process.

6. CONCLUDING REMARK

Bioremediation of EDCs using microbial enzymes are promising processes for water treatment towards green technology process. However, several issues need to be considered; i. the formulation and cost of using LMEs in water treatment; ii. Transformation pathway of EDCs using LMEs need to be outlined in order to know if any of the byproducts are toxic to the environment. Despite the issues raised above, enzymatic treatment does offer an environmentally friendly approach to overcome the health-related issues.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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